



EPA/635/R-24/162bF
www.epa.gov/iris

IRIS Toxicological Review of Formaldehyde (Inhalation) Supplemental Information

[CASRN 50-00-0]

August 2024

Integrated Risk Information System
Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed by the U.S. Environmental Protection Agency, Office of Research and Development and approved for publication. Any mention of trade names, products, or services does not imply an endorsement by the U.S. government or the U.S. Environmental Protection Agency. EPA does not endorse any commercial products, services, or enterprises.

CONTENTS

APPENDIX A. BACKGROUND INFORMATION SUPPLEMENT	A-1
A.1. CHEMICAL PROPERTIES	A-1
A.2. PRODUCTION, SOURCES, AND USES OF FORMALDEHYDE	A-2
A.3. HUMAN INHALATION EXPOSURE TO FORMALDEHYDE.....	A-4
APPENDIX B. SYSTEMATIC REVIEW METHODS DOCUMENTATION.....	B-1
B.1. SUMMARY OF DOCUMENTATION	B-1
B.2. LITERATURE SEARCH AND SCREENING	B-1
B.2.1. Overview	B-1
B.2.2. Sensory Irritation.....	B-2
B.2.3. Pulmonary Function	B-7
B.2.4. Immune-Mediated Conditions, including Allergies and Asthma	B-11
B.2.5. Respiratory Pathology	B-17
B.2.6. Mechanistic Information Relevant to Noncancer Respiratory Effects.....	B-26
B.2.7. Nervous System Effects.....	B-41
B.2.8. Developmental and Reproductive Effects.....	B-48
B.2.9. Cancer.....	B-54
B.2.10. Mechanistic Information Relevant to Cancer	B-66
B.3. STUDY EVALUATION	B-81
B.3.1. Exposure Quality Evaluations.....	B-81
B.3.2. Sensory Irritation.....	B-104
B.3.3. Pulmonary Function	B-128
B.3.4. Immune-Mediated Conditions Including Allergies and Asthma	B-152
B.3.5. Respiratory Pathology	B-185
B.3.6. Mechanistic Information Related to Inflammation and Immune Effects	B-200
B.3.7. Nervous System Effects.....	B-252
B.3.8. Developmental and Reproductive Effects.....	B-282
B.3.9. Cancer.....	B-302
B.3.10. Mechanistic Information Related to Genotoxicity and Cancer.....	B-371
APPENDIX C. HAZARD IDENTIFICATION SUPPLEMENT.....	C-1

Supplemental Information—Formaldehyde (Inhalation)

C.1.	TOXICOKINETICS OF INHALED AND ENDOGENOUS FORMALDEHYDE.....	C-1
C.1.1.	Toxicokinetics of Inhaled Formaldehyde at the Portal of Entry (POE).....	C-1
C.1.2.	Spatial Distribution of Tissue Uptake of Formaldehyde at the Portal of Entry	C-3
C.1.3.	Tissue Penetration of Formaldehyde Within the Upper Respiratory Tract	C-7
C.1.4.	Modifying Factors and Specific Uncertainties Regarding the Toxicokinetics of Inhaled Formaldehyde Within the POE	C-23
C.1.5.	Conclusions Regarding the Toxicokinetics of Inhaled Formaldehyde Within the POE	C-29
C.1.6.	Toxicokinetics of inhaled formaldehyde beyond the portal of entry	C-30
C.1.7.	Levels of Endogenous and Inhaled Formaldehyde in Blood and Distal Tissues	C-30
C.1.8.	Conjugation, Metabolism, and Speciation of Formaldehyde Outside the POE	C-38
C.1.9.	Elimination Pathways of Exogenous and Endogenous Formaldehyde	C-39
C.1.10.	Conclusions Regarding the Toxicokinetics of Inhaled Formaldehyde Outside of the POE.....	C-42
C.1.11.	Toxicokinetics Summary	C-43
C.1.12.	Modeling Formaldehyde Flux to Respiratory Tract Tissue.....	C-44
C.2.	REFLEX BRADYPNEA.....	C-62
C.2.1.	The signs of reflex bradypnea	C-63
C.2.2.	Irritation, reflex bradypnea, and the RD ₅₀	C-65
C.2.3.	Tolerance	C-67
C.2.4.	Reflex bradypnea and interpreting health effects data	C-67
C.2.5.	Summary	C-69
C.3.	GENOTOXICITY	C-70
C.3.1.	Genotoxicity of Formaldehyde in Cell-Free Systems	C-71
C.3.2.	Genotoxicity of Formaldehyde in Prokaryotic Organisms	C-72
C.3.3.	Genotoxicity of Formaldehyde in Nonmammalian Systems	C-78
C.3.4.	Genotoxicity of Formaldehyde in in Vitro Mammalian Cells	C-81
C.3.5.	Genotoxicity of Formaldehyde in Experimental Animals.....	C-103
C.3.6.	Genotoxic Endpoints in Humans	C-120
C.3.7.	Summary Table by Genotoxicity Endpoint.....	C-169
C.4.	SENSORY IRRITATION (SUPPORTING INFORMATION FOR HAZARD)	C-176
C.5.	PULMONARY FUNCTION (SUPPORTING INFORMATION FOR HAZARD)	C-186
C.5.1.	Controlled human exposure studies	C-187

Supplemental Information—Formaldehyde (Inhalation)

C.5.2. Change in pulmonary function measures during a work shift or anatomy lab session.....	C-190
C.6. RESPIRATORY TRACT PATHOLOGY (SUPPORTING INFORMATION FOR HAZARD).....	C-196
C.6.1. Short-term respiratory tract pathology studies in experimental animals	C-196
C.7. MECHANISTIC EVIDENCE RELATED TO POTENTIAL NONCANCER RESPIRATORY HEALTH EFFECTS (SUPPORTING INFORMATION FOR HAZARD)	C-201
C.7.1. Synthesis of Changes in the Upper Respiratory Tract (URT)	C-202
C.7.2. Synthesis of Changes in the Lower Respiratory Tract (LRT)	C-237
C.7.3. Synthesis of Changes in the Blood and Lymphoid Organs	C-251
C.7.4. Integrated Mechanistic Evidence Synthesis by Tissue Compartment	C-267
C.8. CANCER (SUPPORTING INFORMATION FOR HAZARD)	C-285
C.8.1. Cancers other than Upper Respiratory Tract and LHP Cancers	C-285
APPENDIX D. DOSE-RESPONSE ASSESSMENT SUPPLEMENT	D-1
D.1. DOSE-RESPONSE ANALYSIS FOR NONCANCER HEALTH EFFECTS	D-1
D.1.1. Sensory Irritation.....	D-2
D.1.2. Pulmonary Function	D-27
D.1.3. Asthma Exacerbation	D-29
D.1.4. Respiratory Tract Pathology	D-33
D.1.5. Reproductive Toxicity in Males	D-40
D.2. DOSE-RESPONSE ANALYSIS FOR CANCER	D-43
D.2.1. Supporting Information for Upper Respiratory Tract (Nasal) Cancer Estimates based on Epidemiology Data	D-43
D.2.2. Supporting Information for Upper Respiratory Tract (Nasal) Cancer Estimates based on Animal Data	D-46
D.2.3. Supporting Information for Lymphohematopoietic Cancer Estimates	D-126
D.2.4. Estimates Using DNA Adduct Data in Animals and Background Incidence.....	D-136
APPENDIX E. QUALITY ASSURANCE FOR THE IRIS TOXICOLOGICAL REVIEW OF FORMALDEHYDE	E-1
APPENDIX F. RESPONSE TO EXTERNAL COMMENTS	F-1
F.1. NASEM REPORT CHAPTER 2: “METHODS AND ORGANIZATION”; CHARGE QUESTION 1: ASSESSMENT DEVELOPMENT METHODS AND ORGANIZATION.....	F-3
F.1.1. Charge Question(s) and Peer Review Comment Summary.....	F-3
F.1.2. Response to Prior National Research Council (NRC) Reports	F-4
F.1.3. Alignment of the Assessment Methods with the State of Practice	F-5
F.1.4. Other Public Comments on Assessment Methods and Organization	F-32
F.2. NASEM REPORT CHAPTER 3: TOXICOKINETICS; CHARGE QUESTION 2: TOXICOKINETICS	F-35

Supplemental Information—Formaldehyde (Inhalation)

F.2.1. Charge Question and Peer Review Comment Summary.....	F-35
F.2.2. Distribution of Inhaled Formaldehyde; Metabolism, Binding, and Removal of Inhaled Formaldehyde	F-36
F.2.3. EPA's use of Toxicokinetic Data and Dosimetry Models	F-37
F.3. NASEM REPORT CHAPTER 4: NONCANCER HEALTH EFFECTS; CHARGE QUESTION 3: RESPIRATORY SYSTEM (PORTAL-OF-ENTRY) HEALTH EFFECTS, CHARGE QUESTION 4: SYSTEMIC HEALTH EFFECTS, AND CHARGE QUESTION 5: NONCANCER RFC	F-53
F.3.1. Charge Questions on Noncancer Health Effects and the Noncancer RfC	F-53
F.3.2. Sensory Irritation.....	F-54
F.3.3. Pulmonary Function	F-70
F.3.4. Respiratory Pathology	F-78
F.3.5. Allergy and Asthma	F-82
F.3.6. Reproductive and Developmental Toxicity	F-95
F.3.7. Nervous System Effects	F-104
F.3.8. Derivation of the RfC.....	F-109
F.4. NASEM REPORT CHAPTER 5: CANCER; CHARGE QUESTION 6: CANCER AND CHARGE QUESTION 7: IUR FOR CANCER.....	F-125
F.4.1. Hazard Identification	F-125
F.4.2. Dose-Response Analysis of Cancer Effects of Formaldehyde	F-162
F.5. GENERAL PUBLIC COMMENTS AND PUBLIC COMMENTS NOT ADDRESSED UNDER THE TOPIC AREAS ABOVE.....	F-186
APPENDIX G. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES	G-1
REFERENCES	R-1

TABLES

Table A-1. Physicochemical properties of formaldehyde	A-2
Table A-2. Ambient air levels by land use category based on 2018 annual site averages	A-6
Table A-3. Formaldehyde emission rates from various consumer products	A-9
Table A-4. Studies on residential indoor air levels of formaldehyde	A-10
Table B-1. Search terms for sensory irritation	B-2
Table B-2. Studies of sensory irritation effects in humans	B-5
Table B-3. Summary of search terms for pulmonary function	B-7
Table B-4. Studies of pulmonary function effects in humans	B-10
Table B-5. Search terms – allergy-related conditions and asthma	B-11
Table B-6. Studies of immune-mediated conditions in humans, focusing on allergies and asthma	B-14
Table B-7. Search terms for respiratory tract pathology in humans	B-17
Table B-8. Search terms for respiratory tract pathology in animals	B-18
Table B-9. Studies of respiratory tract pathology in humans	B-23
Table B-10. Animal studies of respiratory tract pathology	B-24
Table B-11. Summary of supplemental literature search terms for mechanistic studies relevant to potential noncancer respiratory health effects	B-26
Table B-12. Mechanistic studies relating to respiratory or systemic inflammatory and immune responses	B-31
Table B-13. Summary of search terms for nervous system effects	B-41
Table B-14. Studies of nervous system effects	B-45
Table B-15. Summary of search terms for developmental or reproductive toxicity	B-48
Table B-16. Studies of reproductive and developmental effects	B-52
Table B-17. Summary of search terms for carcinogenicity in humans	B-54
Table B-18. Summary of search terms for respiratory tract cancers in animals	B-55
Table B-19. Summary of search terms for lymphohematopoietic cancers in animals	B-55
Table B-20. Studies of site-specific cancer in humans	B-62
Table B-21. Animal studies of respiratory tract cancers	B-64
Table B-22. Animal studies of lymphohematopoietic cancer	B-65
Table B-23. Search terms for cancer mechanisms of respiratory cancers	B-66
Table B-24. Search terms for cancer mechanisms of LHP cancers	B-68
Table B-25. Mechanistic studies relating to respiratory tract cancers, focusing on genotoxicity	B-71
Table B-26. Mechanistic studies relating to lymphohematopoietic cancers, focusing on genotoxicity	B-76
Table B-27. Inhalation exposure quality: formaldehyde (Note: exposure deficiencies are shaded)	B-83
Table B-28. Evaluation of studies examining sensory irritation in humans: residential studies	B-104
Table B-29. Evaluations of studies examining sensory irritation in humans: school-based studies	B-111
Table B-30. Evaluations of studies examining sensory irritation in humans: controlled human exposure studies	B-112
Table B-31. Evaluation of studies examining sensory irritation in humans: anatomy courses	B-116
Table B-32. Evaluations of studies examining sensory irritation in humans: occupational studies	B-121
Table B-33. Evaluation of formaldehyde - pulmonary function epidemiology studies	B-128
Table B-34. Evaluation of formaldehyde - pulmonary function controlled human exposure studies	B-150
Table B-35. Evaluation of allergy and asthma studies	B-153

Supplemental Information—Formaldehyde (Inhalation)

Table B-36. Evaluation of lower respiratory symptoms in infants and young children (up to age 3 years)	B-179
Table B-37. Evaluation of controlled acute exposure studies among people with asthma	B-181
Table B-38. Respiratory pathology study evaluations: epidemiology studies	B-185
Table B-39. Respiratory pathology study evaluations: controlled exposure studies in humans.....	B-191
Table B-40. Evaluation of controlled inhalation exposure studies examining respiratory pathology in animals.....	B-192
Table B-41. Evaluation of controlled inhalation exposure studies examining cell proliferation and mucociliary function in animals	B-201
Table B-42. URT-specific structural modification, sensory nerve-related changes, or immune and inflammation-related changes.....	B-205
Table B-43. LRT (e.g., lung, trachea, BAL) markers of structural modification, immune response, inflammation, or oxidative stress	B-213
Table B-44. Changes in pulmonary function involving provocation (e.g., bronchoconstrictors; allergens; etc.).....	B-226
Table B-45. Serum (primarily) antibody responses.....	B-231
Table B-46. Serum markers of immune response (other than antibodies), inflammation, or oxidative stress	B-236
Table B-47. Effects on other immune system-related tissues (e.g., bone marrow, spleen, thymus, lymph nodes, etc.)	B-244
Table B-48. Effects on other tissues (data extracted for possible future consideration, but not included in this assessment)	B-249
Table B-49. Evaluation of observational epidemiology studies of formaldehyde—neurological effects	B-252
Table B-50. Evaluation of human controlled exposure studies of formaldehyde – nervous system effects	B-259
Table B-51. Evaluation of controlled inhalation exposure studies examining nervous system in animals.....	B-261
Table B-52. Evaluation of studies pertaining to mechanistic events associated with nervous system effects	B-274
Table B-53. Evaluation of observational epidemiology studies of formaldehyde - reproductive and developmental outcomes	B-282
Table B-54. Study quality evaluation of developmental and reproductive toxicity animal studies	B-295
Table B-55. Categorization of exposure assessment methods by study design.....	B-303
Table B-56. Outcome-specific effect estimates classified with High confidence	B-305
Table B-57. Outcome-specific effect estimates classified with Medium confidence	B-305
Table B-58. Outcome-specific effect estimates classified as not informative.....	B-308
Table B-59. Evaluation of occupational cohort studies of formaldehyde and cancers of the URT (NPC, SN, OHPC) and LHP (HL, MM, LL, ML)	B-309
Table B-60. Evaluation of case-control studies of formaldehyde and cancers of the URT (NPC, SN, OHPC) and LHP (HL, MM, LL, ML)	B-337
Table B-61. Evaluation of controlled inhalation exposure studies examining respiratory tract cancer or dysplasia in animals	B-363
Table B-62. Evaluation of controlled inhalation exposure studies examining lymphohematopoietic cancers in animals.....	B-369
Table B-63. Evaluation of genotoxicity endpoints in epidemiology studies of formaldehyde exposure	B-372

Supplemental Information—Formaldehyde (Inhalation)

Table C-1. Dosimetry and response of formaldehyde in experimental animals by indirect measurements	C-3
Table C-2. Comparison of formaldehyde uptake at the portal of entry with single or repeated inhalation exposure	C-6
Table C-3. ADH3 kinetics in human and rat tissue samples and cultured cells	C-11
Table C-4. Levels of folate intermediates, activity of folate-dependent enzymes, and the rate of oxidation of formate in the liver of various species	C-14
Table C-5. Summary of endogenous and exogenous DNA-protein crosslinks in nasal tissues of rats following inhalation exposure of ¹³ CD ₂ -labeled formaldehyde	C-16
Table C-6. Summary of endogenous and exogenous DNA monoadducts in nasal tissue of monkeys and rats following inhalation exposure of ¹³ CD ₂ -labeled formaldehyde	C-20
Table C-7. Summary of blood and tissue levels of total ^a formaldehyde in humans and experimental animals following inhalation exposure to formaldehyde.....	C-32
Table C-8. Summary of endogenous and exogenous DNA monoadducts in distal tissues of monkeys and rats following inhalation exposure of ¹³ CD ₂ -labeled formaldehyde	C-35
Table C-9. Summary of endogenous and exogenous DNA-protein crosslinks in distal tissues of monkeys and rats following inhalation exposure of ¹³ CD ₂ -labeled formaldehyde	C-38
Table C-10. Summary of excretion study following exposure to formaldehyde by inhalation in rats	C-40
Table C-11. Measured levels of formaldehyde, methanol and ethanol in room air and exhaled breath.....	C-41
Table C-12. Formaldehyde respiratory depression (RD) values for several mouse strains and exposure durations	C-66
Table C-13. Formaldehyde respiratory depression (RD) values for several rat strains and exposure durations.	C-66
Table C-14. Summary of genotoxicity of formaldehyde in cell-free systems	C-71
Table C-15. Summary of genotoxicity of formaldehyde in prokaryotic systems	C-73
Table C-16. Summary of genotoxicity studies for formaldehyde in nonmammalian organisms	C-79
Table C-17. Summary of <i>in vitro</i> genotoxicity studies of formaldehyde in mammalian cells	C-90
Table C-18. Summary of <i>in vivo</i> genotoxicity studies of formaldehyde inhalation exposure in experimental animals	C-110
Table C-19. Summary of <i>in vivo</i> genotoxicity studies of formaldehyde exposure by intraperitoneal and oral routes of exposure in experimental animals.....	C-116
Table C-20. Summary of genotoxicity of formaldehyde in human studies	C-128
Table C-21. Genotoxicity summary table.....	C-170
Table C-22. Summary of epidemiology studies of laboratory exposures to formaldehyde and human sensory irritation	C-176
Table C-23. Summary of epidemiology studies of occupational exposures to formaldehyde and human sensory irritation	C-181
Table C-24. Formaldehyde effects on pulmonary function in controlled human exposure studies	C-187
Table C-25. Studies of pulmonary function measures during a work shift or anatomy lab session.....	C-194
Table C-26. Short-term respiratory pathology studies in animals	C-197
Table C-27. Summary of changes in the upper respiratory tract (URT) resulting from formaldehyde exposure	C-207
Table C-28. Mucociliary function studies in experimental animals	C-214
Table C-29. Mucociliary function studies in humans	C-216
Table C-30. Subchronic or chronic exposure cell proliferation studies in experimental animals.....	C-223
Table C-31. Short-term exposure cell proliferation studies in experimental animals	C-227

Supplemental Information—Formaldehyde (Inhalation)

Table C-32. Summary of changes in the lower respiratory tract (LRT) as a result of formaldehyde exposure	C-243
Table C-33. Summary of changes in LRT cell counts and immune factors as a result of formaldehyde exposure	C-247
Table C-34. Summary of changes in the blood and lymphoid organs as a result of formaldehyde exposure	C-255
Table C-35. Summary of changes in blood cell counts and immune factors as a result of formaldehyde exposure	C-261
Table D-1. Concentration-response information for the central estimate of the effect extracted from (Hanrahan et al., 1984).	D-4
Table D-2. Concentration-response information for the upper bound on the central estimate of the effect extracted from (Hanrahan et al., 1984)	D-5
Table D-3. Extracted data from (Liu et al., 1991) and (Sexton et al., 1986) used to estimate additional data point for dose-response of sensory irritation	D-9
Table D-4. Modeling summary for irritation symptoms in (Kulle et al., 1987; Kulle, 1993)	D-12
Table D-5. Incidence and severity of sensory irritation symptoms in humans after controlled formaldehyde exposure	D-13
Table D-6. CatReg hypothesis test results for irritation datasets when intercept and slope parameters are stratified by sex	D-15
Table D-7. CatReg hypothesis test results for irritation datasets when intercept and slope parameters are stratified by endpoint, eye and nose/throat irritation	D-16
Table D-8. Summary of CatReg modeling results for sensory irritation	D-16
Table D-9. Incidence and severity of sensory irritation symptoms in humans after controlled formaldehyde exposure	D-18
Table D-10. BMD modeling summary for eye irritation in males and females	D-18
Table D-11 BMD modeling summary of nose or throat irritation in females	D-19
Table D-12. Modeling summary for irritation symptoms in (Andersen, 1979; Andersen and Molhave, 1983)	D-21
Table D-13. Sensory irritation symptom data from Andersen and Molhave (1983, 1979)	D-21
Table D-14. BMD modeling summary of conjunctival irritation and dryness in the nose and throat dryness in males and females; 0 % background prevalence	D-21
Table D-15. BMD modeling summary of conjunctival irritation and dryness in the nose and throat in males and females; 3% background prevalence.....	D-23
Table D-16. BMD modeling summary of conjunctival irritation and dryness in the nose and throat in males and females; 6% background prevalence.....	D-24
Table D-17. BMD modeling summary of conjunctival irritation and dryness in the nose and throat in males and females; 9% background prevalence.....	D-26
Table D-18. Modeled effect estimates for night-time symptoms of an asthma attack; data from (Venn et al., 2003).....	D-30
Table D-19. Incidence of squamous metaplasia in nasal cavity of F344 rats.....	D-33
Table D-20. Benchmark dose modeling of rat respiratory histopathological effects.....	D-35
Table D-21. Endpoints selected for dose-response modeling for reproductive and developmental toxicity in animals	D-40
Table D-22. Summary of BMDS modeling for serum testosterone in male Wistar rats exposed to formaldehyde by inhalation for 13 weeks (Ozen et al., 2005); BMR = 1 SD change from the control mean.....	D-41
Table D-23. Summary of BMDS modeling for relative testis weight in male Wistar rats exposed to formaldehyde by inhalation for 13 weeks (Ozen et al., 2002); BMR = 10% RD.....	D-42

Supplemental Information—Formaldehyde (Inhalation)

Table D-24. Summary of BMDS modeling for relative testis weight in male Wistar rats exposed to formaldehyde by inhalation for 13 weeks (Ozen et al., 2002); BMR = 1 SD change from the control mean.....	D-42
Table D-25. Extra risk calculation for environmental exposure to 0.0550 ppm formaldehyde (the LEC ₀₀₅ for NPC incidence) using a log-linear exposure-response model based on the cumulative exposure trend results of Beane Freeman (2013), as described in Section 5.2.1	D-44
Table D-26. Data used in dose-response modeling of SCC in F344 rat nose	D-46
Table D-27. Hypothesis testing of parameter equivalence for modeling Monticello et al. (1996); Kerns et al. (1983) studies	D-55
Table D-28. Kaplan-Meier adjusted tumor incidence in the pooled F344 SCC data.....	D-60
Table D-29. Multistage-Weibull model fit to combined Kerns and Monticello data, dropping high dose.....	D-67
Table D-30. Comparison of parameter estimates in Table D-29 with other Multistage-Weibull model fits to the same data	D-68
Table D-31. Modeled formaldehyde flux on F344 rat nasal lining (Source: (Kimbell et al., 2001b)).....	D-70
Table D-32. Evaluation of assumptions and uncertainties in the CIIT model for nasal tumors in the F344 rat.....	D-75
Table D-33. PBPK models for formaldehyde-DPX	D-80
Table D-34. Comparison of (Conolly et al., 2000) and (Subramaniam et al., 2007) DPX concentrations, averaged over 1 week (pmol/mm ³)	D-84
Table D-35. Influence of control data in modeling formaldehyde-induced cancer in the F344 rat	D-85
Table D-36. Variation in number of cells across nasal sites in the F344 rat	D-93
Table D-37. Parameter specifications and estimates for clonal growth models of nasal SCC in the F344 rat using alternative characterization of cell replication and death rates.....	D-109
Table D-38. Parameter specifications and estimates for clonal growth models of nasal SCC in the F344 rat using cell replication and death rates as characterized in Conolly et al. (2003)	D-110
Table D-39. Comparison of statistical confidence bounds on added risk for two models	D-112
Table D-40. Summary of evaluation of major assumptions and results in (Conolly et al., 2004).....	D-114
Table D-41. Extrapolation of parameters for enzymatic metabolism to the human in Conolly et al. (2000)	D-116
Table D-42. Relative risk estimates for mortality from multiple myeloma (ICD-8 code 203), leukemia (ICD-8 codes 204–207), myeloid leukemia (ICD-8 code 205), and other/unspecified leukemia (ICD-8 code 207) by level of formaldehyde exposure for different exposure metrics.....	D-126
Table D-43. Regression coefficients for leukemia, myeloid leukemia, and myeloid plus other/unspecified leukemias mortality from NCI trend test models of cumulative exposure	D-127
Table D-44. Extra risk estimates for myeloid plus other/unspecified leukemia mortality from various levels of continuous lifetime exposure to formaldehyde	D-130
Table D-45. EC ₀₀₅ , LEC ₀₀₅ , and inhalation unit risk estimates for myeloid plus other/unspecified leukemia mortality from formaldehyde exposure based on log-linear trend analyses of cumulative exposure data from the Beane Freeman et al. (2009) study	D-131
Table D-46. EC ₀₀₅ , LEC ₀₀₅ , and inhalation unit risk estimates for myeloid plus other/unspecified leukemia incidence from formaldehyde exposure based on Beane Freeman et al. (2009) log-linear trend analyses for cumulative exposure	D-132

Table D-47. EC ₀₀₅ and LEC ₀₀₅ estimates for mortality and incidence and incidence unit risk estimates for all leukemia and for myeloid leukemia using alternate approaches (all person-years)	D-132
Table D-48. Exposure-response modeling (all person-years) and (incidence) unit risk estimate derivation results for different leukemia groupings.....	D-133
Table G-1. Hazard conclusions and toxicity values developed by other national and international health agencies	G-1

FIGURES

Figure A-1. Chemical structure of formaldehyde.....	A-1
Figure A-2. Formaldehyde Ambient Concentrations Contribution by Sector.....	A-6
Figure A-3. Range of formaldehyde air concentrations (ppb) in different environments.....	A-14
Figure B-1. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and sensory irritation in humans.	B-3
Figure B-2. Sensory irritation literature tree (interactive version here).....	B-4
Figure B-3. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and pulmonary function in humans.	B-8
Figure B-4. Pulmonary function effects in humans literature tree (interactive version here).	B-9
Figure B-5. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and respiratory and immune-mediated conditions.	B-12
Figure B-6. Asthma and immune effects in humans literature tree (interactive version here).	B-13
Figure B-7. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and respiratory tract pathology in humans.....	B-19
Figure B-8. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and respiratory tract pathology in animals.	B-20
Figure B-9. Human respiratory tract pathology literature tree (interactive version here).	B-21
Figure B-10. Animal respiratory tract pathology literature tree (interactive version here).....	B-22
Figure B-11. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and mechanistic data associated with potential noncancer effects on the respiratory system.	B-29
Figure B-12. Mechanistic inflammation and immune effects literature tree (interactive version here).....	B-30
Figure B-13. Literature search documentation for sources of primary data pertaining to formaldehyde exposure and nervous system effects.....	B-43
Figure B-14. Nervous system effects literature tree.....	B-44
Figure B-15. Literature search documentation for sources of primary data pertaining to formaldehyde exposure and developmental and reproductive toxicity.	B-50
Figure B-16. Reproductive and developmental effects literature tree.....	B-51
Figure B-17. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and upper respiratory or lymphohematopoietic cancers in humans.....	B-56
Figure B-18. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and upper respiratory tract (nasal) cancers in animals.	B-57

Supplemental Information—Formaldehyde (Inhalation)

Figure B-19. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and lymphohematopoietic (LHP) cancers in animals.	B-58
Figure B-20. Human cancer literature tree.	B-59
Figure B-21. Animal respiratory tract cancer literature tree.	B-60
Figure B-22. Animal lymphohematopoietic cancer literature tree.	B-61
Figure B-23. Mechanistic respiratory tract cancer literature tree.	B-69
Figure B-24. Mechanistic lymphohematopoietic cancer literature tree.	B-70
Figure C-1. Schematic of the rat upper respiratory tract depicting the gradient of formaldehyde concentration formed following inhalation exposure, both from anterior to posterior locations, as well as across the tissue depth..	C-2
Figure C-2. Metabolism of formaldehyde.	C-10
Figure C-3. Compartmentalization of mammalian one-carbon metabolism.	C-13
Figure C-4. Metabolic incorporation and covalent binding of formaldehyde in rat respiratory tract. ³ H/ ¹⁴ C ratios in macromolecular extracts from rat respiratory mucosa (A) and olfactory mucosa (B) following 6-hour exposure to ¹⁴ C- and ³ H-labeled formaldehyde (0.3, 2, 6, 10, and 15 ppm, corresponding to 0.37, 2.46, 7.38, 12.3, 18.42 mg/m ³ , respectively).	C-18
Figure C-5. Endogenous and dietary sources of formaldehyde production.	C-27
Figure C-6. ³ H/ ¹⁴ C ratios in macromolecular extracts from rat bone marrow following 6-hour exposure to ¹⁴ C- and ³ H-labeled formaldehyde (0.3, 2, 6, 10, and 15 ppm, corresponding to 0.37, 2.46, 7.38, 12.3, 18.42 mg/m ³ , respectively).	C-35
Figure C-7. Reconstructed nasal passages of F344 rat, rhesus monkey, and human.	C-46
Figure C-8. Illustration of interspecies differences in airflow and verification of CFD simulations with water-dye studies.	C-47
Figure C-9. Lateral view of nasal wall mass flux of inhaled formaldehyde simulated in the F344 rat, rhesus monkey, and human.	C-48
Figure C-10. Lateral view of nasal wall mass flux of inhaled formaldehyde simulated at various inspiratory flow rates in a human model.	C-49
Figure C-11. Single-path model simulations of surface flux per ppm of formaldehyde exposure concentration in an adult male human.	C-53
Figure C-12. Pressure drop versus volumetric airflow rate predicted by the CIIT CFD model compared with pressure drop measurements made in two hollow molds (C1 and C2) of the rat nasal passage (Cheng et al., 1990) or in rats in vivo (Gerde et al., 1991).	C-55
Figure C-13. Formaldehyde-DPX dosimetry in the F344 rat.	C-57
Figure C-14. Flux of highly reactive gas across the nasal lining as a function of normalized distance from the nostril for 5 adults and 2 children.	C-59
Figure C-15. Signs of Reflex Bradypnea.	C-63
Figure C-16. An oscillograph that compares the respiratory cycle for mice exposed to an URT irritant (lower tracing) to an air control group (upper tracing).	C-64
Figure C-17. Formaldehyde effects on minute volume in naïve and formaldehyde-pretreated male B6C3F1 mice and F344 rats.	C-64
Figure C-18. The potential impact of reflex bradypnea on fetal development.	C-69
Figure C-19. Plots of change in FEF at 25–75% of FVC across a work shift or anatomy lab session by study with study details.	C-192
Figure C-20. Plots of change in FEV1 across a work shift or anatomy lab session by study with study details.	C-193

Supplemental Information—Formaldehyde (Inhalation)

Figure C-21. Plots of change in FVC across a work shift or anatomy lab session by study with study details.....	C-194
Figure C-22. Nasal cell proliferation in rats exposed to formaldehyde.	C-222
Figure C-23. Mechanistic events for respiratory effects of formaldehyde based on <i>robust</i> or <i>moderate</i> evidence.	C-269
Figure C-24. Mechanistic events for respiratory effects of formaldehyde based on <i>robust</i> , <i>moderate</i> , or <i>slight</i> evidence.	C-270
Figure C-25. Possible sequences of mechanistic events identified based on the most reliable evidence available.....	C-273
Figure D-1. Regression of prevalence of “burning eyes” versus indoor formaldehyde concentration (ppm) in mobile homes (approximately 1-hour air samples).	D-3
Figure D-2. Plot of the central estimate of the prevalence odds by residential concentration-response information from Table D-1.	D-4
Figure D-3. Plot of the upper bound on the central estimate of the prevalence odds by residential concentration-response information from Table 2.	D-6
Figure D-4. Plot of the prevalence odds by residential concentration-response information from the central estimate of from Hanrahan et al. (1984) combined with complementary information from the central estimate of from Liu et al. (1991).	D-10
Figure D-5. Plot of the prevalence odds by residential concentration-response information from the central estimate of from (Hanrahan et al., 1984) combined with information from the central estimate of from (Liu et al., 1991) with third degree polynomial function fit to these data.	D-11
Figure D-6. Selected BMDS model results (Probit model) for eye irritation (incidence) versus ppm formaldehyde exposure (ppm) in male and female adults (Kulle et al., 1993; 1987). BMD = BMC.....	D-19
Figure D-7. Selected BMDS model results (Multistage 3° model) for nose or throat irritation (incidence) versus ppm formaldehyde exposure (dose) in female adults (Kulle et al., 1993; 1987). BMD = BMC.....	D-20
Figure D-8. Selected BMDS model results (LogLogistic Model) for conjunctival irritation and dryness in the nose and throat dryness (incidence) versus mg/m ³ formaldehyde exposure (dose) in male and female adults assuming a 0% background prevalence (Andersen and Molhave, 1983, 1979). BMD = BMC.	D-23
Figure D-9. Selected BMDS model results (LogLogistic model) for conjunctival irritation and dryness in the nose and throat dryness (incidence) versus mg/m ³ formaldehyde exposure (dose) in male and female adults assuming a 3% background prevalence (Andersen and Molhave, 1983, 1979). BMD = BMC.	D-24
Figure D-10. Selected BMDS model results (LogLogistic model) for conjunctival irritation and dryness in the nose and throat dryness (incidence) versus mg/m ³ formaldehyde exposure (dose) in male and female adults assuming a 6% background prevalence (Andersen and Molhave, 1983, 1979). BMD = BMC.	D-26
Figure D-11. Selected BMDS model results (Hill model) for conjunctival irritation and dryness in the nose and throat dryness (incidence) versus mg/m ³ formaldehyde exposure (dose) in male and female adults assuming a 9% background prevalence (Andersen and Molhave, 1983, 1979). BMD = BMC.....	D-27
Figure D-12. Midsagittal section of rat nose showing section levels from (Kerns et al., 1983) (nostril is to the left).	D-33

Supplemental Information—Formaldehyde (Inhalation)

Figure D-13. Lateral view of contour plot of formaldehyde flux to the rat (on the top) and human nasal lining (on the bottom) using CFD modeling (Kimbell et al., 2001b) (nostril is to the right).....	D-36
Figure D-14. All model fits (top panel) and best fitting log-probit model (bottom panel) for level 1 squamous metaplasia (F344 rat).	D-37
Figure D-15. All model fits (top panel) and best fitting Multistage (k=1) model (bottom panel) for basal hyperplasia (Wistar rat).	D-38
Figure D-16. All model fits (top panel) and best fitting log-probit model (bottom panel) for squamous metaplasia (Wistar rat).....	D-39
Figure D-17. Plot of mean response (serum testosterone, serum testosterone, (Ozen et al., 2005) by formaldehyde concentration (mg/m ³), with the fitted curve for the Power model.	D-41
Figure D-18. Dose response for the best fitting multistage Weibull time-to-tumor model (Model MSW1) of pooled rat nasal tumor plot is at the specified time risk estimates were derived for the benchmark dose calculation.....	D-62
Figure D-19. Probability vs time for the best fitting MSW distribution calculated at each exposure concentration.	D-63
Figure D-20. Probabilities from best fitting MSW model plotted against probabilities from the nonparametric model.	D-64
Figure D-21. Probability vs time for MSW distribution of model MSW2.....	D-65
Figure D-22. Probabilities from MSW model MSW2 plotted against probabilities from the nonparametric model.	D-66
Figure D-23. Dose response of normal (α_N) and initiated (α_I) cell division rate in Conolly et al. (2003).	D-73
Figure D-24. ULLI data for pulse and continuous labeling studies.....	D-90
Figure D-25. Logarithm of normal cell replication rate α_N versus formaldehyde flux (in units of pmol/mm ² -hour) for the F344 rat nasal epithelium.....	D-93
Figure D-26. Logarithm of normal cell replication rate versus formaldehyde flux with simultaneous confidence limits for the ALM.....	D-94
Figure D-27. Logarithm of normal cell replication rate versus formaldehyde flux with simultaneous confidence limits for the PLM.	D-95
Figure D-28. Various dose-response models of normal cell replication rate; N1.....	D-98
Figure D-29. Various dose-response models of normal cell replication rate; N2.....	D-99
Figure D-30. Various dose-response models of normal cell replication rate; N3.....	D-99
Figure D-31. Various dose-response models of normal cell replication rate; N4.....	D-100
Figure D-32. Various dose-response models of normal cell replication rate; N5.....	D-100
Figure D-33. Various dose-response models of normal cell replication rate; N6.....	D-101
Figure D-34. BBDR models for the rat—models with positive added risk.....	D-107
Figure D-35. BBDR rat models resulting in negative added risk.	D-107
Figure D-36. Models resulting in positive added rat risk: Dose response for normal and initiated cell replication.....	D-108
Figure D-37. Models resulting in negative added rat risk: Dose response for normal and initiated cell replication.....	D-109
Figure D-38. Effect of choice of NTP bioassays for historical controls on human risk.....	D-118
Figure D-39. Variations to the hockey-stick model for division rates of initiated cells in rats.	D-120
Figure D-40. Variations to the J-shaped model for division rates of initiated cells in rats.	D-121
Figure D-41. Very similar model estimates of probability of fatal tumor in rats for three models in Figure D-39.....	D-122

Supplemental Information—Formaldehyde (Inhalation)

Figure D-42. Cell proliferation data from Meng et al. (2010).	D-124
Figure D-43. Graphs of the additional human risks estimated by applying these modified models for α_i , using all NTP controls, compared to those obtained using the original (Conolly et al., 2004) model.	D-125
Figure D-44. Schematic of the bottom-up approach.	D-141

ABBREVIATIONS

AC50	activity concentration at 50%	CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
ACA	activated aerosol carbon	CF	cystic fibrosis
ADAF	Age-dependent adjustment factors	CFD	computational fluid dynamic
ADH	alcohol dehydrogenase	CFU	colony forming units
ADME	absorption, distribution, metabolism, and excretion	CHO	Chinese hamster ovary (cell line cells)
AEGL	Acute Exposure Guidelines Levels	CI	confidence interval
AGT	angiotensinogen	CIIT	Chemical Industry Institute of Toxicology
AIC	Akaike's information criterion	CL	confidence limit
ALDH	aldehyde dehydrogenase	CML	chronic myeloid leukemia
ALM	Anterior lateral meatus	CNS	central nervous system
ALS	Amyotrophic lateral sclerosis	COI	conflict of interest
ALT	alanine aminotransferase	COPD	chronic obstructive pulmonary disease
AML	acute myeloid leukemia	CPAD	Chemical and Pollutant Assessment Division
AMS	anterior medial septum	CPHEA	Center for Public Health and Environmental Assessment
ANCOVA	Analysis of Covariance	CYP450	cytochrome P450
ANOVA	Analysis of Variance	DAF	dosimetric adjustment factor
AOP	adverse outcome pathway	DCFH	dichlorofluorescein diacetate
AST	aspartate aminotransferase	DMSO	dimethylsulfoxide
atm	atmosphere	DNA	deoxyribonucleic acid
ATSDR	Agency for Toxic Substances and Disease Registry	DPX	DNA-protein crosslinks
BAL	bronchoalveolar lavage	EA	ethyl acetate
BALT	bronchus-associated lymphoid tissue	EPA	Environmental Protection Agency
BBDR	biologically based dose-response	ER	extra risk
BCME	Bis(chloromethyl)ether	ETS	Environmental tobacco smoke
BDNF	Brain-derived neurotrophic factor	FA	Formaldehyde
BFU	Burst-forming unit	FAD	flavin adenine dinucleotide
BM	bone marrow	FANCA	Fanconi anemia complementation group A
BMC	benchmark concentration	FDA	Food and Drug Administration
BMCL	benchmark concentration lower confidence limit	FDH	formaldehyde dehydrogenase
BMD	benchmark dose	FEF	forced expiratory flow
BMDL	benchmark dose lower confidence limit	FEMA	Federal Emergency Management Agency
BMDS	Benchmark Dose Software	FEV ₁	forced expiratory volume of 1 second
BMI	Body Mass Index	FISH	Fluorescence In Situ Hybridization
BMR	benchmark response	FSH	follicle stimulating hormone
BOEL	Binding Occupational Exposure Limit	FVC	forced vital capacity
BUN	blood urea nitrogen	GD	gestation day
BW	body weight	GDH	glutamate dehydrogenase
BW ^{3/4}	body weight scaling to the 3/4 power	GGT	γ-glutamyl transferase
CA	chromosomal aberration	GLP	Good Laboratory Practice
CAA	Clean Air Act	GM	granulocyte macrophage
CAS	Chemical Abstracts Service	GSH	glutathione
CASRN	Chemical Abstracts Service registry number	GST	glutathione-S-transferase
CBMN	cytokinesis-block micronucleus assay	HAP	hazardous air pollutant
CD	Cluster of Differentiation		
CE	capillary electrophoresis		

Supplemental Information—Formaldehyde (Inhalation)

HAWC	Health Assessment Workspace Collaborative	PK	pharmacokinetic
Hb/g-A	animal blood:gas partition coefficient	PND	postnatal day
Hb/g-H	human blood:gas partition coefficient	POD	point of departure
HBCD	hexabromocyclododecane	POD _[AD]	duration-adjusted POD
HEC	human equivalent concentration	QSAR	quantitative structure-activity relationship
HED	human equivalent dose	RD	relative deviation
HERO	Health and Environmental Research Online	RfC	inhalation reference concentration
HPRT	hypoxanthine-guanine phosphoribosyl transferase	RfD	oral reference dose
i.p.	intraperitoneal	RGDR	regional gas dose ratio
i.v.	intravenous	RNA	ribonucleic acid
IAP	IRIS Assessment Plan	ROBINS I	Risk of Bias in Nonrandomized Studies of Interventions
IARC	International Agency for Research on Cancer	SAR	structure-activity relationship
IRIS	Integrated Risk Information System	SCE	sister chromatid exchange
IUR	inhalation unit risk	SD	standard deviation
LC ₅₀	median lethal concentration	SDH	sorbitol dehydrogenase
LD ₅₀	median lethal dose	SE	standard error
LOAEL	lowest-observed-adverse-effect level	SGOT	serum glutamic oxaloacetic transaminase, also known as AST
LOEL	lowest-observed-effect level	SGPT	serum glutamic pyruvic transaminase, also known as ALT
MeSH	Medical Subject Headings	TK	toxicokinetics
MN	micronuclei	TLV	threshold limit values
MNPCE	micronucleated polychromatic erythrocyte	TSCATS	Toxic Substances Control Act Test Submissions
MOA	mode of action	TTP	time-to-pregnancy
MTD	maximum tolerated dose	TWA	time-weighted average
NCI	National Cancer Institute	UDS	unscheduled DNA synthesis
NMD	normalized mean difference	UF	uncertainty factor
NOAEL	no-observed-adverse-effect level	UF _A	animal-to-human uncertainty factor
NOEL	no-observed-effect level	UF _D	database deficiencies uncertainty factor
NTP	National Toxicology Program	UF _H	human variation uncertainty factor
NZW	New Zealand White (rabbit breed)	UF _L	LOAEL-to-NOAEL uncertainty factor
OAR	Office of Air and Radiation	UF _S	subchronic-to-chronic uncertainty factor
OECD	Organisation for Economic Co-operation and Development	VOC	volatile organic compounds
OLEM	Office of Land and Emergency Management	WBC	white blood cells
ORD	Office of Research and Development	WHO	World Health Organization
OSF	oral slope factor	WOS	Web of Science
PBPK	physiologically based pharmacokinetic	XP	xeroderma pigmentosum
PECO	populations, exposures, comparators, and outcomes		

APPENDIX A. BACKGROUND INFORMATION SUPPLEMENT

Appendix A introduces potentially useful context for this assessment. These summaries do not provide comprehensive descriptions of the available information on these topics and are not intended for use in decision purposes. Readers are encouraged to refer to source materials cited below, contemporary publications on these topics, and specific assessments on these topic areas.

A.1. CHEMICAL PROPERTIES

Formaldehyde (CASRN 50-00-0) is the first of the series of aliphatic aldehydes and is a gas at room temperature. Its molecular structure is depicted in Figure A-1. It is noted for its reactivity and versatility as a chemical intermediate. It readily undergoes polymerization, is highly flammable, and can form explosive mixtures with air. It decomposes at temperatures above 150°C ([IPCS, 2002](#)).

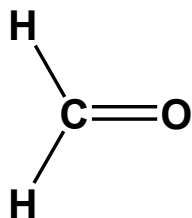


Figure A-1. Chemical structure of formaldehyde.

At room temperature, pure formaldehyde is a colorless gas with a strong, pungent, suffocating, and highly irritating odor ([NLM, 2019](#)). Formaldehyde is readily soluble in water, alcohols, ether, and other polar solvents ([IPCS, 2002](#)). A synopsis of its physicochemical properties is given in Table A-1.

Table A-1. Physicochemical properties of formaldehyde

Name	Formaldehyde
International Union for Pure and Applied Chemistry name	Formaldehyde
Synonyms	Formic aldehyde Methanal Methyl aldehyde Methylene oxide Oxomethane Oxymethylene
Chemical Abstracts Service Index name	Formaldehyde
Chemical Abstracts Service Registry Number	50-00-0
Formula	HCHO
Molecular weight	30.03
Density	Gas: 1.067 (air = 1) Liquid: 0.815 g/mL at –20°C
Vapor pressure	3,883 mm Hg at 25°C
Log K _{ow}	–0.75 to 0.35
Henry's law constant	3.4×10^{-7} atm-m ³ /mol at 25°C 2.2×10^{-2} Pa-m ³ /mol at 25°C
Conversion factors (25°C, 760 mm Hg)	1 ppm = 1.23 mg/m ³ (v/v) 1 mg/m ³ = 0.81 ppm (v/v)
Boiling point	–19.5°C at 760 mm Hg
Melting point	–92°C
Flash point	60°C; 83°C, closed cup for 37%, methanol-free aqueous solution; 50°C closed cup for 37% aqueous solution with 15% methanol
Explosive limits	73% upper; 7% lower by volume in air
Autoignition temperature	300°C
Solubility	Very soluble in water; soluble in alcohols, ether, acetone, benzene
Reactivity	Reacts with alkalis, acids, and oxidizers

Sources: [Walker \(1975\)](#); [IPCS \(2002\)](#); [Gerberich and Seaman \(2013\)](#); [ATSDR \(1999\)](#); [ACGIH \(2001\)](#).

A.2. PRODUCTION, SOURCES, AND USES OF FORMALDEHYDE

Formaldehyde has both commercial and industrial uses. Formaldehyde has been produced commercially since the early 1900s and, in recent years, has been ranked in the top 25 highest volume chemicals produced in the U.S. ([NTP, 2010](#); [ATSDR, 1999](#)). Based on EPA's Chemical Data Reporting (CDR) the national production volume for formaldehyde was 3.9 billion pounds/year in

2011 and between 1 and 5 billion pounds/year for the years 2012 through 2015

(<https://chemview.epa.gov/chemview/#>).

Approximately 55% of the consumption of formaldehyde is in the production of industrial resins (NTP, 2010). Formaldehyde is a chemical intermediate used in the production of some plywood adhesives, abrasive materials, insulation, foundry binders, brake linings made from phenolic resins, surface coatings, molding compounds, laminates, wood adhesives made from melamine resins, phenolic thermosetting, resin curing agents, explosives made from hexamethylenetetramine, urethanes, lubricants, alkyd resins, acrylates made from trimethylolpropane, plumbing components from polyacetal resins, and controlled-release fertilizers made from urea formaldehyde concentrates (IPCS, 1989), as cited in (ATSDR, 1999). Formaldehyde is used in smaller quantities for the preservation and embalming of biological specimens. It is also used as a germicide, an insecticide, and a fungicide in some products. It is found (as an ingredient or impurity) in some cosmetics and personal hygiene products, such as some soaps, shampoos, hair preparations, deodorants, sunscreens, dry skin lotions, and mouthwashes, mascara and other eye makeup, cuticle softeners, nail creams, vaginal deodorants, and shaving creams (NTP, 2010; IPCS, 2002; ATSDR, 1999).

Formaldehyde is commonly produced as an aqueous solution called formalin, which is used in industrial processes and usually contains about 37–40% formaldehyde and 12–15% methanol. Methanol is added to formalin to slow polymerization that leads eventually to precipitation as paraformaldehyde. Paraformaldehyde has the formula $(\text{CH}_2\text{O})_n$, where n is 8 to 100. It is essentially a solid form of formaldehyde and therefore has some of the same uses as formaldehyde (Kiernan, 2000). When heated, paraformaldehyde sublimates as formaldehyde gas. This characteristic makes it useful as a fumigant, disinfectant, and fungicide, such as for the decontamination of laboratories, agricultural premises, and barbering equipment. Long-chain polymers (e.g., Delrin plastic) are less inclined to release formaldehyde, but they have a formaldehyde odor and require additives to prevent decomposition.

The major sources of anthropogenic emissions of formaldehyde are motor vehicles, power plants, manufacturing plants that produce or use formaldehyde or substances that contain formaldehyde (i.e., adhesives), petroleum refineries, coking operations, incineration, wood burning, and tobacco smoke. Among these anthropogenic sources, the greatest volume source of formaldehyde is automotive exhaust from engines not fitted with catalytic converters (NEG, 2003). The Toxic Release Inventory (TRI) data for 2016 show total releases of 19.4 million pounds with about 13 million being stored via underground injection (EPA TRI Explorer, https://enviro.epa.gov/triexplorer/tri_release.chemical).

Formaldehyde is formed in the lower atmosphere by photochemical oxidation of hydrocarbons or other formaldehyde precursors that are released from combustion processes (ATSDR, 1999). Formaldehyde can also be formed by a variety of other natural processes, such as

decomposition of plant residues in the soil, by photochemical processes in sea water, and forest fires ([NLM, 2019](#)).

The input of formaldehyde into the environment is counterbalanced by its removal by several pathways. Formaldehyde is removed from the air by direct photolysis and oxidation by photochemically produced hydroxyl and nitrate radicals. Measured or estimated half-lives for formaldehyde in the atmosphere range from 1.6 to 19 hours, depending upon estimates of radiant energy, the presence and concentrations of other pollutants, and other factors ([ATSDR, 1999](#)). Given the generally short daytime residence times for formaldehyde, there is limited potential for long-range transport ([IPCS, 2002](#)). In cases where organic precursors are transported long distances, however, secondary formation of formaldehyde may occur far from the anthropogenic sources of the precursors.

Formaldehyde is released to water from the discharges of both treated and untreated industrial wastewater from its production and from its use in the manufacture of formaldehyde-containing resins ([ATSDR, 1999](#)). Formaldehyde is also a possible by-product from using ozone and/or hydrogen peroxide for drinking-water disinfection. In water, formaldehyde is rapidly hydrated to form a glycol, and the equilibrium favors the glycol.

A.3. HUMAN INHALATION EXPOSURE TO FORMALDEHYDE

Exposure assessments are not conducted or included as part of IRIS toxicological reviews, and authoritative sources on exposure assessment should be consulted. Most recently (as of the time of this assessment), draft formaldehyde exposure assessments are underway in EPA's TSCA Office (see <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-evaluation-formaldehyde#re-findings>). The overview section on human exposure to formaldehyde included in this Appendix is intended to provide context for the analyses of hazard identification and dose-response presented in this assessment. General population exposure to formaldehyde can occur via inhalation, ingestion, and dermal contact, with inhalation exposure representing the primary exposure route. Only inhalation exposure is discussed below. Formaldehyde exposure can occur occupationally via three main scenarios:

- The production of aqueous solutions of formaldehyde (formalin) and their use in the chemical industry (e.g., for the synthesis of various resins), as a preservative in medical laboratories and embalming fluids, and as a disinfectant.
- Release from formaldehyde-based resins in which it is present as a residue and/or through their hydrolysis and decomposition by heat (e.g., during the manufacture of wood products, textiles, synthetic vitreous insulation products, and plastics). In general, the use of phenol-formaldehyde resins results in much lower emissions of formaldehyde than those of urea- and melamine-based resins.
- The pyrolysis or combustion of organic matter (e.g., in engine exhaust gases or during firefighting) ([IARC, 2006](#)).

Occupational exposures occur not only during the production of products containing formaldehyde, but also during the use of these products in construction and decoration ([Kim et al., 2011](#)). Industries with the greatest potential for exposure include health services, business services, printing and publishing, manufacture of chemicals and allied products, manufacture of apparel and allied products, manufacture of paper and allied products, personal services, machinery (except clerical), transport equipment, and furniture and fixtures ([IARC, 1995](#)). Exposure levels for the workers of various professions in a selected number of studies range from 49 to 4,280 $\mu\text{g}/\text{m}^3$ (40 to 3,480 ppb), with plywood particle board production workers having the highest exposures ([Kim et al., 2011](#)).

In recent years, concerns have been raised regarding occupational exposures resulting from the use of semi-permanent professional hair straightening products. In 2010, responding to requests from hair salon employees to the National Institute of Occupational Safety and Health (NIOSH), a study of hair smoothing treatment products marketed as formaldehyde free was conducted. The CDC study ([2011](#)) found that the formaldehyde content in a total of 105 samples of these products ranged from 6.8 to 11.8%, with an average of 8.8%. Air samples taken in seven hair salons during smoothing treatments showed 8-hour time-weighted average concentrations of formaldehyde ranging from 7.4 $\mu\text{g}/\text{m}^3$ (6 ppb) to 407.1 $\mu\text{g}/\text{m}^3$ (331 ppb) ([CDC, 2011](#)). Air concentrations vary depending on factors such as room ventilation, ceiling height, room size, and duration of the treatment ([CDC, 2011](#)). Another study by ([Pierce et al., 2011](#)) collected air samples during the use of four commercially available hair smoothing products. The hair stylist 8-hour time-weighted average concentrations of formaldehyde ranged from 24.6 $\mu\text{g}/\text{m}^3$ (20 ppb) to 196.8 $\mu\text{g}/\text{m}^3$ (160 ppb) for one treatment per day and 61.5 $\mu\text{g}/\text{m}^3$ (50 ppb) to 922.5 $\mu\text{g}/\text{m}^3$ (750 ppb) for four consecutive treatments ([Pierce et al., 2011](#)). Time weighted average concentrations decreased as the distance from the treatment location increased ([Pierce et al., 2011](#)).

EPA's AirToxScreen (<https://www.epa.gov/AirToxScreen>; note: a previous version was the National Air Toxics Assessment) provides modeled formaldehyde concentrations based on emissions inventories and meteorological data for areas such as counties, states and the nation and includes the contiguous US, Alaska, Hawaii, Puerto Rico, and Virgin Islands. The range of estimated county mean outdoor air concentrations is 0.1–4.3 $\mu\text{g}/\text{m}^3$. The breakout by Sector is illustrated in Figure A-2.

Ambient air monitoring data for formaldehyde are available from EPA's Ambient Monitoring Archive for HAPs which includes data from the Air Quality System database and other data sources (<https://www.epa.gov/amtic/amtic-air-toxics-data-ambient-monitoring-archive>). Measurement data are collected from National Air Toxic Trends Sites (NATTS) and other sites across the country operated by state, local, and tribal agencies that are not part of the NATTS network. Data for the year 2018 come from 100 monitors located in 27 states and the District of Columbia. The annual means for these monitors range from 0.25–11.06 $\mu\text{g}/\text{m}^3$ (0.20–9.01 ppb) and have an overall average of 2.97 $\mu\text{g}/\text{m}^3$ (2.42 ppb). The annual means were derived by EPA through

Supplemental Information—Formaldehyde (Inhalation)

averaging all available daily data from each site that has at least three valid quarters for the year (i.e., a valid quarter is a quarter that contains at least seven daily averages) (<https://www.epa.gov/system/files/documents/2021-08/annual-average-statistics-documentation-2018.pdf>). Table A-2 presents the data by land use category based on the annual means from each site for 2018. The land use is established in the Air Quality System database from the site description.

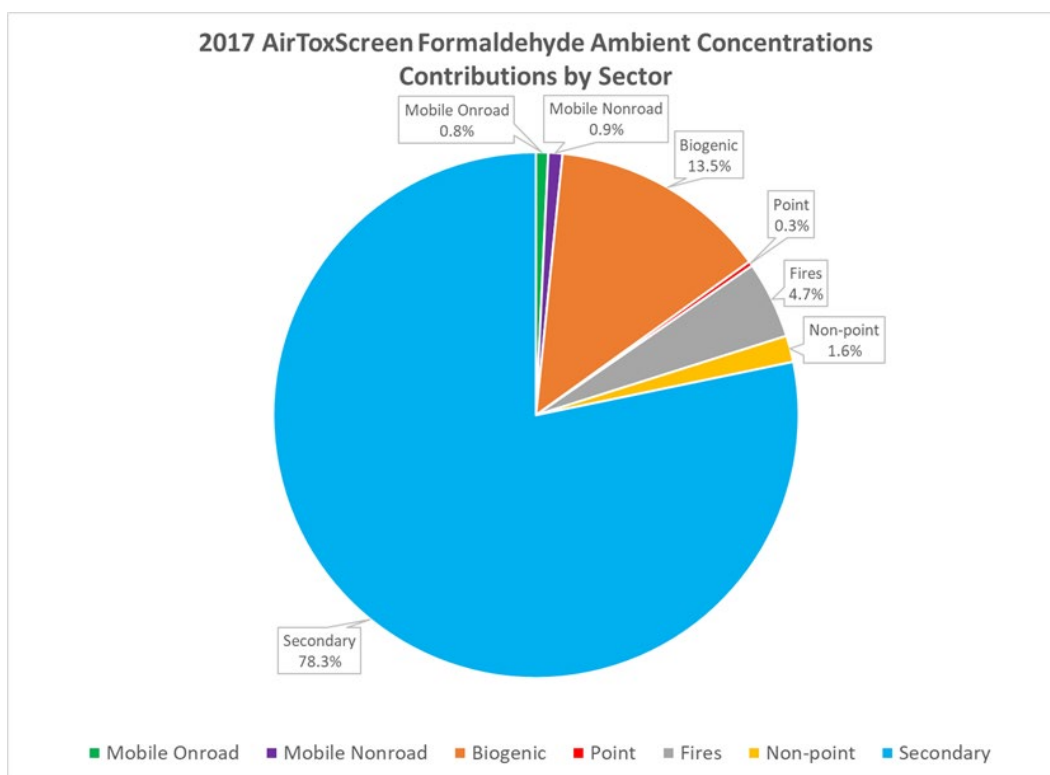


Figure A-2. Formaldehyde Ambient Concentrations Contribution by Sector.

Source: Based on 2017 AirToxScreen (EPA/OAR).

Table A-2. Ambient air levels by land use category based on 2018 annual site averages

	Annual formaldehyde ambient air concentrations by category ($\mu\text{g}/\text{m}^3$)					
	Agriculture	Commercial	Forest	Industrial	Mobile	Residential
Number of annual averages	5	31	4	11	6	43
Mean	2.02	2.88	1.98	3.42	3.80	3.00
Minimum	1.40	0.25	1.03	1.74	2.02	0.88
Maximum	2.61	4.84	3.40	8.25	5.71	11.06

Source: EPA's Ambient Monitoring Archive for HAPs which includes data from the Air Quality System and other data sources at <https://www.epa.gov/amtic/amtic-air-toxics-data-ambient-monitoring-archive>.

Supplemental Information—Formaldehyde (Inhalation)

In general, ambient levels of formaldehyde in outdoor air are significantly lower than those measured in the indoor air of workplaces or residences ([IARC, 1995](#); [ATSDR, 1999](#)). Indoor sources of formaldehyde in air include volatilization from pressed wood products, carpets, fabrics, insulation, permanent press clothing, latex paint, and paper bags, along with emissions from gas burners, kerosene heaters, and cigarettes. [Kim et al. \(2015b\)](#) suggested that air fresheners, scented candles, and electric diffusers may also contribute to indoor concentrations of formaldehyde. Indoor air levels are affected by the age of the source materials, temperature, humidity, and ventilation rates ([Parthasarathy et al., 2011](#); [IARC, 2006](#)). Release rates of formaldehyde from consumer products have been published in the literature. Table A-3 presents a selected number of products and their respective emission rates in $\mu\text{g}/\text{m}^2\text{-hour}$.

In general, the major indoor air sources of formaldehyde can be described in two ways: (1) those sources that have the highest emissions when the product is new with decreasing emission over time, as with the first set in the examples above; and (2) those sources that are reoccurring or frequent such as the second set of examples above. Several studies were found in the literature that investigated indoor air concentrations of formaldehyde in various housing types. Median indoor air concentrations in various European countries in both commercial and residential buildings ranged from $10 \mu\text{g}/\text{m}^3$ to $50 \mu\text{g}/\text{m}^3$ ([Sarigiannis et al., 2011](#)). A summary of residential indoor air data in the U.S. and Canada is provided in Table A-4. These are organized by manufactured (i.e., mobile homes/trailers with wheels that are designed to be moved) and conventional housing and in chronological order, beginning with the most recent studies. Results vary depending on housing characteristics and date of study. In general, higher concentrations are found in manufactured houses.

Even though formaldehyde levels in construction materials have declined, indoor inhalation concerns persist. For example, as shown in Table A-4, studies have measured formaldehyde levels in manufactured homes. [ATSDR \(2007\)](#) reported on air sampling in 96 unoccupied trailers provided by the Federal Emergency Management Agency (FEMA) used as temporary housing for people displaced by Hurricane Katrina (see Table A-4). Formaldehyde levels in closed trailers averaged $1,279 \pm 849 \mu\text{g}/\text{m}^3$ (mean $\pm$ standard deviation [SD]) (1.04 ± 0.69 ppm), with a range of $12\text{--}4,500 \mu\text{g}/\text{m}^3$ ($0.01\text{--}3.66$ ppm). The levels decreased to an average of $480 \pm 324 \mu\text{g}/\text{m}^3$ (0.39 ± 0.27 ppm), with a range of $0.00\text{--}2,005 \mu\text{g}/\text{m}^3$ ($0.00\text{--}1.63$ ppm) when the air conditioning was turned on. Levels also decreased to an average of $111 \pm 98 \mu\text{g}/\text{m}^3$ (0.09 ± 0.08 ppm), with a range of $12\text{--}603 \mu\text{g}/\text{m}^3$ ($0.01\text{--}0.49$ ppm) when the windows were opened. ([ATSDR, 2007](#)) found an association between temperature and formaldehyde levels; higher temperatures were associated with higher formaldehyde levels in trailers with the windows closed. They also noted that different commercial brands of trailers yielded different formaldehyde levels.

In December 2007 and January 2008, the Centers for Disease Control and Prevention (CDC) measured formaldehyde levels in a stratified random sample of 519 FEMA-supplied occupied travel trailers, park models, and mobile homes (“trailers”) ([Henry, 2008](#)). At the time of the study,

sampled trailers were in use as temporary shelters for Louisiana and Mississippi residents displaced by hurricanes Katrina and Rita. The geometric mean level of formaldehyde in sampled trailers was $95 \mu\text{g}/\text{m}^3$ (77 ppb), and the range was $3.7\text{--}726 \mu\text{g}/\text{m}^3$ (3–590 ppb) (see Table A-4).

Another study by ([Maddalena et al., 2008](#)) measured indoor air concentrations for a range of volatile organic compounds (VOCs), including formaldehyde in four unoccupied temporary housing units (i.e., mobile homes) under steady state ventilation conditions. A morning and an afternoon measurement were taken for each unit. The overall average air concentration of formaldehyde for the four mobile homes was $569 \mu\text{g}/\text{m}^3$. This is consistent with values measured by ([ATSDR, 2007](#)) and CDC ([Henry, 2008](#)). Consistently higher air concentrations of formaldehyde were measured in the afternoon samples.

Air concentrations of formaldehyde were lower for conventional housing as shown in Table A-4. Mean values from studies published between 1980 and 2008 ranged from 6.2 to $>1,230 \mu\text{g}/\text{m}^3$. Although no conclusions could be drawn based on the age of the study alone, some of the studies in Table A-4 suggest that air concentrations are influenced by the age of the house and season of the year. Lower air concentrations were observed as the age of the house increased. Higher concentrations were generally observed during the summer months.

[Salthammer et al. \(2010\)](#) present a thorough review of formaldehyde sources and levels found in the indoor environment. Based on an examination of international studies carried out in 2005 or later they conclude that the average exposure of the population to formaldehyde is 20 to $40 \mu\text{g}/\text{m}^3$ under normal living conditions. Figure A-3 summarizes the range of formaldehyde air concentrations in various environments. The dotted line represents the WHO guidelines of $100 \mu\text{g}/\text{m}^3$. More recently, ([Branco et al., 2015](#)) measured hourly mean formaldehyde concentrations as high as $204 \mu\text{g}/\text{m}^3$ in nursery schools in Portugal.

Data on formaldehyde levels in outdoor and indoor air were collected under Canada's National Air Pollution Surveillance program ([IPCS, 2002](#); [Health Canada, 2001](#)). The effort included four suburban and four urban sites sampled in the period 1990–1998. A Monte Carlo analysis applied to the pooled data ($n = 151$) was used to estimate the distribution of time-weighted 24-hour air exposures. This study suggested that mean levels in outdoor air were $3.3 \mu\text{g}/\text{m}^3$ (2.7 ppb) and mean levels in indoor air were $35.9 \mu\text{g}/\text{m}^3$ (29.2 ppb) ([Health Canada, 2001](#)). The simulation analysis also suggested that general population exposures averaged $33\text{--}36 \mu\text{g}/\text{m}^3$ (27–30 ppb).

Since the early- to mid-1980s, manufacturing processes and construction practices have been changed to reduce levels of indoor formaldehyde emissions ([ATSDR, 1999](#)). A 2008 law enacted by the California Air Resource Board (Final Regulation Order: Airborne Toxic Control Measure to Reduce Formaldehyde Emissions from Composite Wood Products; <http://www.arb.ca.gov/regact/2007/compwood07/fro-final.pdf>) has limited the amount of formaldehyde that can be released by specific composite wood products (i.e., hardwood plywood, particle board, and medium density fiberboard) sold, supplied, or manufactured for use in

Supplemental Information—Formaldehyde (Inhalation)

California. For this reason, the mean indoor air levels presented by ([Health Canada, 2001](#)) (based on samples collected from 1989–1995) may overestimate current levels.

Table A-3. Formaldehyde emission rates from various consumer products

Products	Emission Rate ($\mu\text{g}/\text{m}^2\text{-hr}$)	Reference
Pressed wood products	ND–1,500	Pickrell et al. (1983)
New clothing	0.63–31.25	Pickrell et al. (1983)
Insulation products	2.17–25.83	Pickrell et al. (1983)
Paper plates and cups	3.13–41.67	Pickrell et al. (1983)
Fabrics	ND–14.58	Pickrell et al. (1983)
Carpets	ND–2.71	Pickrell et al. (1983)
Carpets with urethane foam backing	411–6 ^a	Yu and Crump (1998)
Textile carpet	83–36 ^a	Yu and Crump (1998)
Carpet with synthetic/PVC fibers	120–11 ^a	Yu and Crump (1998)
Carpet assembly	153,000–783 ^a	Yu and Crump (1998)
Carpet underlay	8,110–12 ^a	Yu and Crump (1998)
Vinyl/PVC flooring	22,280–91 ^a	Yu and Crump (1998)
Linoleum flooring	220–22 ^a	Yu and Crump (1998)
Vinyl tiles	91–45 ^a	Yu and Crump (1998)
Rubber floorings	1,400 ^b	Yu and Crump (1998)
Soft plastic flooring	590 ^b	Yu and Crump (1998)
Cork floor tiles	805–7 ^a	Yu and Crump (1998)
Mineral wool insulation batt	15–12 ^b	Yu and Crump (1998)
Glass wool fibrous insulation	4–0.08	Yu and Crump (1998)
Extruded polystyrene thermal insulants	1,400–22 ^a	Yu and Crump (1998)
Extruded polyethylene duct and pipe insulants	0.8–0.28 ^b	Yu and Crump (1998)
Plastic laminated board	0.4 ^b	Yu and Crump (1998)
Vinyl and fiber glass wallpaper	300 ^b	Yu and Crump (1998)
PVC foam wallpaper	230	Yu and Crump (1998)
PVC wall covering	100	Yu and Crump (1998)
Vinyl coated wallpaper	95–20	Yu and Crump (1998)
Vinyl wallpaper	40	Yu and Crump (1998)
Wallpaper	100–31	Yu and Crump (1998)
Vapor barriers (bituminous tar)	6.3 ^c	Yu and Crump (1998)

Supplemental Information—Formaldehyde (Inhalation)

Products	Emission Rate (µg/m ² -hr)	Reference
Black rubber trim for jointing	103	Yu and Crump (1998)
Vinyl covering	46–30 ^d	Yu and Crump (1998)
Textile wall and floor coverings	1,600 ^b	Yu and Crump (1998)
Acoustic partitions	158–6 ^a	Yu and Crump (1998)
Office chair	1,060–100 ^a	Yu and Crump (1998)
Particle board	1,500–2,167 ^e 200–28 ^a	Pickrell et al. (1984) Yu (Yu and Crump, 1998)
Plywood	1,292–1,375 ^e 1,450–44	Pickrell et al. (1984) Yu and Crump (1998)
Bare urea-formaldehyde wood products (¼–¾")	8.6–1,580 ^f	Kelly et al. (1999)
Coated urea-formaldehyde wood products	<2.7–460 ^f	Kelly et al. (1999)
Permanent press fabric	42–215 ^f	Kelly et al. (1999)
Decorative laminates	4.2–51 ^f	Kelly et al. (1999)
Fiberglass products	16–32 ^f	Kelly et al. (1999)
Bare phenol-formaldehyde wood products	4.1–9.2 ^f	Kelly et al. (1999)
Paper grocery bags	<0.5 ^f	Kelly et al. (1999)
Paper towels	<0.6 ^f	Kelly et al. (1999)
Latex paint	326–854 ^b	Kelly et al. (1999)
Fingernail hardener	178,000–215,500 ^b	Kelly et al. (1999)
Nail polish	20,700 ^b	Kelly et al. (1999)
Commercially applied urea-formaldehyde floor finish	421–1,050,000 ^b	Kelly et al. (1999)

^a The first number in the range indicates initial emissions; the second number indicates emissions after some time (e.g., hours, days, months).

^b Values represent initial emissions.

^c 124 days old.

^d <98 days old.

^e Range indicates different test conditions in temperature and relative humidity.

^f Emission rates represent typical conditions, defined as 70 °F, 50% Relative Humidity, and 1 air change per hour.

Table A-4. Studies on residential indoor air levels of formaldehyde

Location (year measured)	N ^a	Concentration mean (range); µg/m ³	Reference
Manufactured housing			

Supplemental Information—Formaldehyde (Inhalation)

Location (year measured)	N^a	Concentration mean (range); $\mu\text{g}/\text{m}^3$	Reference
LA & MS, FEMA-supplied temporary housing units (Dec. 2007–Jan. 2008)	519 ^b	95 (3.7–726) ^c	Henry (2008)
FEMA 4 temporary housing units (2007)	4 ^b	569 (331–926)	Maddalena et al. (2008)
Baton Rouge, LA, 96 FEMA-supplied temporary housing units (2006) Baseline ^d Ventilation with air conditioning and bathroom vents only Ventilation with open windows and vents	96 852 863	1,279 (12–4,500) 480 (0–2,005) 111 (12–603)	ATSDR (2007)
Florida, new manufactured house (2000)	NR	95 (NR)	Hodgson et al. (2002) ^e
United States, East and Southeast (1997–98)	4	42 (26–58)	Hodgson et al. (2000) ^e
California, mobile homes (1984–85)	470	86–111(NR)	Sexton et al. (1989) ^f
United States (NR) Complaint mobile homes Newer mobile homes Older mobile homes	>500 260	123–1,107 (0–5,166) 1,032 308	Gammage and Hawthorne (1985)
Texas, mobile homes whose residents requested testing (1979–82) Homes < 1 year old Homes > 1 year old	443 ^b	NR (ND–9,840) ≥ 2,460 for 27% of homes ≥ 2,460 for 11.5% of homes	Norsted et al. (1985) ^f
United States (NR)	430 ^b	> 1,230 for 4% of samples 615–1,230 for 18% of samples 123–615 for 64% of samples < 123 for 14% of samples	Breyse (1984) ^g
United States (NR)	431 ^b	470 (12–3,599)	Ulsamer et al. (1982) ^g
United States (NR) Complaint homes, WA, < 2 year old Complaint homes, WA, 2–10 year old Complaint homes, MN, < 2 year old Complaint homes, MN, 2–10 year old Complaint homes, WI, < 2 year old Complaint homes, WI, 2–7 year old Random sample, WI, < 2 year old	110 ^b 77 ^b 66 ^b 43 ^b 38 ^b 9 ^b NR	950 (NR) 581 (NR) 1,041 (NR) 339 (NR) 891 (NR) 560 (NR) 661 (NR)	Stone et al., 1981 ^g
Wisconsin, complaint homes, 0.2–12 year old (NR)	65 ^b	590 ^h (NR)	Dally et al. (1981) ^g
Conventional housing or unspecified			
California (2011–2013)	352 ^b	21 (NR)	Vardoulakis et al. (2020)
Cincinnati, Ohio (2011) (median, IQR) Low-income homes, renovated and nonrenovated, all measurements	96	20 (14–33)	Coombs et al. (2016)

Supplemental Information—Formaldehyde (Inhalation)

Location (year measured)	N^a	Concentration mean (range); µg/m³	Reference
Quebec City, Canada (2008–2011)	83 ^b	37 (NR)	Vardoulakis et al. (2020)
Summer Field, CA (2006)	52 ^b	36 (4.7–143.6)	Offermann et al. (2008)
Québec, Canada (2005)	96 ^b	30 (9.6–90)	Gilbert et al. (2006)
Prince Edward Island, Canada (winter 2002)	59 ^b	39.0 (5.5–87.5)	Gilbert et al. (2005)
Los Angeles, CA; Houston, TX, and Elizabeth, NJ (summer 1999–spring 2001)	398	22 ± 7.1 ⁱ	Weisel et al. (2005)
New York City, NY (46 houses) (1999), Los Angeles, CA (41 houses) (2000) NYC (winter) NYC (summer) LA (winter) LA (fall)	37 41 40 33	12 ± 4.7 (5.2–22) 21 ± 11 (5.8–51) 21 ± 11 (7.9–59) 16 ± 6.2 (8.2–32)	Sax et al. (2004)
Canada (1989–1995) Northwest Territories; Windsor, Ontario; Hamilton, Ontario; Trois-Rivières, Québec; Saskatoon, Saskatchewan	151	36 (12–144)	Environment Canada (2000)
United States, East and Southeast, site-built houses (1997–1998)	7	44 ^j (17–71)	Hodgson et al. (2000) ^e
Arizona (Jun. 1995–Feb. 1998)	189	21 ^h (max. 408)	Graf et al. (1999)
Louisiana, 53 houses: 75% urban; 25% rural (NR)	419	460 (ND–6,599)	Lemus et al. (1998) ^e
Boston, MA (1993) winter, 4 residences summer, 9 residences	14 26	13.7 (7.4–19.8) 19.8 (7.3–66.2)	Reiss et al. (1995) ^e
Maryland (1995) Newly built house 30 days after installation pressed wood	1 ^b	<94 55	Hare et al. (1996)
Colorado (1992–93) Prior to occupancy After occupancy for 5 months	9	26 (8.0–66) 49 (33.0–81.2)	Lindstrom et al. (1995) ^e
New Jersey, 6 residential houses (1992)	36	67.1 (33–125)	Zhang et al. (1994)
Arizona, houses (NR)	202 ^b	32 (max. 172)	Krzyzanowski et al. (1990) ^d
United States, residential, various locations (1981–84)	273	44.0 ^h (NR)	Shah and Singh (1988) ^b
San Francisco, CA, Bay Area (1984) Kitchen Main bedroom	48 45	50 (NR) 44 (NR)	Sexton et al. (1986) ^b
United States (NR) Homes with UFFI Homes with UFFI	>1,200 131	62–148 (123–4,182) 31–86 (12–209)	Gammage and Hawthorne (1985)
Pullman, WA, houses (NR)	NR	6.2–89 (NR)	Lamb et al. (1985) ^f

Supplemental Information—Formaldehyde (Inhalation)

Location (year measured)	N ^a	Concentration mean (range); $\mu\text{g}/\text{m}^3$	Reference
United States (NR) UFFI houses Non-UFFI houses and apartments	244 ^b 59 ^b	> 1,230 for 2.8% of samples 615–1,230 for 1.9% of samples 123–615 for 24.1% of samples < 123 for 71.2% of samples > 1,230 for 1.8% of samples 615–1,230 for 1.8% of samples 123–615 for 36.3% of samples < 123 for 60.1% of samples	Breyse (1984)^g
United States (1982) Houses 0–30 year old Houses 0–5 year old Houses 5–15 year old Houses > 15 year old Houses 0–5 year old spring summer autumn Houses 5–15 year old spring summer autumn Houses > 15 year old spring summer autumn	40 ^b 18 ^b 11 ^b 11 ^b 18 ^b 11 ^b 11 ^b	75.9 $\pm$ 95.0 ⁱ 103.0 $\pm$ 112.1 ⁱ 52.0 $\pm$ 52.0 ⁱ 39.0 $\pm$ 52.0 ⁱ 107.0 $\pm$ 114.0 ⁱ 137 $\pm$ 125 ⁱ 58.0 $\pm$ 68.0 ⁱ 53.0 $\pm$ 49.0 ⁱ 60.0 $\pm$ 59.0 ⁱ 41.9 $\pm$ 43.1 ⁱ 44.0 $\pm$ 63.0 ⁱ 36.0 $\pm$ 46.0 ⁱ 32.0 $\pm$ 28.0 ⁱ	Hawthorne et al. (1983)^g
United States (1983) Energy-efficient new houses Low-ventilation modernized houses	20 ^b 16 ^b	76 (NR) 37 (NR)	Grimsrud et al. (1983)^g
United States (1981) Houses without UFFI Houses with UFFI	41 ^b 636 ^b	40 (12–98) 150 (12–4,200)	Ulsamer et al. (1982)^g
United States (1980–81) Houses averaging 2 year old air-tight construction mechanical ventilation Houses averaging 6 year old (loose construction)	9 ^b 1 ^b	44 $\pm$ 22 ⁱ 33 $\pm$ 20 ⁱ 17 (NR)	Offerman et al., 1982 ^g
United States (1978–79)	13 ^b	120 ^h (NR)	Dally et al. (1981)^g
United States (1979) Energy-efficient house Unoccupied house without furniture Unoccupied house with furniture Occupied house day night	2 ^b	98 (40–150) 81 $\pm$ 7.0 ⁱ 225 $\pm$ 16.0 ⁱ 263 $\pm$ 26.0 ⁱ 141 $\pm$ 44.0 ⁱ	Berk et al. (1980)^g

Supplemental Information—Formaldehyde (Inhalation)

Note: Concentrations were converted from ppb to $\mu\text{g}/\text{m}^3$ for consistency ($1 \text{ ppb} = 1.23 \mu\text{g}/\text{m}^3$).

ND = not detected; NR = not reported; UFFI = urea formaldehyde foam insulation; IQR = interquartile range; FEMA = Federal Emergency Management Agency.

^a Number of samples unless denoted with footnote (b).

^b Number of houses.

^c Geometric mean.

^d Baseline refers to initial levels measured 4 days prior to intervention phase of the study during which ventilation via air conditioning or open windows was provided.

^e Cited in [\(IARC\) \(2006\)](#).

^f Cited in [ATSDR \(1999\)](#).

^g Cited in [IPCS \(1989\)](#).

^h Median.

ⁱ Standard deviation.

Source: Adapted from [NTP \(2010\)](#) and other sources as noted.

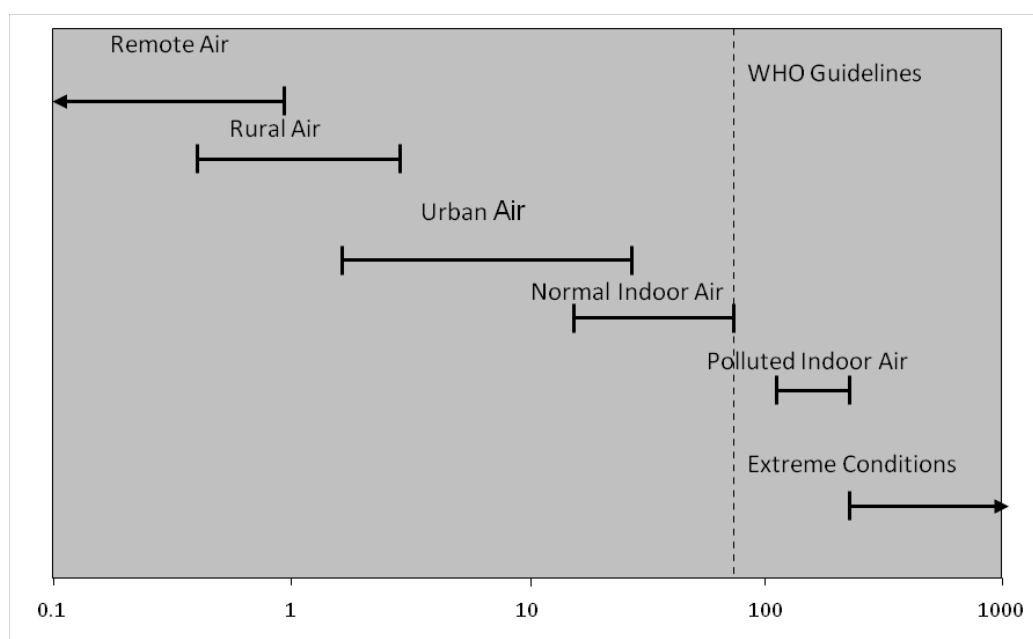


Figure A-3. Range of formaldehyde air concentrations (ppb) in different environments.

Notes: Graph is in logarithmic scale; “Normal indoor conditions,” “polluted indoor conditions,” and “extreme conditions” were not defined.

Source: [Salthammer et al. \(2010\)](#).

In addition, the Canadian indoor air data may overestimate formaldehyde levels in U.S. homes, because many residential homes in Canada use wood burning stoves more frequently and have tighter construction (due to colder winters), leading to less dilution of indoor emissions. The outdoor air levels, however, appear to have remained fairly constant over recent years, and the median outdoor level from the Canadian study ($2.8 \mu\text{g}/\text{m}^3$) (2.3 ppb) is very similar to the median of the U.S. monitoring data ($2.83 \mu\text{g}/\text{m}^3$) (2.3 ppb) in 1999.

Supplemental Information—Formaldehyde (Inhalation)

Indoor air measurements combined with information about daily activity diaries have been used as surrogate of personal exposures. A recent study conducted with 41 children ages 9–12 years old in Australia concluded that although indoor air measurements from stationary monitors tended to slightly overestimate personal exposures, they were a good surrogate of personal exposures to children ([Lazenby et al., 2012](#)). The mean exposure from personal monitors ranged from <5 to 34 $\mu\text{g}/\text{m}^3$ (<4–26.3 ppb) with a mean of 13.7 $\mu\text{g}/\text{m}^3$ (11.1 ppb) ([Lazenby et al., 2012](#)).

APPENDIX B. SYSTEMATIC REVIEW METHODS DOCUMENTATION

B.1. SUMMARY OF DOCUMENTATION

This Appendix complements the methods outlined in Sections 2.2 and 2.3 of the Toxicological Review, namely the literature search and screening and the study evaluation portions of the assessment, respectively. This Appendix provides the documentation of applying those methods to the formaldehyde inhalation evidence base for literature search and screening (Appendix B.2) and study evaluation (Appendix B.3). For this assessment, the searches and study evaluations were specific to each health effect- or mechanism- specific topic, as described in Section 2 of the Toxicological Review. Thus, although there is overlap (e.g., for epidemiology studies or experimental animal studies irrespective of health topic), they are documented separately by topic.

This Appendix also includes the results of a systematic evidence map (SEM) developed in 2021 (see Section 2.2 of the Toxicological Review). The results of that SEM are similarly subdivided by health effect- or mechanisms-specific topic. The specific aims of the 2021 SEM were: to:

- Identify epidemiological (i.e., human), toxicological (i.e., experimental animal), and mechanistic literature using an identical literature search approach as was used to develop a 2017 Step 1 draft IRIS formaldehyde-inhalation assessment (see Section 2.2).
- Tag supplemental (not primary research) studies.
- Create a literature inventory of studies meeting PECO criteria. The literature inventory summarizes basic features of study design, health system(s), and endpoints assessed.
- Assess PECO-relevant studies, within each health effect category, to determine if they are *possibly impactful* (i.e., likely, based on the study design and tested exposure levels, to have a potential impact on the hazard conclusions or toxicity values), and document the reasons in a literature inventory.

B.2. LITERATURE SEARCH AND SCREENING

B.2.1. Overview

As described in Section 2.2.1 of the Toxicological Review, initial searches (computerized searches augmented by secondary search approaches) were conducted in 2012, except for the search for mechanisms related to respiratory health effects, (Appendix B.2.6), which was initially conducted in 2014. Annual updates were performed annually through 2016 in support of development of a completed, 2017 draft assessment. In 2021, a separate systematic evidence map

(SEM) was developed to identify the relevant literature from 2016 through 2021, in particular studies with the potential to impact hazard or toxicity value conclusions. Thus, Appendix B.2.2 through B.2.10 below separately describes the search strings used, the search and screening results for literature searches through 2016, and the literature search results and documentation of the screening from the 2021 SEM. Details of the methods are provided in the corresponding subsections of Section 2.2 in the Toxicological Review.

The literature search results and tagging (i.e., the screening decisions by the two reviewers based on the PECO criteria in the Toxicological Review) are separately documented in HERO (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/4051).¹

B.2.2. Sensory Irritation

As detailed in Section 2.2.2 of the Toxicological Review, the sensory irritation review focused on human studies of symptoms of irritation, primarily ocular, nasal, and throat symptoms. Animal and mechanistic studies were not a focus of this search. The search strings are documented in Table B-1, the literature search results through 2016 are documented in Figure B-1, and the literature search results through 2021 identified by the SEM are documented in Figure B-2, with the SEM screening decisions documented in the subsequent section.

Search Strings

Table B-1. Search terms for sensory irritation

Database, search parameters	Terms
PubMed No date restriction	(Formaldehyde[majr] OR paraformaldehyde[majr] OR formalin[majr]) AND (irritation OR irritant OR irritants)
Web of Science No date restriction	TS= (Formaldehyde OR paraformaldehyde OR formalin) AND TS=(irritation OR irritant OR irritants)

¹A separate HERO page was previously used for documentation in prior drafts of this assessment: (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/31). This page included highly variable screening documentation applied as systematic review methods were evolving within the IRIS Program in parallel with development of the draft formaldehyde assessment. The current HERO page (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/4051) consolidates and refines those search and screening tags into a structure that is more transparent, user-friendly, and consistent with current practice. The total number of studies is lower in the current HERO page. In addition to improvements to HERO (e.g., duplicate removal), the older page includes studies not identified as part of the current systematic reviews; for example, the older page documents the cited references from prior formaldehyde assessments (e.g., the draft 2010 IRIS assessment), including background studies not used in the current assessment.

Literature Search Results through 2016

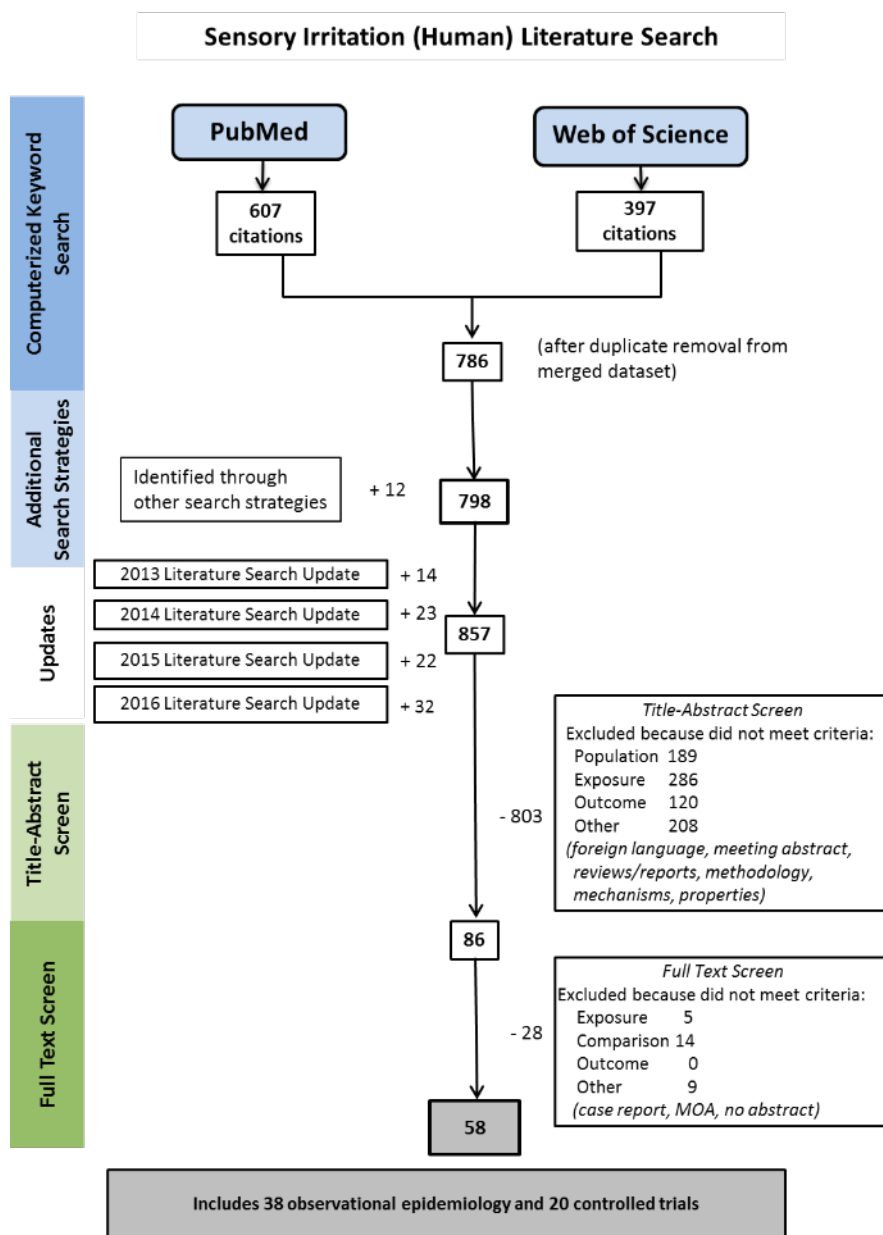


Figure B-1. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and sensory irritation in humans.

Literature Search Results from the Systematic Evidence Map Update through 2021

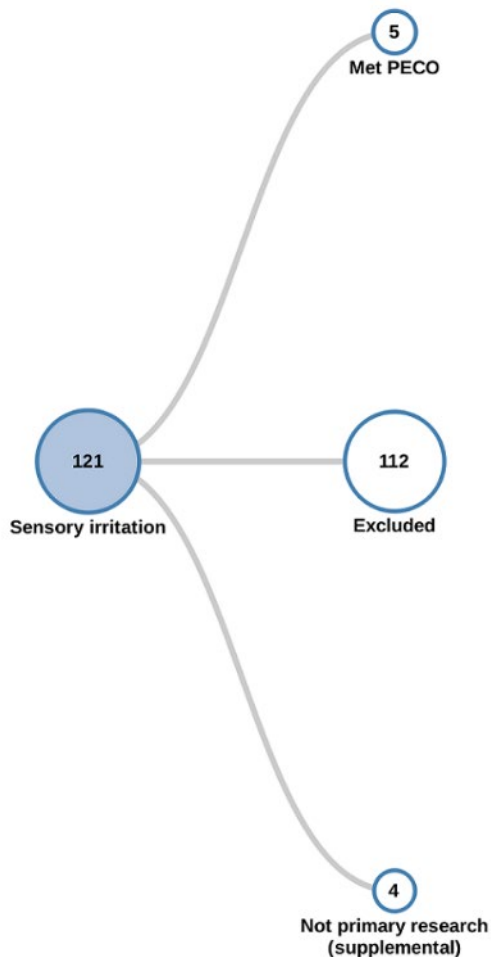


Figure B-2. Sensory irritation literature tree (interactive version [here](#)).

A total of 121 citations were retrieved for the assessment of sensory irritation in humans and five studies met PECO criteria.

Screening for Inclusion from the 2021 Systematic Evidence Map Update

Of the five studies meeting PECO criteria, one of the identified studies was deemed to be *possibly impactful* ([Saowakon et al., 2015](#)) but already had been included in the 2017 draft (Table B-2), and thus zero additional studies were included.

Table B-2. Studies of sensory irritation effects in humans

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
Humans					
Saowakon et al. (2015)	Not extracted	Not extracted	Not extracted	Possibly impactful	Already identified and considered in 2017 draft
Aung et al. (2021)	Occupational Myanmar cross-sectional	Air sampling, adults, medical students, and instructors in anatomy dissection rooms	Unpleasant odor, eye irritation, nasal irritation symptoms	Not impactful	High exposure levels, adults, health effects well-established
Deng et al. (2020) only abstract available (full text Chinese)	Occupational China cross-sectional	Air sampling, adults, medical students in anatomy dissection rooms	Subjective symptoms (e.g., itchy eyes, nasal congestion, runny nose)	Not impactful	High exposure levels, adults, health effects well-established
Sakellaris et al. (2020)	Occupational Europe (Portugal, Spain, Italy, Greece, France, Hungary, the Netherlands, Finland) cross-sectional	Air sampling, adults, office building occupants	Eye irritation (dry eyes, watering, or itchy eyes, burning or irritated eyes), respiratory symptoms (blocked or stuffy nose, runny nose, dry/irritated throat, cough	Not impactful	Adults, health effects well-established
Thetkathuek et al. (2016)	Occupational, Chacheongsao Province, Thailand	Air sampling, adults, medium-density fiberboard furniture workers	Respiratory irritation symptoms	Not impactful	High exposure levels, adults, health effects well-established

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
	cross-sectional				

Rows for studies judged as “not impactful” are shaded gray.

B.2.3. Pulmonary Function

As detailed in Section 2.2.3 of the Toxicological Review, the pulmonary function review focused on human studies of standard, quantitative pulmonary function measures, including spirometric measures, FEV₁, FVC, and FEF_{25–75}, as well as PEF measured using a flowmeter. Animal and mechanistic studies were not a focus of this search. The search strings are documented in Table B-3, the literature search results through 2016 are documented in Figure B-3, and the literature search results through 2021 identified by the SEM are documented in Figure B-4, with the SEM screening decisions documented in the subsequent section.

Search Strings

Table B-3. Summary of search terms for pulmonary function

Database, search parameters	Terms
PubMed No date restriction	(Formaldehyde[majr] OR paraformaldehyde[majr] OR formalin[majr]) AND ("pulmonary function" OR "lung function" OR "spiometr*")
Web of Science No date restriction	TS=(Formaldehyde OR paraformaldehyde OR formalin) AND TS=(pulmonary function OR lung function OR spirometry)

Abbreviations: Majr= major topic (filter); TS= the requested "topic" is included as a field tag.

Literature Search Results through 2016

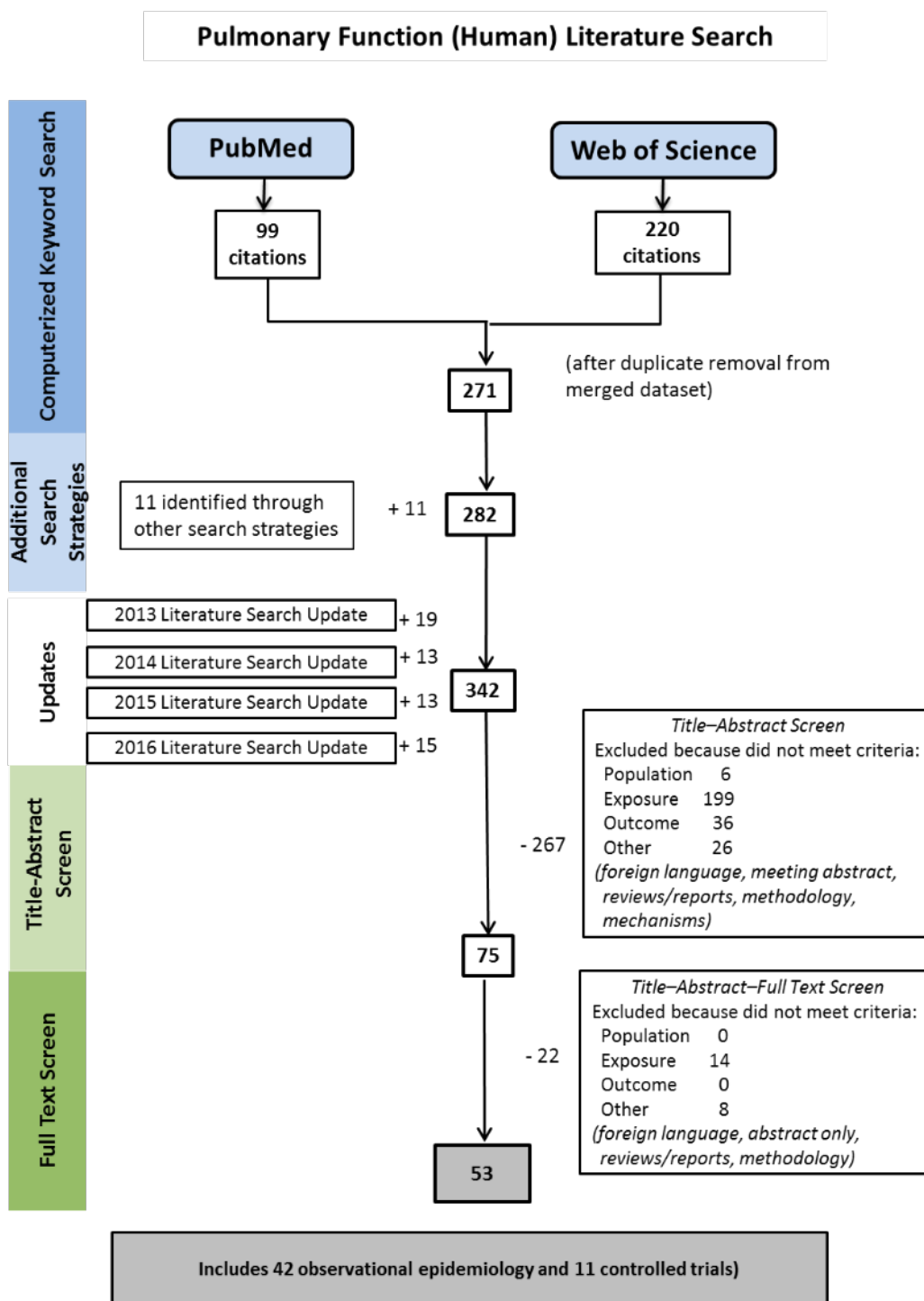


Figure B-3. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and pulmonary function in humans.

Literature Search Results from the Systematic Evidence Map Update through 2021

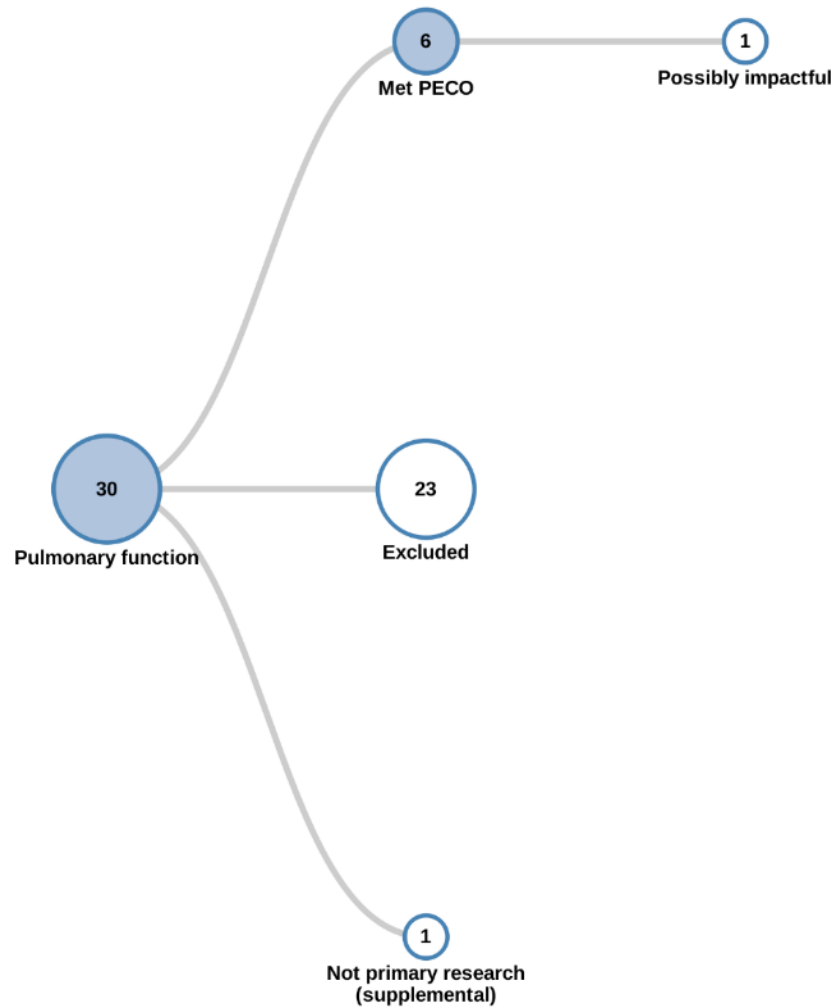


Figure B-4. Pulmonary function effects in humans literature tree (interactive version [here](#)).

A total of 30 citations were retrieved for the assessment of pulmonary function effects in humans and six studies met the PECO criteria.

Screening for Inclusion from the 2021 Systematic Evidence Map Update

Of the six studies meeting the PECO criteria, one study, ([Saowakon et al., 2015](#)), was deemed to be *possibly impactful* (see Table B-4), but already had been included in the 2017 draft; thus, zero (0) additional studies were included.

Table B-4. Studies of pulmonary function effects in humans

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
Humans					
Saowakon et al. (2015)	Not extracted			Possibly impactful	Already identified and considered in 2017 draft
Fsadni et al. (2018)	Schools-based Malta cross-sectional	Air sampling, children, school children	Pulmonary function tests (not specified)	Not impactful	Important details were not provided
Asgedom et al. (2019)	Occupational Ethiopia cross-sectional	Air sampling, adults, particleboard workers	Lung function (FVC, FEV1, FEF 25-75%)	Not impactful	High exposure levels, adults
Deng et al. (2020) only abstract evaluated (full text Chinese)	Occupational China cross-sectional	Air sampling, adults, medical students in anatomy dissection rooms	FEV1, FEV1/FVC, PEF, FEF 25%-75%, MEF25%, FEF50%-75%	Not impactful	High exposure levels, adults
Neghab et al. (2017)	Occupational Shiraz, Iran cross-sectional	Air sampling, adults, kitchen workers exposed to cooking fumes	VC, FVC, FEV1, PEF, FEV1/FVC, FEV1/VC	Not impactful	High exposure levels, adults
Zarei et al. (2017)	Occupational Tehran, Iran cross-sectional	Air sampling, adults, foundry coremakers	FVC, FEV1, FEV1/FVC, peak expiratory flow (PEF), mid forced expiratory flow (FEF25-75%)	Not impactful	High exposure levels, adults

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

FEF_{25-75%} - mid forced expiratory flow, FEF_{50-75%} - forced expiratory flow 50-75%, FEV₁- Forced expiratory volume in one second, FVC – forced vital capacity, PEF - peak expiratory flow, MEF_{25%} - mean flow at 25%, VC -vital capacity.

B.2.4. Immune-Mediated Conditions, including Allergies and Asthma

As detailed in Section 2.2.4 of the Toxicological Review, the immune-mediated conditions review focused on hypersensitivity (allergy) and on asthma. The searches ultimately used to support the evidence synthesis and integration decisions focused on identifying studies of exposed humans. Although experimental animal studies of hypersensitivity were initially included in searches through 2016 (the 2021 SEM did not include this search), given the conclusion that these studies were most appropriately reviewed as mechanistic information (see Section 3.2.3 of the Toxicological Review), these studies were tagged as potentially relevant supplemental material and considered as part of the overarching mechanistic evaluation documented in Appendix B.2.6 (note that these studies were also identified by the searches specific to that mechanistic evaluation). The search strings are documented in Table B-5, the literature search results through 2016 are documented in Figure B-5, and the literature search results through 2021 identified by the SEM are documented in Figure B-6, with the SEM screening decisions documented in the subsequent section.

Search Strings

Table B-5. Search terms – allergy-related conditions and asthma

Database, Initial search date	Terms
PubMed 10/31/2012 No date restriction	formaldehyde and (asthma or wheeze or respiratory or allergy or immune or sensitization) NOT ("formalin test" OR "formaldehyde fixation" OR "formalin fixation" OR "formalin fixed" OR "formaldehyde fixed" OR "formalin-induced" OR "formalin-evoked")
Web of Science 11/5/2012 No date restriction	(TS=formaldehyde and TS=asthma) OR (TS=formaldehyde and TS=allergy) OR (TS=formaldehyde and TS=immune) OR (TS=formaldehyde and TS=respiratory) OR (TS=formaldehyde and TS=sensitization) OR (TS=formaldehyde and TS=wheeze)
Toxline 11/2/2012 No date restriction	formaldehyde @AND @OR (immune allergy asthma respiratory wheeze sensitization)

Literature Search Results through 2016

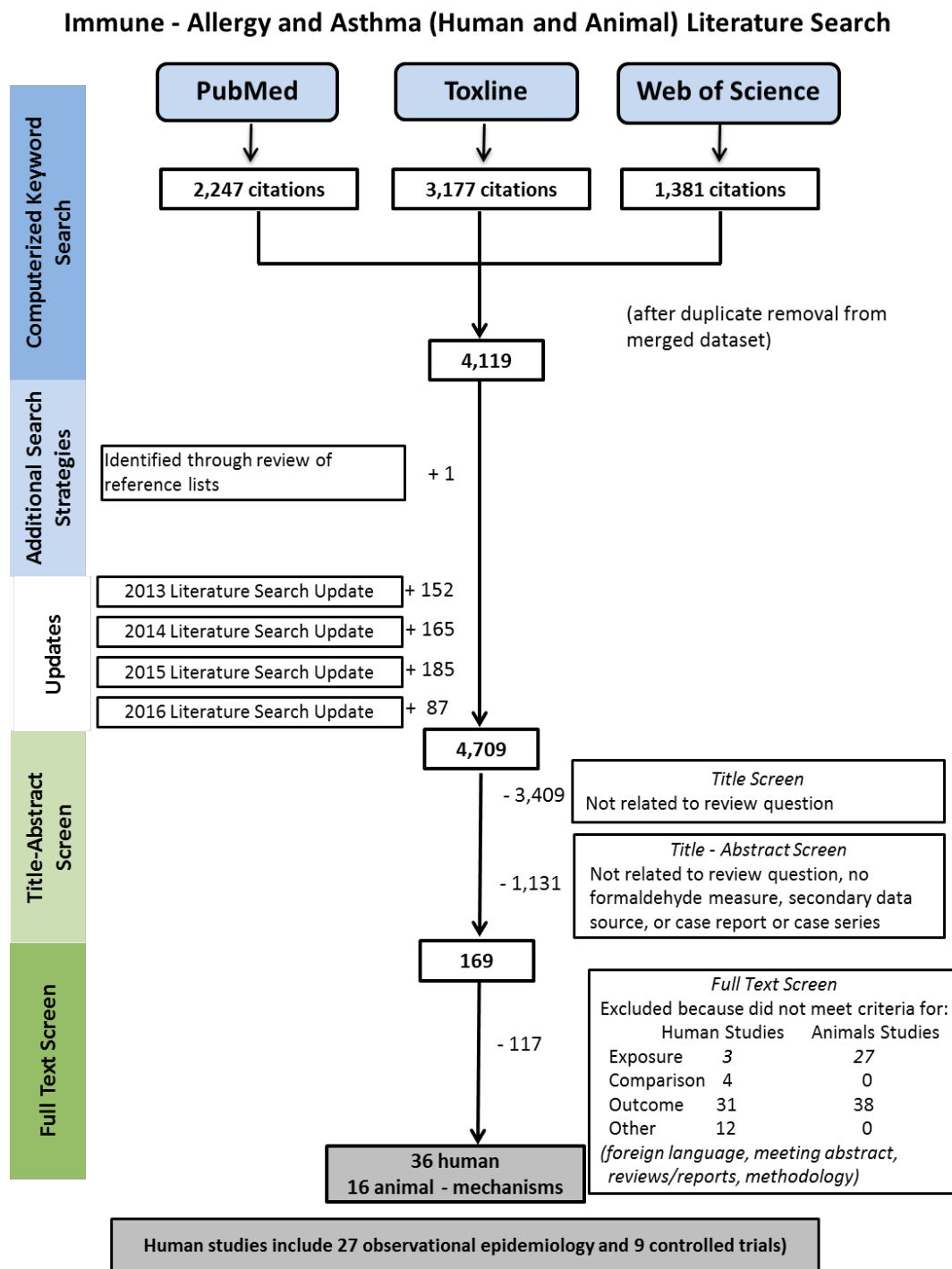


Figure B-5. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and respiratory and immune-mediated conditions. [Note: animal mechanistic studies were ultimately used in the analysis described in Appendix B.2.6, so were tagged as supplemental for this search]

Literature Search Results from the Systematic Evidence Map Update through 2021

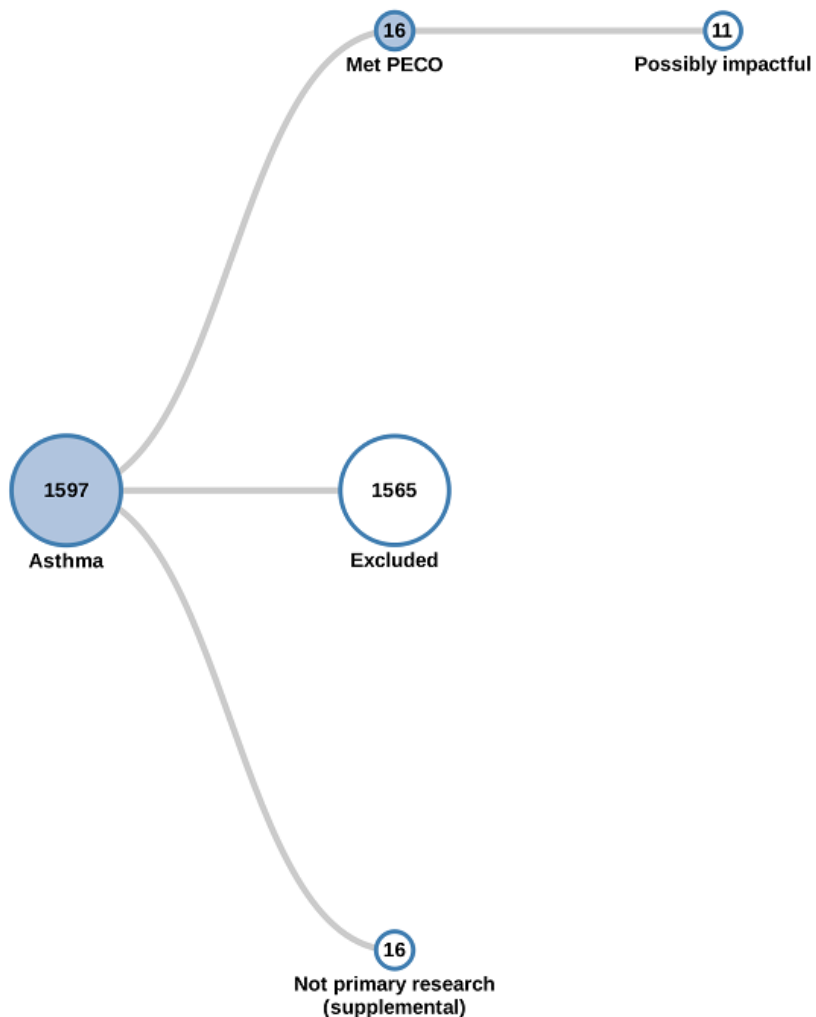


Figure B-6. Asthma and immune effects in humans literature tree (interactive version [here](#)).

A total of 1,597 citations were retrieved for the assessment of asthma and immune effects in humans and 16 studies met the PECO criteria.

Screening for Inclusion from the 2021 Systematic Evidence Map Update

Of the 16 studies meeting PECO criteria, 11 studies were deemed to be *possibly impactful* (see Table B-6).

Table B-6. Studies of immune-mediated conditions in humans, focusing on allergies and asthma

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
Humans					
Branco et al. (2020a)	School-based Northern Portugal cross-sectional	Air sampling, children, preschoolers/primary school students	Asthma (reported, diagnosed), wheezing (active)	Possibly impactful	School-based – children; indoor formaldehyde concentrations between 10–80 µg/m ³
Huang et al. (2017)	Population-based Shanghai, China case-control	Air sampling in residence, children	Current rhinitis	Possibly impactful	Population-based – children; indoor formaldehyde concentrations between 10–80 µg/m ³
Isa et al. (2020a)	School-based Selangor, Malaysia cross-sectional	Air sampling in classroom, children	Rhinitis (past 12 months), skin allergy (past 12 months)	Possibly impactful	School-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m ³
Lajoie et al. (2014)	Population-based Quebec, Canada intervention study	Air sampling, children, ventilation intervention study	Change in prevalence of asthma symptoms and medical care	Possibly impactful	Population-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m ³
Li et al. (2019)	Population-based Hong Kong cohort	Air sampling, birth to 18 mo	Wheeze (new onset)	Possibly impactful	Population-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m ³
Liu et al. (2018a)	Population-based Changchun, China case-control	Air sampling in residence, children	Asthma diagnosis	Possibly impactful	Population-based – children; indoor formaldehyde concentrations between 10–80 µg/m ³
Madureira et al. (2016)	Population-based Porto, Portugal case-control	Air sampling in residence, children	Current asthma	Possibly impactful	Population-based – children; indoor formaldehyde concentrations between 10–80 µg/m ³
Neamtiu et al. (2019)	School-based Alba County, Romania	Air sampling in classroom, children	Asthma-like symptoms (difficult breathing, dry cough, wheezing in past week), allergy-like symptoms	Possibly impactful	School-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m ³

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
	cross-sectional		(skin conditions such as rash, itch, eczema; eye disorders such as red, dry, swollen, itching, burning, or sensation of "sand in eyes"; rhinitis such as itching nose, sneezes, stuffy or blocked nose)		
Norbäck et al. (2017)	School-based Johor Bahru, Malaysia cross-sectional	Air Sampling, children	Rhinitis	Possibly impactful	School-based – children; indoor formaldehyde concentrations between 10–80 µg/m ³
Yon et al. (2019)	School-based Seongnam City, Korea cohort	Air sampling in classroom, children	Current asthma, rhinitis, rhinitis severity	Possibly impactful	School-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m ³
Yu et al. (2017)	Population-based Hong Kong cohort	Air sampling in residence, birth to 18 mo	Wheeze (new onset)	Possibly impactful	Population-based – children; mean indoor formaldehyde concentrations between 10–80 µg/m ³
Asgedom et al. (2019)	Occupational Ethiopia cross-sectional	Air sampling, adults, particleboard workers	Respiratory symptoms (cough, cough with sputum production, phlegm, wheezing, shortness of breath)	Not impactful	Occupational exposure - adults
Dumas et al. (2020)	Occupational United States cohort	Occupational history and job-task-exposure-matrix, adults, health workers (female nurses)	Self-reported incident physician-diagnosed asthma	Not impactful	Occupational exposure – adults
El-Feky et al. (2020)	Occupational Egypt cross-sectional	Industry/ production type, adults, factory workers	Chronic bronchitis, respiratory symptoms and signs, respiratory rate, nasal symptoms, eye symptoms, skin manifestations	Not impactful	Occupational exposure – adults

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
Fsadni et al. (2018)	School-based Malta cross-sectional	Air sampling in classroom, children	Wheezing, rhinitis, eczema, acoustic rhinometry, nasal lavage	Not impactful	Only qualitative results reported, e.g., whether statistically significant and directional arrow
Thetkathuek et al. (2016)	Occupational Chacheongsao Province, Thailand cross-sectional	Air sampling, adults, medium density fiberboard workers	Difficulty breathing, chest discomfort, wheeze	Not impactful	Occupational exposure - adults

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

B.2.5. Respiratory Pathology

As detailed in Section 2.2.5 of the Toxicological Review, the respiratory tract pathology review focused on histopathological endpoints and signs of pathology in nasal and respiratory tissues. Separate searches were performed to identify studies in humans, focusing on evidence of pathology in nasal tissues, and in experimental animals, focusing on any respiratory tissue pathology. Signs such as changes in mucociliary function, cellular proliferation, mucus flow rate, and rhinitis were tracked as supplemental and included in the discussion of mechanistic information for respiratory effects (Appendix B.2.6). The search strings are documented in Table B-7 (human evidence) and Table B-8 (animal studies), the literature search results through 2016 are documented in Figure B-7 (human evidence) and Figure B-8 (animal studies), and the literature search results through 2021 identified by the SEM are documented in Figure B-9 (human studies) and B-10 (animal studies), with the SEM screening decisions documented in the subsequent sections.

Search Strings

Human Studies

Table B-7. Search terms for respiratory tract pathology in humans

Database, Initial Search Date	Terms
PubMed 12/18/2012 No date limitation	(Formaldehyde[majr] OR paraformaldehyde[majr] OR formalin[majr]) AND (Hyperplasia OR metaplasia OR nasal mucosa OR occupational diseases OR respiratory tract diseases OR rhinitis OR mucociliary) AND (epidemiology OR epidemiological OR epidemiologic OR cohort OR retrospective studies OR retrospective OR prospective studies OR prospective OR cross-sectional OR case-control OR cross-sectional study OR prevalence study OR occupational)
Web of Science 12/19/2012 No date limitation	TS= (Formaldehyde OR paraformaldehyde OR formalin) AND TS= (Hyperplasia OR metaplasia OR nasal mucosa OR occupational diseases OR respiratory tract diseases OR rhinitis OR mucociliary) and TS= (epidemiology OR epidemiological OR epidemiologic OR cohort OR retrospective studies OR retrospective OR prospective studies OR prospective OR cross-sectional OR case-control OR cross-sectional study OR prevalence study OR occupational)
Toxline 05/03/2013 No date limitation	(Formaldehyde OR Paraformaldehyde OR Formalin) AND (Hyperplasia OR metaplasia OR nasal mucosa OR occupational diseases OR respiratory tract diseases OR rhinitis OR mucociliary) AND (epidemiology OR epidemiological OR epidemiologic OR cohort OR retrospective studies OR retrospective OR prospective studies OR prospective OR cross-sectional OR case-control OR cross-sectional study OR prevalence study OR occupational)

Animal Studies

Table B-8. Search terms for respiratory tract pathology in animals

Database, initial search date	Terms
PubMed 10/18/2012 Search up through 9/30/2012	Formaldehyde* AND (animals OR dog OR dogs OR canine OR canines OR beagle OR beagles OR "guinea pig" OR "guinea pigs" OR Cavia OR hamster OR hamsters OR Cricetinae OR Mesocricetus OR mice OR mouse OR Mus OR monkey OR monkeys OR Macaca OR primate OR primates OR rabbit OR rabbits OR hare OR hares OR rat OR rats OR Rattus OR Rana or rodent OR rodents OR Rodentia) AND (alveol* OR bronchial OR bronchi OR buccal OR laryngeal OR larynx OR lung OR mouth OR nasal OR nasopharyngeal OR nasopharynx OR nose OR pharyngeal OR pharynx OR pulmonary OR respiratory OR sinonasal OR sinus OR trachea*) AND (edema OR oedema OR cancer OR carcinogens OR carcinogenesis OR carcinogenicity OR carcinoma OR "cell proliferation" OR cilia OR dysplas* OR epithelial OR epithelium OR goblet OR histopath* OR hyperplas* OR hypertrophy* OR metaplas* OR mucociliary OR mucos* OR mucous OR mucus OR necrosis OR neopla* OR olfactory OR patholog* OR rhinitis OR squamous OR transitional OR tumor OR tumour OR turbinate OR ulceration) NOT human
Web of Science 10/18/2012 Search up through 9/30/2012	Topic=Formaldehyde* AND (animals OR dog OR dogs OR canine OR canines OR beagle OR beagles OR "guinea pig" OR "guinea pigs" OR Cavia OR hamster OR hamsters OR Cricetinae OR Mesocricetus OR mice OR mouse OR Mus OR monkey OR monkeys OR Macaca OR primate OR primates OR rabbit OR rabbits OR hare OR hares OR rat OR rats OR Rattus OR Rana or rodent OR rodents OR Rodentia) AND (alveol* OR bronchial OR bronchi OR buccal OR laryngeal OR larynx OR lung OR mouth OR nasal OR nasopharyngeal OR nasopharynx OR nose OR pharyngeal OR pharynx OR pulmonary OR respiratory OR sinonasal OR sinus OR trachea*) AND (edema OR oedema OR cancer OR carcinogens OR carcinogenesis OR carcinogenicity OR carcinoma OR "cell proliferation" OR cilia OR dysplas* OR epithelial OR epithelium OR goblet OR histopath* OR hyperplas* OR hypertrophy* OR metaplas* OR mucociliary OR mucos* OR mucous OR mucus OR necrosis OR neopla* OR olfactory OR patholog* OR rhinitis OR squamous OR transitional OR tumor OR tumour OR turbinate OR ulceration) NOT human
Toxline 10/21/2012 Search up through 9/30/2012	formaldehyde AND (animal OR "nasal cavity" OR nose OR "respiratory tract" OR "cell proliferation" OR mucociliary OR histopathology OR pathology OR cancer OR tumor) NOT (human OR humans OR epidemiology OR epidemiological OR occupation* OR work* OR antinocicepti* OR nocicepti* OR pain OR sensory OR "formalin test" OR bacteria OR bacterial) (including synonyms and CAS numbers, but excluding PubMed records)

Literature Search Results through 2016

Human Studies

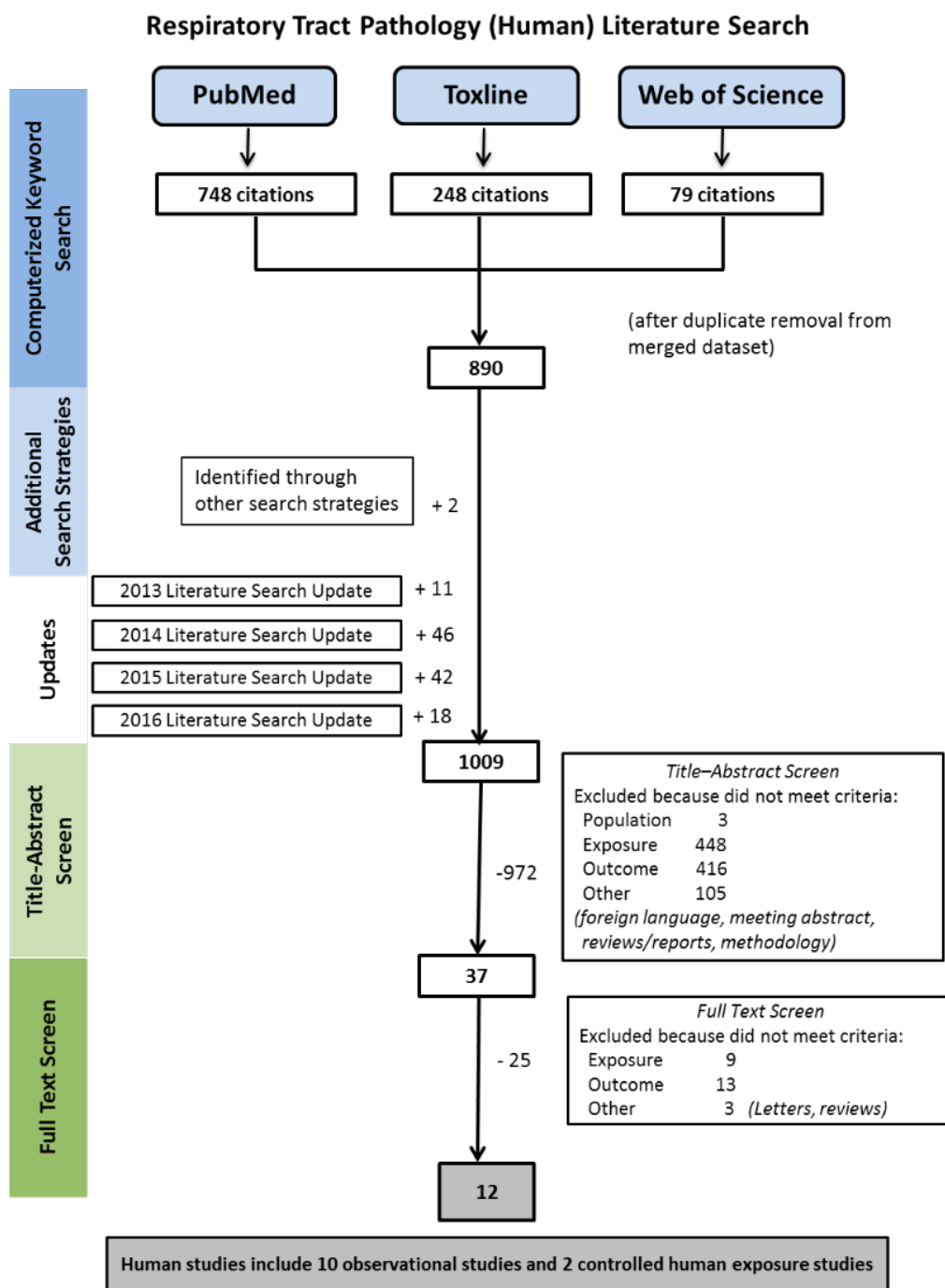


Figure B-7. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and respiratory tract pathology in humans. [Note: an additional controlled human exposure study that met PECO for the human respiratory tract pathology search (totaling 3) was identified by the sensory irritation search and evaluated (see Section B.3.5)].

Animal Studies

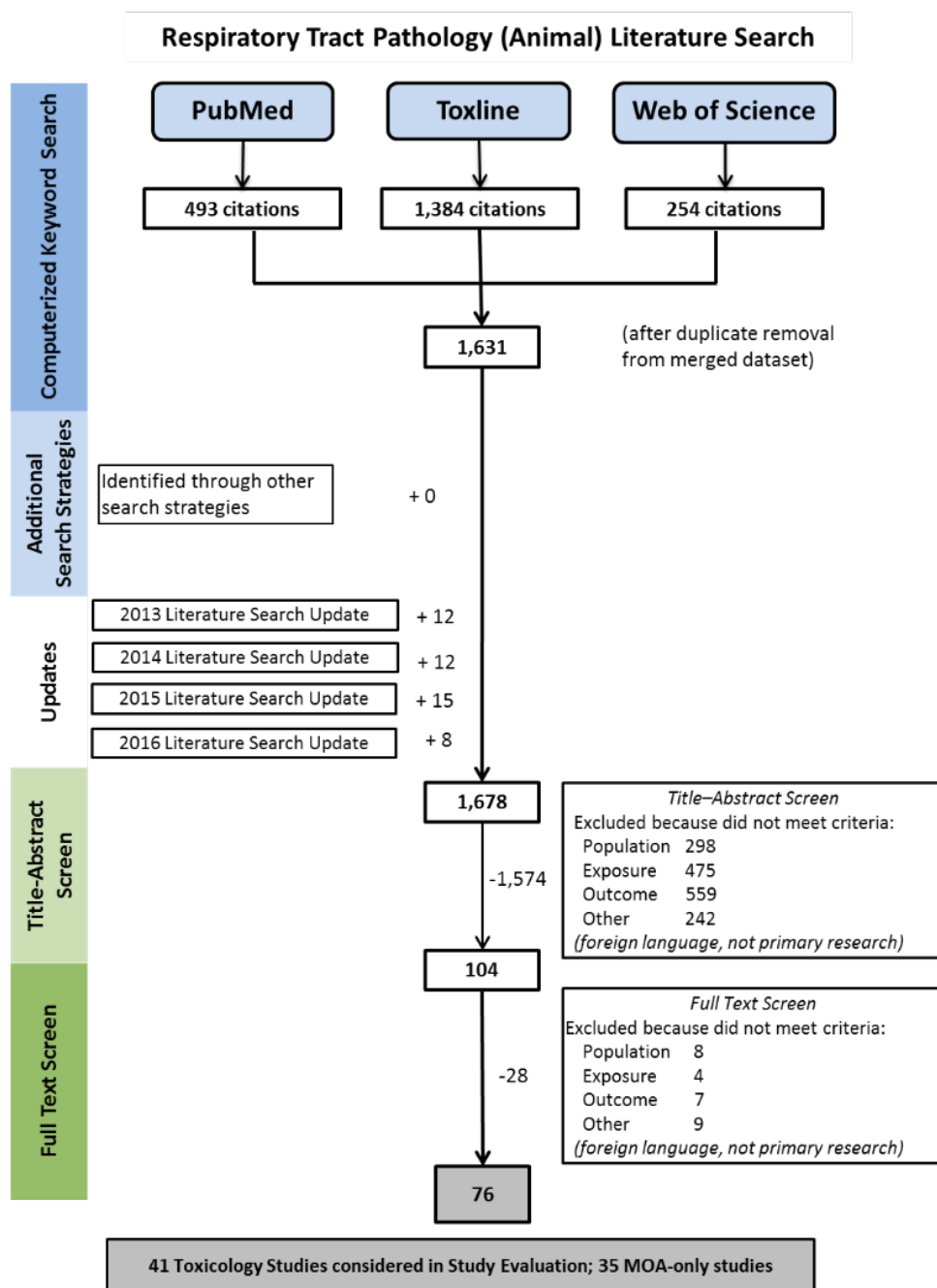


Figure B-8. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and respiratory tract pathology in animals [Note: the MOA studies of cellular proliferation and mucociliary function were ultimately considered in study evaluation and thus tracked as meeting the PECO criteria, including ~10 of the MOA-only studies; other MOA studies were considered as Mechanistic information, see Section B.2.6].

Literature Search Results from the Systematic Evidence Map Update through 2021

Human Studies



Figure B-9. Human respiratory tract pathology literature tree (interactive version [here](#)).

A total of 579 citations were retrieved for the assessment of respiratory tract pathology in humans and one study met the PECO criteria.

Animal Studies

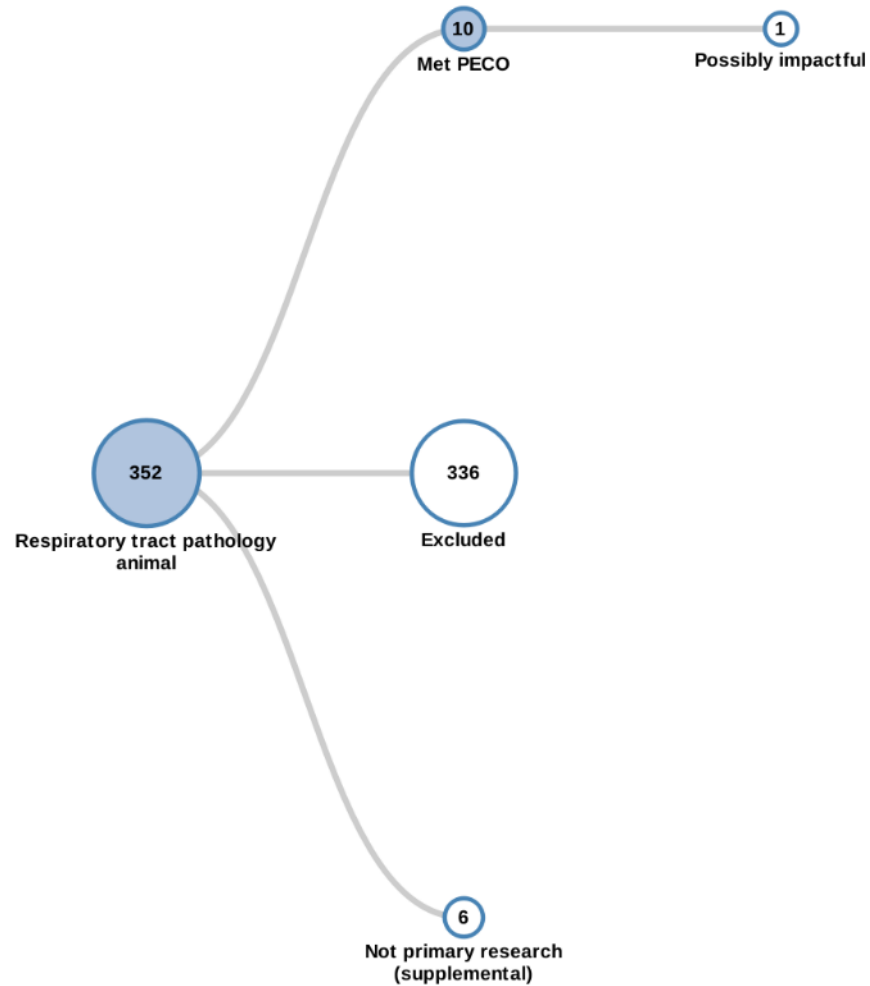


Figure B-10. Animal respiratory tract pathology literature tree (interactive version [here](#)).

A total of 352 citations were retrieved for the assessment of respiratory tract pathology in animals and ten studies met the PECO criteria.

Screening for Inclusion from the 2021 Systematic Evidence Map UpdateHuman Studies

This single human study meeting PECO criteria was deemed to be not impactful (see Table B-9); thus, zero additional studies were included.

Table B-9. Studies of respiratory tract pathology in humans

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
Humans					
Bruno et al. (2018)	Occupational Rome, Italy cross-sectional	Air sampling, adults, Laboratory pathology workers	Nasal cytology (muciparous metaplasia)	Not impactful	Adults, health effects well- established

Rows for studies judged as “not impactful” are shaded gray.

Animal Studies

Of the 10 animal studies meeting PECO criteria (see Table B-10), 1 ([Morgan et al., 2017](#)) was deemed to be *possibly impactful*. ([Morgan et al., 2017](#)) was identified in the literature search update and deemed to be *possibly impactful*, but already had been included in the 2017 draft; thus, zero (0) additional studies were included.

Table B-10. Animal studies of respiratory tract pathology

Reference	Study design	Exposure ^a	Endpoints	Impactful?	Rationale
Animal Studies					
Morgan et al. (2017)	Mouse (Trp53 haploinsufficient), Male Subchronic (8 wk; 6 hr/d, 5 d/wk), then held for 32 wk	Paraformaldehyde 0, 7.5 or 15 ppm (0, 9.2, 18.5 mg/m ³) Inhalation	All major tissues and gross lesions were collected for histopathology (including squamous metaplasia in respiratory tissues)	Possibly impactful	Already included in 2017 draft
Aydemir et al. (2017)	Rat (Wistar), both sexes Subchronic (6 wk; 8 hr/d, 5 d/wk)	Formalin 0, 6 ppm (0, 7.38 mg/m ³) Inhalation	Lung hematoxylin and eosin staining for qualitative review of inflammation and tissue morphology	Not impactful	Formalin; qualitative endpoints
Cheng et al. (2016)	Mouse (Kunming), male Short-term (up to 7 d; continuous)	Formalin 0, 0.08, 0.8 mg/m ³ Inhalation	Hematoxylin and eosin staining for inflammation and edema	Not impactful	Formalin; primarily qualitative endpoints
Abreu et al. (2016)	Mouse (C57BL/6), both sexes Acute (8 hr)	Unspecified test article 0, 0.2, 1.0, 3.0 ppm (0, 0.25, 1.23, 3.69 mg/m ³) Inhalation	Lung morphology and nasal ciliation; histological inflammatory cell counts in lung and scoring in nose	Not impactful	Unknown test article; acute
Lima et al. (2015)	Rat (Fischer), male Short-term (5 d; 20-min × 3/d)	Unspecified test article 0, 1, 5, 10% Inhalation	Trachea histology and morphometric analyses, including mucus production	Not impactful	Unknown test article; high levels; brief exposures
Liu et al. (2018b)	Rat (Sprague Dawley), male Short-term (4 wk; 8 hr/d)	Formalin 0, 0.5, 5, 10 mg/m ³ Inhalation	Lung histopathological architecture measurements	Not impactful	Formalin
Payani et al. (2019)	Rat (Wistar), male Short-term (21 d; 1 hr/d)	Unspecified test article 0, 40% Inhalation (vapor)	Pulmonary histopathology	Not impactful	Unknown test article; high levels; brief exposures

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Endpoints	Impactful?	Rationale
Sapmaz et al. (2017)	Rat (Sprague Dawley), male Short-term (4 wk; 8 hr/d) or Subchronic (13 wk; 8 hr/d)	Paraformaldehyde 0, 5, 10 ppm (0, 6.2, 12.3 mg/m ³) Inhalation	Hematoxylin and eosin staining (airway inflammation; morphology; scored injury); trachea thickness	Not impactful	High levels; primarily qualitative endpoints
Sholapuri et al. (2020)	Rat (Wistar), male Short-term (21 d; 1 hr/d)	Formalin 0, 40% Inhalation	Lung histopathology	Not impactful	Formalin; high levels; brief exposures
Song et al. (2017)	Mouse (Balb/c), male Short- term (18 d; 3 hr/d)	Formalin 0, 2.44 ppm (0, 3.00 mg/m ³) Inhalation	Airway inflammation histology	Not impactful	Formalin; No formaldehyde-only control (without ovalbumin [OVA])

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

^a Use of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

B.2.6. Mechanistic Information Relevant to Noncancer Respiratory Effects

As detailed in Section 2.2.6 of the Toxicological Review, the searches for mechanistic information related to noncancer respiratory system effects included a specific focus on identifying studies relevant to potential inflammation- and immune-related changes. This search encompassed mechanistic information related to changes in the respiratory tract, blood, and lymphoid tissues that might not have been captured by health effect-specific systematic searches. As outlined in Section 2 of the Toxicological Review, the specific search strings below were augmented with studies identified as supplemental through other health effect-specific searches, including hypersensitivity-related responses in animal studies (see also Appendix B.2.4) and information on cellular proliferation and mucociliary function (see also Appendix B.2.5). The search strings are documented in Table B-11, the literature search results through 2016 are documented in Figure B-11, and the literature search results through 2021 identified by the SEM are documented in Figure B-12, with the SEM screening decisions documented in the subsequent section.

Search Strings

Table B-11. Summary of supplemental literature search terms for mechanistic studies relevant to potential noncancer respiratory health effects

Database	Search (no date limit thru 8/31/2014)
PubMed searched 9/4/2014	(*formaldehyde OR formalin) AND ("Adaptive immunity" OR asthma OR "atopic dermatitis" OR immune OR "innate immunity" OR redox OR allergic OR allergy OR "mucosal immunity" OR Eosinophil* OR Inflammation OR "Lung function test" OR "Nitric oxide" OR Wheezing OR rhinosinusitis OR lymphocyte OR bronchiolitis OR glucocorticoid OR IgE OR basophil OR "histamine-releasing factor" OR "mast cell" OR "reactive nitrogen species" OR "reactive oxygen species" OR "oxidative stress" OR isoprostane OR "Airway remodeling" OR phagocytosis OR "toll-like" OR "respiratory immunity" OR autoimmune OR interleukin OR "immune system" OR "allergic rhinitis" OR "chronic obstructive pulmonary disease" OR copd OR corticosteroids OR "Chronic bronchitis" OR fibrocyte OR hematopoie* OR "Epithelial injury" OR "epithelial repair" OR Th17 OR "Airway hyperresponsiveness" OR "Airway smooth muscle" OR "airway hyperreactivity" OR "Bronchoalveolar lavage" OR neutrophil OR cytokine OR Bronchiectasis OR th2 OR th9 OR "t cell" OR leukotriene OR "Bronchial epithelial cell" OR "Dendritic cell" OR Endothelin OR "growth factor" OR Lipoxins OR Prostaglandin OR cyclooxygenase OR "matrix metalloproteinase" OR ovalbumin OR "tumor necrosis factor" OR Phosphodiesterase OR "Bronchopulmonary dysplasia" OR Adipokine OR Eicosanoid OR bronchoconstriction OR Phospholipase OR Hyperpnoea OR bronchiectasis OR "corticosteroid responsiveness" OR "Type 2" OR "muscarinic receptor antagonism" OR "obstructive airway" OR Immunomodulation OR lipocalins OR allergen OR corticosteroids OR "Vascular endothelial growth factor" OR bronchiectasis OR immunodeficiency OR "Muscarinic receptor" OR *inflammatory OR Complement OR "Myeloid suppressor cell" OR immunoglobulin OR mucin OR Autophagy OR Leukocyte OR macrophage OR BALT OR "extracellular lining fluid") NOT (nocicept* OR pain OR "formalin test" OR "formalin-induced" OR "formaldehyde-fixed" or "formalin-fixed" OR "paraformaldehyde-fixed" OR "formaldehyde fixation" OR "formalin fixation" OR "10% formalin" OR "10% buffered formalin" OR "10% neutral buffered formalin" OR vaccin* OR inactivated OR "formalin-killed" or "formaldehyde-killed" OR dental OR formalinized)

Supplemental Information—Formaldehyde (Inhalation)

Database	Search (no date limit thru 8/31/2014)
Web of Science searched 9/5/2014	(TS=("formaldehyde" OR "formalin") AND TS=("Adaptive immunity" OR "asthma" OR "atopic dermatitis" OR "immune" OR "innate immunity" OR "redox" OR "allergic" OR "allergy" OR "mucosal immunity" OR Eosinophil* OR "Inflammation" OR "Lung function test" OR "Nitric oxide" OR "Wheezing" OR "rhinosinusitis" OR "lymphocyte" OR "bronchiolitis" OR "glucocorticoid" OR "IgE" OR "basophil" OR "histamine-releasing factor" OR "mast cell" OR "reactive nitrogen species" OR "reactive oxygen species" OR "oxidative stress" OR "isoprostane" OR "Airway remodeling" OR "phagocytosis" OR "toll-like" OR "respiratory immunity" OR "autoimmune" OR "interleukin" OR "immune system" OR "allergic rhinitis" OR "chronic obstructive pulmonary disease" OR "copd" OR "corticosteroids" OR "Chronic bronchitis" OR "fibrocyte" OR hematopoie* OR "Epithelial injury" OR "epithelial repair" OR "Th17" OR "Airway hyperresponsiveness" OR "Airway smooth muscle" OR "airway hyperreactivity" OR "Bronchoalveolar lavage" OR "neutrophil" OR "cytokine" OR "Bronchiectasis" OR "th2" OR "th9" OR "t cell" OR "leukotriene" OR "Bronchial epithelial cell" OR "Dendritic cell" OR "Endothelin" OR "growth factor" OR "Lipoxins" OR "Prostaglandin" OR "cyclooxygenase" OR "matrix metalloproteinase" OR "ovalbumin" OR "tumor necrosis factor" OR "Phosphodiesterase" OR "Bronchopulmonary dysplasia" OR "Adipokine" OR "Eicosanoid" OR "bronchoconstriction" OR "Phospholipase" OR "Hyperpnoea" OR "bronchiectasis" OR "corticosteroid responsiveness" OR "Type 2" OR "muscarinic receptor antagonism" OR "obstructive airway" OR "Immunomodulation" OR "lipocalins" OR "allergen" OR "corticosteroids" OR "Vascular endothelial growth factor" OR "bronchiectasis" OR "immunodeficiency" OR "Muscarinic receptor" OR *inflammatory OR "Complement" OR "Myeloid suppressor cell" OR "immunoglobulin" OR "mucin" OR "Autophagy" OR "Leukocyte" OR "macrophage" OR "BALT" OR "extracellular lining fluid")) NOT TS=(nocicept* OR "pain" OR "formalin test" OR "formalin-induced" OR "formaldehyde-fixed" OR "formalin-fixed" OR "paraformaldehyde-fixed" OR "formaldehyde fixation" OR "formalin fixation" OR "10% formalin" OR "10% buffered formalin" OR "10% neutral buffered formalin" OR vaccin* OR "inactivated" OR "formalin-killed" or "formaldehyde-killed" OR "dental" OR "formalinized") Indexes=SCI-EXPANDED, CPCI-S, BKCI-S, BKCI-SSH Timespan=All years
Toxline searched 9/3/2014	Part 1 @SYN0+@AND+(@OR+"Adaptive+immunity"+asthma+"atopic+dermatitis"+immune+"innate+immunity"+redox+allergic+allergy+"mucosal+immunity"+Eosinophil*+Inflammation+"Lung+function+test"+"Nitric+oxide"+Wheezing+rhinosinusitis+lymphocyte+bronchiolitis+glucocorticoid+IgE+basophil+"histamine-releasing+factor"+"mast+cell"+"reactive+nitrogen+species"+"oxidative+stress"+isoprostane+"Airway+remodeling"+phagocytosis+"toll-like"+"respiratory+immunity"+autoimmune+interleukin+"immune+system"+"allergic+rhinitis"+"chronic+obstructive+pulmonary+disease")+(@OR+formaldehyde+formalin+@term+@rn+50-00-0)+@NOT+(@OR+nocicept*+pain+"formalin+test"+"formalin-induced"+"formaldehyde-fixed"+"formalin-fixed"+"paraformaldehyde-fixed"+"formaldehyde+fixation"+"formalin+fixation"+"buffered+formalin"+"neutral+buffered+formalin"+vaccin*+inactivated+"formalin-killed"+"formaldehyde-killed"+dental+formalinized)+@NOT+@org+pubmed+pubdart+"NIH+reporter" @SYN0+@AND+(@OR+"Adaptive+immunity"+asthma+"atopic+dermatitis"+immune+"innate+immunity"+redox+allergic+allergy+"mucosal+immunity"+Eosinophil*+Inflammation+"Lung+function+test"+"Nitric+oxide"+Wheezing+rhinosinusitis+lymphocyte+bronchiolitis+glucocorticoid+IgE+basophil+"histamine-releasing+factor"+"mast+cell"+"reactive+nitrogen+species"+"oxidative+stress"+isoprostane+"Airway+remodeling"+phagocytosis+"toll-like"+"respiratory+immunity"+autoimmune+interleukin+"immune+system"+"allergic+rhinitis"+"chronic+obstructive+pulmonary+disease")+(@OR+formaldehyde+formalin+@term+@rn+50-00-

Supplemental Information—Formaldehyde (Inhalation)

Database	Search (no date limit thru 8/31/2014)
	0)+@NOT+(@OR+nocicept*+pain+"formalin+test"+"formalin-induced"+"formaldehyde-fixed"+"formalin-fixed"+"paraformaldehyde-fixed"+"formaldehyde+fixation"+"formalin+fixation"+"buffered+formalin"+"neutral+buffered+formalin"+vaccin*+inactivated+"formalin-killed"+"formaldehyde-killed"+dental+formalinized)+@AND+@org+"nih+reporter" Part 2 @SYN0+@AND+(@OR+copd+corticosteroids+"Chronic+bronchitis"+fibrocyte+hematopoie*+"Epithelial+injury"+"epithelial+repair"+Th17+"Airway+hyperresponsiveness"+"Airway+smooth+muscle"+"airway+hyperreactivity"+"Bronchoalveolar+lavage"+neutrophil+cytokine+Bronchiectasis+th2+th9+"t+cell"+leukotriene+"Bronchial+epithelial+cell"+"Dendritic+cell"+Endothelin+"growth+factor"+Lipoxins+Prostaglandin+cyclooxygenase+"matrix+metalloproteinase"+ovalbumin+"tumor+necrosis+factor"+Phosphodiesterase+"Bronchopulmonary+dysplasia"+Adipokine+Eicosanoid+bronchoconstriction+Phospholipase+Hyperpnoea+bronchiectasis+"corticosteroid+responsiveness"+"Type+2"+"muscarinic+receptor+antagonism"+"obstructive+airway"+Immunomodulation+lipoalins+allergen+corticosteroids+"Vascular+endothelial+growth+factor"+bronchiectasis+immunodeficiency+"Muscarinic+receptor"+inflammatory+Complement+"Myeloid+suppressor+cell"+immunoglobulin+mucin+Autophagy+Leukocyte+macrophage+BALT+"extracellular+lining+fluid")+(@OR+formaldehyde+formalin+@term+@rn+50-00-0)+@NOT+(@OR+nocicept*+pain+"formalin+test"+"formalin-induced"+"formaldehyde-fixed"+"formalin-fixed"+"paraformaldehyde-fixed"+"formaldehyde+fixation"+"formalin+fixation"+"buffered+formalin"+"neutral+buffered+formalin"+vaccin*+inactivated+"formalin-killed"+"formaldehyde-killed"+dental+formalinized)+@NOT+@org+pubmed+pubdart+"NIH+reporter" @SYN0+@AND+(@OR+copd+corticosteroids+"Chronic+bronchitis"+fibrocyte+hematopoie*+"Epithelial+injury"+"epithelial+repair"+Th17+"Airway+hyperresponsiveness"+"Airway+smooth+muscle"+"airway+hyperreactivity"+"Bronchoalveolar+lavage"+neutrophil+cytokine+Bronchiectasis+th2+th9+"t+cell"+leukotriene+"Bronchial+epithelial+cell"+"Dendritic+cell"+Endothelin+"growth+factor"+Lipoxins+Prostaglandin+cyclooxygenase+"matrix+metalloproteinase"+ovalbumin+"tumor+necrosis+factor"+Phosphodiesterase+"Bronchopulmonary+dysplasia"+Adipokine+Eicosanoid+bronchoconstriction+Phospholipase+Hyperpnoea+bronchiectasis+"corticosteroid+responsiveness"+"Type+2"+"muscarinic+receptor+antagonism"+"obstructive+airway"+Immunomodulation+lipoalins+allergen+corticosteroids+"Vascular+endothelial+growth+factor"+bronchiectasis+immunodeficiency+"Muscarinic+receptor"+inflammatory+Complement+"Myeloid+suppressor+cell"+immunoglobulin+mucin+Autophagy+Leukocyte+macrophage+BALT+"extracellular+lining+fluid")+(@OR+formaldehyde+formalin+@term+@rn+50-00-0)+@NOT+(@OR+nocicept*+pain+"formalin+test"+"formalin-induced"+"formaldehyde-fixed"+"formalin-fixed"+"paraformaldehyde-fixed"+"formaldehyde+fixation"+"formalin+fixation"+"buffered+formalin"+"neutral+buffered+formalin"+vaccin*+inactivated+"formalin-killed"+"formaldehyde-killed"+dental+formalinized)+@AND+@org+"nih+reporter"

Abbreviations: Majr = major topic (filter); TS = the requested "topic" is included as a field tag.

Literature Search Results through 2016

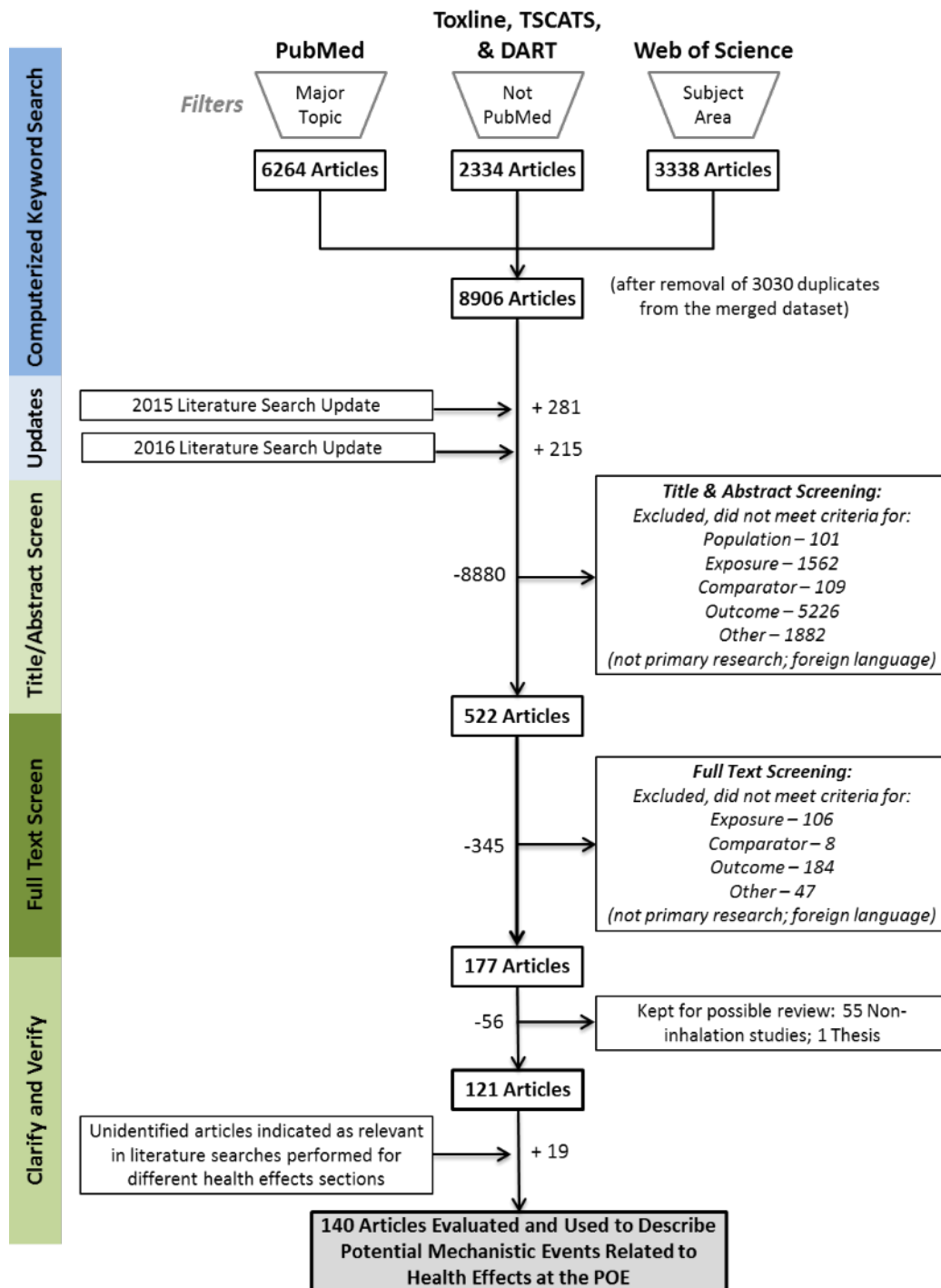


Figure B-11. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and mechanistic data associated with potential noncancer effects on the respiratory system.

Literature Search Results from the Systematic Evidence Map Update through 2021

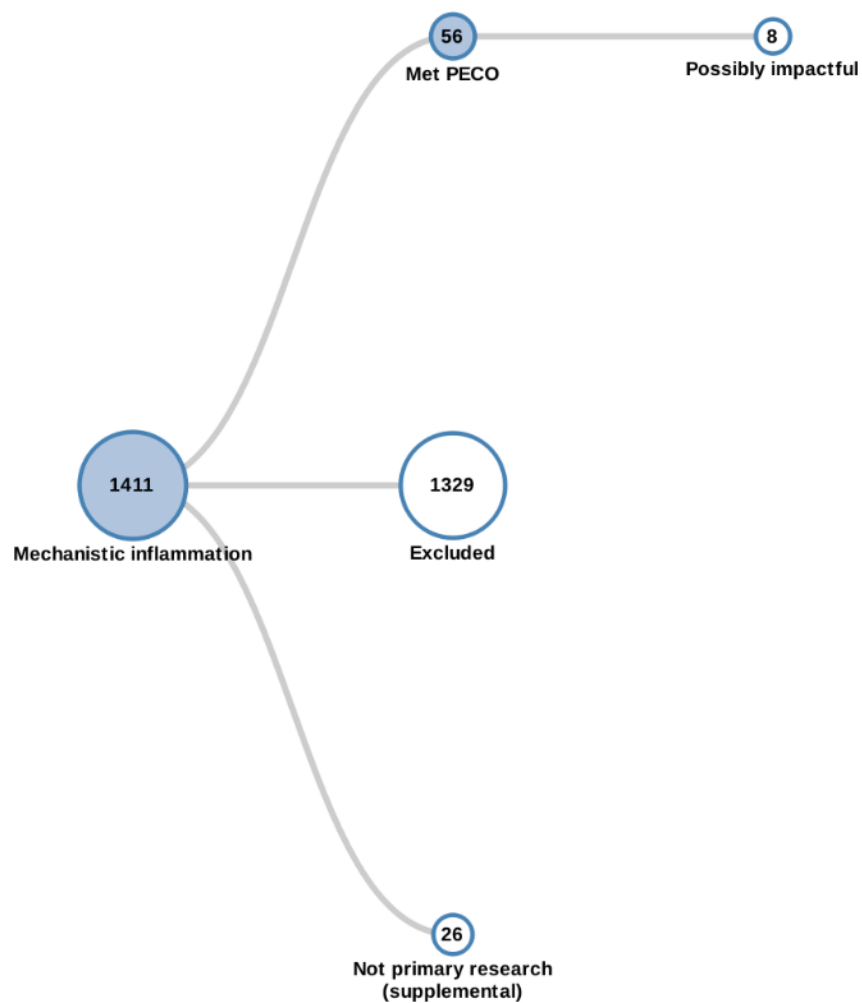


Figure B-12. Mechanistic inflammation and immune effects literature tree (interactive version [here](#)).

A total of 1,411 citations were retrieved for the assessment of mechanistic information on inflammation and immune responses (in the respiratory system or at systemic sites) and 56 studies met the PECO criteria.

Screening for Inclusion from the 2021 Systematic Evidence Map Update

Of the 56 studies that met the PECO criteria (see Table B-12), eight were deemed to be *possibly impactful* (note: one *possibly impactful* study is repeated under both the animal and in vitro/ex vivo sections). ([Sapmaz et al., 2015](#); [Murta et al., 2016](#); [Morgan et al., 2017](#); [Lima et al., 2015](#); [da Silva et al., 2015](#); [Aydin et al., 2014](#); [Abreu et al., 2016](#)) and ([Bellisario et al., 2016](#)) were identified in the literature search update and included in the inventory table although they had been included in the 2017 draft Toxicological Review of Formaldehyde (Inhalation). In vitro/ex vivo study designs and a study of endogenous formaldehyde biology also were included.

Table B-12. Mechanistic studies relating to respiratory or systemic inflammatory and immune responses

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
Human Studies					
Bassig et al. (2016)	Occupational Guangdong, China Cross-sectional	Air sampling Adult formaldehyde factory workers	WBC counts in blood, with subtype analyses of cells of both myeloid and lymphoid lineage (include CD4 T cell subtyping and cell activation markers)	Possibly impactful	PBL sub-population analyses and lineage are key endpoints of concern
Costa et al. (2019)	Occupational Portugal Cross-sectional	Air sampling Adult anatomy-pathology laboratory workers	Lymphocyte counts, subpopulations analyses	Possibly impactful	PBL sub-population analyses and lineage are key endpoints of concern
Augenreich et al. (2020)	Occupational Boone, North Carolina, USA Cohort	Air sampling Adult medical students in anatomy dissection rooms	Circulating markers of oxidative stress and inflammation; brachial artery dilation (arm), reactive hyperemia (leg), blood pressure/pulse/heart rate	Not impactful	ROS measures not key endpoints of concern
Bellisario et al. (2016)	Occupational Torino, Italy cross-sectional	Air sampling, adults, Female surgical nurses	Biomarkers of oxidative stress (urinary malondialdehyde and 15-F2t-isoprostane)	Not impactful	Already included in 2017 draft; ROS measures not key endpoints of concern

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
Bruno et al. (2018)	Occupational Rome, Italy Cross-sectional	Air sampling Adult pathology laboratory workers	Counts of neutrophils, eosinophils, lymphocytes, macrophages, ratio of mucous-secreting cells and ciliated cells in the middle portion of the inferior turbinate	Not impactful	Cell counts (without sub-analyses) not key endpoints of concern
Ghelli et al. (2020)	Occupational Turin, Italy Cohort	Air sampling Adult (female) hospital workers	ROS measures in urine and inflammatory markers and cytokines in blood. Genotyped for CYP1A1, GSTT1, GSTM1, TNFa, and IL-6 polymorphisms	Not impactful	ROS and cytokine-related measures not key endpoints of concern
Isa et al. (2020a)	School-based Selangor, Malaysia Cross-sectional	Air sampling School children	Fractional exhaled nitric oxide (FeNO, an airway ROS/inflammation marker)	Not impactful	ROS markers not key endpoints of concern
Isa et al. (2020b)	School-based Hulu Langat, Selangor, Malaysia Cross-sectional	Air sampling, children, Suburban and urban school children	Inflammatory cytokine markers in sputum; exhaled FeNO	Not impactful	ROS and cytokine-related measures not key endpoints of concern
Yon et al. (2019)	School-based Seongnam City, Korea Cohort	Air sampling School children	Serum formaldehyde-specific IgE; airway function; and exhaled FeNO	Not impactful	ROS and antibody-related measures not key endpoints of concern
Animal Studies					
Liu et al. (2017)	Mouse (ICR), male Subchronic (20 wk; 2 hr/d)	Unspecified test article 0, 1, 10 mg/m ³ Inhalation	Bone marrow cell MN; polychromatic erythrocytes (PCE)/normochromatic erythrocyte (NCE) ratio (immature/mature RBCs)	Possibly Impactful	Key endpoints of concern; longer duration study is rare (note: presumed use of formalin limits interpretation)
Ma et al. (2020)	Mouse (Balb/c), male Subchronic (8 wk; 8 hr/d, 7 d/wk)	Formaldehyde in water (methanol free) 0, 2 mg/m ³ Inhalation	DNA damage (comet assay) in peripheral tissues (e.g., spleen; thymus); % of CD4+ T cells, CD8+ T cells, ratio of CD4+/CD8+ cells, and CD4 and CD8 cell phenotyping spleen	Possibly impactful	Key endpoints of concern regarding immune cell health and function

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
			weights, percentage of the DN (double negative), DP (double positive), CD4SP (single positive) and CD8SP cell populations in the isolated thymocytes, cytotoxicity in CD4SP and CD8SP cells, Runx (Runx 1,2,3, C), Runx1, Runx3, and ThPOK expression in the DP cells, ROS		
Morgan et al. (2017)	Mouse (Trp53 haploinsufficient), Male Subchronic (8 wk; 6 hr/d, 5 d/wk), then held for 32 wk	Paraformaldehyde 0, 7.5 or 15 ppm (0, 9.23, 18.5 mg/m ³) Inhalation	Hematology	Possibly impactful	Already included in 2017 draft
Park et al. (2020)	Mouse (BALB/c), female Short-term (2 wk; 4 hr/d, 5 d/wk)	Fresh formaldehyde solution (methanol-free) 0, 1.38, 5.36 mg/m ³ Inhalation	Splenic cytokines, T cell populations and Th1/Th2 balance, differentiation markers	Possibly impactful	T cell subpopulation analyses are key endpoints of concern
Zhao et al. (2020)	Mouse (Balb/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 3 mg/m ³ Inhalation	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies in nose, lung, spleen, and bone marrow	Possibly impactful (POE tissues); Not impactful (systemic tissues)	Key endpoints of concern (note: formalin; in vitro are of less concern for POE tissues)
Aydemir et al. (2017)	Rat (Wistar albino), both sexes Subchronic (6 wk; 8 hr/d, 5 d/wk)	Formalin 0, 6 ppm (0, 7.4 mg/m ³) Inhalation (note: i.p. not PECO relevant)	Blood DNA damage (comet assay) and ROS markers	Not impactful	Formalin; high level
Aydin et al. (2014)	Rat (Sprague-Dawley), male Short-term (4 wk)	Formalin 0, 5.27, 10.02 ppm (0, 6.48, 12.3 mg/m ³)	Serum and lung total antioxidant and oxidant status, and oxidative stress index; serum glucose, protein, albumin, lipids,	Not impactful	Already included in 2017 draft; Note: ROS and serum

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
		Inhalation	cholesterol, HDL, LDL, triglyceride, T protein; lung irisin levels and immunostaining		lipid-related measures not key endpoints of concern
Bernardini et al. (2020)	Mouse (Swiss), male Short-term (4 wk; 4 hr/d, 5 d/wk)	Unspecified test article 0, 0.5, 1, 5, 10 ppm (0, 0.62, 1.23, 6.15, 12.3 mg/m ³) Inhalation	Lung histopathology; BAL cell counts and inflammatory and ROS markers; global methylation in blood and bone marrow	Not impactful	Unknown test article; not key endpoints of concern
Cheng et al. (2016)	Mouse (Kunming), male Short-term (3 or 7 d; continuous)	Formalin 0, 0.08, 0.8 mg/m ³ Inhalation	Serum CD4+, CD8+, and CD4/CD8 T cell counts	Not impactful	Formalin
Abreu et al. (2016)	Mouse (C57BL/6), female Acute (single exposure, assessed 8 hr later)	Unspecified test article 0, 0.2, 1, 3 ppm (0, 0.25, 1.23, 3.69 mg/m ³) Inhalation	Lung mechanics and morphology, inflammatory cell counts and cytokines, and ROS markers	Not impactful	Already included in 2017 draft; Note: unknown test article; acute
da Silva et al. (2015)	Rat (Wistar), male Short-term (3 d; 90-min/d)	Unspecified test article 0, 1 % Inhalation	BAL cell counts (WBCs, Mono., Lympho., Neutro., Eosin.), cytokines, and myeloperoxidase activity (inflammation); lung morphometrics, microvascular permeability, and mRNA levels	Not impactful	Already included in 2017 draft; Note: unknown test article; high levels
Duan et al. (2018)	Mouse (BALB/c), male Short-term (18 d; 5 hr/d)	Formalin 0, 1 mg/m ³ Inhalation	Pulmonary eosinophil cationic protein (histopathology), ROS markers, nuclear factor kappa B activation, and cytokine and growth factor levels	Not impactful	Formalin; no saline plus formaldehyde control group
Duan et al. (2020)	Mouse (Balb/c), male Short-term (21 d; 6 hr/d)	Formalin 0, 0.5 mg/m ³ Inhalation	Airway IgE, cytokines and inflammatory factors, Th1/Th2 balance, mucus secretion, histopathology, and lung function	Not impactful	Formalin; not key endpoints of concern
Ge et al. (2020a)	Mouse (Balb/c), male	Formalin 0,0.5, 3 mg/m ³	CBC; Myeloid progenitor cell (BFU-E and CFU-GM) colony counts and cytokines;	Not impactful	Formalin

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
	Short-term (2 wk; 8 hr/d, 5 d/wk)	Inhalation	circulating ROS and cytokine markers; bone marrow histology, ROS, and gene expression of cell cycle and DNA damage markers		
Han et al. (2016)	Rat (Sprague-Dawley), male Subchronic (6 wk; 2 hr/d, 5 d/wk beginning at PND3)	Paraformaldehyde 0, 0.83, 1.16 ppm (0, 1.02, 1.43 mg/m ³) Inhalation	Serum IgE, thymus Th1 and Th2 cytokines, body weight	Not impactful	Nonspecific antibodies and cytokines not key endpoints of concern
Jin et al. (2021)	Mouse (C57BL/6J), both sexes Short-term (4 d; 6 hr/d)	Unspecified test article 0, 5 ppm (0, 6.15 mg/m ³) Inhalation	Respiratory parameters (e.g., rate) during exposure; serum lipids; serum cell counts and soluble factors (CBC)	Not impactful	Unknown test article; not key endpoints of concern
Kang et al. (2018)	Mouse (BALB/c), male Short-term (18 d; 5 hr/d)	Formalin 0, 1 mg/m ³ Inhalation	Serum IgE, IgG; airway hyperreactivity, ROS markers, nuclear factor kappa B and MAPK activation; cytokine levels, and mast cell degranulation	Not impactful	Formalin; not key endpoints of concern
Leal et al. (2018)	Mouse (C57BL6), male Short-term (2 wk; 1 hr/d, 5 d/wk)	Unspecified test article 0, 0.92 mg/m ³ Inhalation	Lung cytokines and elasticity measures	Not impactful	Unknown test article; not key endpoints of concern
Li et al. (2017)	Mouse (Balb/c or C57BL/6), male Short-term (25 d; 6 hr/d)	Formalin 0, 0.5, 3 mg/m ³ Inhalation	Bronchial responsiveness (to methacholine), BAL cytokines and cell counts (total, eosin., lympho., neutro.); Serum OVA-specific IgE, IgG1, and IgG2a	Not impactful	Formalin; not key endpoints of concern
Lima et al. (2015)	Rat (Fischer), male Short-term (5 d; 20-min x3/d)	Unspecified test article 0, 1, 5, 10 % Inhalation	Trachea histology and morphometric analyses, including mucus production, glycogen, ROS markers, and inflammatory cell counts.	Not impactful	Already included in 2017 draft; Note: Unknown test article; high levels
Liu et al. (2018b)	Rat (Sprague Dawley), male	Formalin 0, 0.5, 5, 10 mg/m ³ Inhalation	Lung autophagy, histopathology and BAL cytokines	Not impactful	Formalin; not key endpoints of concern

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
	Short-term (4 wk; 8 hr/d)				
Macedo et al. (2016b)	Rat (Wistar), male Short-term (3 d; 90-min/d)	Formalin 0, 1 % Inhalation	BAL ROS markers and cellular oxidative burst; lung tissue antioxidant enzyme measures	Not impactful	Formalin; high levels
Murta et al. (2016)	Rat (Fischer), male Short-term (5 d; 20-min × 3/d)	unspecified 0, 1, 5, 10 %, inhalation	BALF cell counts (WBCs, macrophages, lymphocytes, neutrophils, eosinophils), inflammatory and ROS markers, and neutrophil ROS production Lung tissue inflammatory markers, H&E staining and morphometry	Not impactful	Already included in 2017 draft; Note: unknown test article; high levels
Payani et al. (2019)	Rat (Wistar, albino), male Short-term (21 d; 1 hr/d)	Unspecified test article 0, 40 % Inhalation	Lung ROS markers	Not impactful	Unknown test article; high levels
Sapmaz et al. (2015)	Rat (Sprague-Dawley), male Short-term (4 wk; 8 hr/d)	Paraformaldehyde 0, 5, 10 ppm (0, 6.15, 12.3 mg/m ³) Inhalation	Serum total IgA, IgM, IgG, complement C3	Not impactful	Already included in 2017 draft; Note: Nonspecific antibody-related measures not key endpoints of concern
Sholapuri et al. (2020)	Rat (Wistar), male Short-term (21 d; 1 hr/d)	Formalin 0, 40 % Inhalation	Hematology parameters (CBC); BAL histamine; lung histology	Not impactful	Formalin; high levels
Song et al. (2017)	Mouse (Balb/c), male Short-term (25 d)	Formalin 0, 2.44 ppm (0, 3 mg/m ³) Inhalation	Serum levels of cytokines, neuropeptides, ROS, and IgE; leukocyte counts and cellular antioxidant levels.	Not impactful	Formalin; No formaldehyde-only control (without OVA);
Wei et al. (2017b)	Mouse (BALB/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 3 mg/m ³ Inhalation	Complete blood cell count; bone marrow - myeloid progenitor formation assay, ROS assay, IL-3 and GM-CSF ELISA, systemic	Not impactful	Formalin; short-term (otherwise

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
			toxicity, bone marrow cellularity, apoptosis assay		impactful endpoints)
Wei et al. (2017a)	Mouse (BALB/c), male Short-term (2 wk; 5 d/wk), followed by 7 d recovery	Formalin 0, 3 mg/m ³ Inhalation	Complete blood cell count, bone marrow histopathology, myeloid progenitor colony-forming cell assay, ROS and cytokine measures, and DNA-protein crosslinks	Not impactful	Formalin; short-term (otherwise impactful endpoints)
Wen et al. (2016)	Mouse (Balb/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 3 mg/m ³ Inhalation	Cell counts (WBCs, lymphocytes, monocytes, neutrophils, RBCs, platelets); serum antibody (total) level; ROS markers; PBL proliferation; serum hemagglutination titer and delayed-type hypersensitivity (both after sheep RBC injection)	Not impactful	Formalin (limits interpretability of systemic effects)
Wu et al. (2020)	Mouse (Balb/C), male Short-term (21 d; 5 hr/d)	Formalin 0, 0.8 mg/m ³ Inhalation	Pulmonary function; lung histopathology; airway hyperresponsiveness; lung IgE and cytokine (including Th1/Th2) levels	Not impactful	Formalin; not key endpoints of concern
Zhang et al. (2018b)	Mouse (Balb/c), male Short-term (7, 14, or 28 d, 2 4hr/d for constant and 12 hr/d for intermittent)	Unspecified test article 0, 0.8 (intermittent) or 0, 0.4 (constant) ppm (0, 0.49, or 0.98 mg/m ³) Inhalation	BAL cell counts (total, eosin., neutro., lympho.); lung tissue ROS markers, histology, and cytokine and inflammatory marker immunohistochemistry	Not impactful	Unknown test article; not key endpoints of concern
In Vitro/Ex Vivo Studies					
Zhao et al. (2020)	Mouse (Balb/c), male Ex vivo primary lung and nose cells (systemic cells not PECO-relevant) Acute (1 hr)	Formalin 0, 50, 100, 200, 400 µM In media	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies	Possibly impactful	Key endpoints of concern (note: formalin; in vitro are of less concern for POE tissues)
An et al. (2019)	Human immortalized bronchial epithelial cells (in vitro)	Unspecified test article 0, 20, 40, 60, 80, 100, 120 µM	Cell proliferation, ROS production, and markers of cell division/proliferation and ROS	Not impactful	Unknown test article; in vitro; acute

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
	experiments in LHP-relevant cells were excluded) Acute (2 hr)	In media			
Arslan-Acaroz and Bayşu-Sozibilir (2020)	Human immortalized lung epithelial cells Acute (4 hr)	Unspecified test article 0, 100 µM, In media	Cell viability and ROS markers	Not impactful	Unknown test article; in vitro; acute
Boncler et al. (2019)	Human immortalized lung epithelial cells (other in vitro experiments in this study excluded as not PECO relevant) Acute (24 hr)	Unspecified test article 0, 63, 126, 378, 504, 630 µmol/L In media	Cell viability and mitochondrial membrane potential	Not impactful	Unknown test article; in vitro; acute
Cui et al. (2016)	Human immortalized lung cells or Mouse (Balb/c) nasal instillation Acute up to 48 hr	Unspecified test article 0, 200 µM In media or instilled	Cell signaling and gene expression, ROS, and cellular currents	Not impactful	Unknown test article; acute
Gostner et al. (2016)	Human immortalized, lung epithelial cells Short-term (3 d)	Unspecified test article 0, 0.1, 0.5 ppm (0, 0.12, 0.62 mg/m ³) Gaseous exposure at the air:liquid interface	Cell viability; gene expression	Not impactful	Unknown test article; not key endpoints of concern
Jude et al. (2016)	Human primary airway smooth muscle (HASM) cells Acute (1 hr, assessed at 24 hr)	Formalin 0, 0.2, 0.8, 2 ppm (0, 0.25, 0.98, 2.46 mg/m ³) Vapor delivered to cells	Agonist-induced calcium mobilization, cytotoxicity, ROS markers and cytokines in co-cultures; cabachol-induced airway narrowing in slices	Not impactful	Formalin; in vitro; acute
Kim et al. (2018)	Human immortalized endometrial adenocarcinoma cells Short-term (6 d)	Unspecified test article 10 ⁻¹¹ to 10 ⁻³ M In media	ROS production, protein expression of markers associated with cell transformation and proliferation	Not impactful	Unknown test article; in vitro

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
	[Note: study included due to use of this cell line to examine mechanisms associated with epithelial cell-cell interactions]				
Li et al. (2008)	Human immortalized tracheal epithelial cells Acute (4 or 24 hr)	Unspecified test article 0, 20, 50, 100, 200 µM In media	Cell viability and expression of MAPK-responsive genes	Not impactful	Unknown test article; in vitro; acute
Liu et al. (2019)	Human immortalized bronchial epithelial cells Acute (24 hr)	Unspecified test article 0, 40, 80, 160 µmol/L In media	Apoptosis, PI3K-Akt pathway signaling markers	Not impactful	Unknown test article; in vitro; acute
Mi et al. (2019)	Human pulmonary alveolar epithelial cells in artificial airway Acute (2, 4, or 6 hr)	Unspecified test article 0.025 and 40 µM (0.025 µM = ~0.3 ppm) Nitrogen carrier-mediated delivery directly into cells	ROS and cytokine markers	Not impactful	Unknown test article; acute
Nazarpour-Noshadi et al. (2020)	Human immortalized lung epithelial cells Acute/short-term (24, 48, and 72 hr)	Unspecified test article 0, 25, 50, 100, 150, 200, 300 µM In media	Cellular viability and DNA damage markers	Not impactful	Unknown test article; in vitro
Vitoux et al. (2018)	Human immortalized conjunctival epithelial cells Acute (15–30 min, assess at 1 or 24 hr)	Formalin 0, 100, 1,200 µg/m ³ Airflow over cells	Expression of inflammatory cytokines	Not impactful	Formalin; in vitro; acute
Zhang et al. (2019)	Human immortalized lung bronchial cells Acute (3, 6, 12, or 24 hr)	Formalin 0, 5, 10, 20, 40, 80 µmol/L In media	ROS and cytotoxicity markers m	Not impactful	Formalin; in vitro; acute

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
Zhang et al. (2020b)	Human Immortalized bronchial epithelial cells	Formalin 0, 10, 40, 80 µM 24 hr	DNA damage - comet assay; apoptosis; mitochondria-mediated apoptosis; reactive oxygen species levels	Not impactful	Formalin; in vitro; endpoints well-established in vitro
Models, Endogenous Formaldehyde, or Other Studies					
Dingler et al. (2020)	Mouse (C57BL/6 background), ALDH2 and ALDH5 WT, single, and double KO, both sexes (note: also includes primary cultures of human PBLs, fibroblasts, and buccal cells not deemed PECO-relevant)	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde detoxification processes in normal function)	Genotoxicity in peripheral blood cells and bone marrow (MN assay, SCE); bone marrow stem cell and progenitor cell quantification, lineage characterization, and B cell maturation; thymic development and cell lineage characterization; complete blood cell count, cell cycle profiling	Possibly impactful	No formaldehyde inhalation but included as a reference study for discussion of potential sources of susceptibility relating to formaldehyde detoxification; cell production from bone marrow is a key endpoint of concern

Abbreviations: WBC = white blood cell; ROS = reactive oxygen species; BAL = bronchoalveolar lavage (F = fluid); RBC = red blood cell; PBL = peripheral blood leukocyte; CBC = complete blood cell (count).

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

^a Use of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

B.2.7. Nervous System Effects

As detailed in Section 2.2.7 of the Toxicological Review, the review of nervous system effects focused on studies in humans or animals that examined objective, apical effects on the nervous system, including structural, behavioral, chemical, and electrophysiological changes, as well as mechanistic studies. A single search strategy (with different PECO screening criteria) was used to capture studies in humans and animals, as well as relevant in vivo mechanistic studies. As described in Section 2 of the Toxicological Review, studies of symptoms were not included. The search strings are documented in Table B-13, the literature search results through 2016 are documented in Figure B-13, and the literature search results through 2021 identified by the SEM are documented in Figure B-14, with the SEM screening decisions documented in the subsequent section.

Search Strings

Table B-13. Summary of search terms for nervous system effects

Database, Search Parameters	Terms
PubMed No date restriction	(formaldehyde [majr] OR paraformaldehyde) AND (neuron OR neurons OR neurono* OR neurolo* OR neuronal OR neurotox* OR neurophys* OR neurochem* OR neurotrans* OR neuropsych* OR neuropath* OR neuromusc* OR nerve OR nerves OR nervous OR electrophys* OR “evoked potential” OR *encephalog* OR encephalop* OR *sensory OR sensori* OR “central nervous system” OR CNS OR brain OR spine OR spinal OR spino* OR *axon* OR *synapt* OR *synaps* OR *myelin* OR dendrite* OR *behavior* OR learn* OR memory OR *motor OR *motion OR operant OR habituat* OR *coordination OR weakness OR righting OR reflex OR psychologic* OR mood OR sleep* OR visual OR audit* OR touch OR taste OR sound OR smell OR “pain sensitivity” OR nociception OR olfact* OR *glia* OR oligoden* OR astrocyte* OR balance OR sensation OR sensitization OR tremor* OR convuls* OR seizure* OR grip OR gait OR paralysis OR posture OR mobility OR rearing OR splay OR stereotypy OR conditioning OR avoidance OR approach OR neuropath* OR attent* OR aggress* OR arous*)
	NOT (“formalin test” OR “formaldehyde fixation” OR “formalin fixation” OR “formalin fixed” OR “formaldehyde fixed” OR “formalin-induced” OR “formalin-evoked”) [Note: for quality control, ≈10% (50) of the 451 excluded article titles were scanned in PubMed: none were relevant]
Web of Science No date restriction Lemmatization “off”	SU= ("Anatomy & Morphology" OR "Behavioral Sciences" OR "Biochemistry & Molecular Biology" OR "Cell Biology" OR "Developmental Biology" OR "Life Sciences Biomedicine Other Topics" OR "Neurosciences & Neurology" OR Pathology OR Pediatrics OR Physiology OR "Public, Environmental & Occupational Health" OR "Reproductive Biology" OR "Research & Experimental Medicine" OR Toxicology OR "Veterinary Sciences" OR Psychology) AND TS= (formaldehyde OR paraformaldehyde OR formalin) AND TS= (neuron OR neurons OR neurono* OR neurolo* OR neuronal OR neurotox* OR neurophys* OR neurochem* OR neurotrans* OR neuropsych* OR neuropath* OR neuromusc* OR nerve OR nerves OR nervous OR electrophys* OR “evoked potential” OR *encephalog* OR encephalop* OR *sensory OR sensori* OR “central nervous system” OR CNS OR brain OR spine OR spinal OR spino* OR

Supplemental Information—Formaldehyde (Inhalation)

Database, Search Parameters	Terms
	axon OR *synapt* OR *synaps* OR *myelin* OR dendrite* OR *behavior* OR learn* OR memory OR *motor OR *motion OR operant OR habituat* OR *coordination OR weakness OR righting OR reflex OR psychologic* OR mood OR sleep* OR visual OR audit* OR touch OR taste OR sound OR smell OR “pain sensitivity” OR nociception OR olfact* OR *glia* OR oligoden* OR astrocyte* OR balance OR sensation OR sensitization OR tremor* OR convuls* OR seizure* OR grip OR gait OR paralysis OR posture OR mobility OR rearing OR splay OR stereotypy OR conditioning OR avoidance OR approach OR neuropath* OR attenti* OR aggressi* OR arous*)
	NOT TS= ("formalin test" OR "formaldehyde fixation" OR "formalin fixation" OR "formalin fixed" OR "formaldehyde fixed" OR "formalin-induced" OR "formalin-evoked") [Note: for quality control, ≈2% (80) of the 3,825 excluded article titles were scanned in WoS: none were relevant].
ToxNet (Toxline and DART) No date restriction	formaldehyde AND (neuro* OR neurotox*) (including synonyms and CAS numbers, but excluding PubMed records)
TCATS2 Restricted to 01/01/2010 and newer	“formaldehyde” OR CAS Number: “50-00-0”

Literature Search Results through 2016

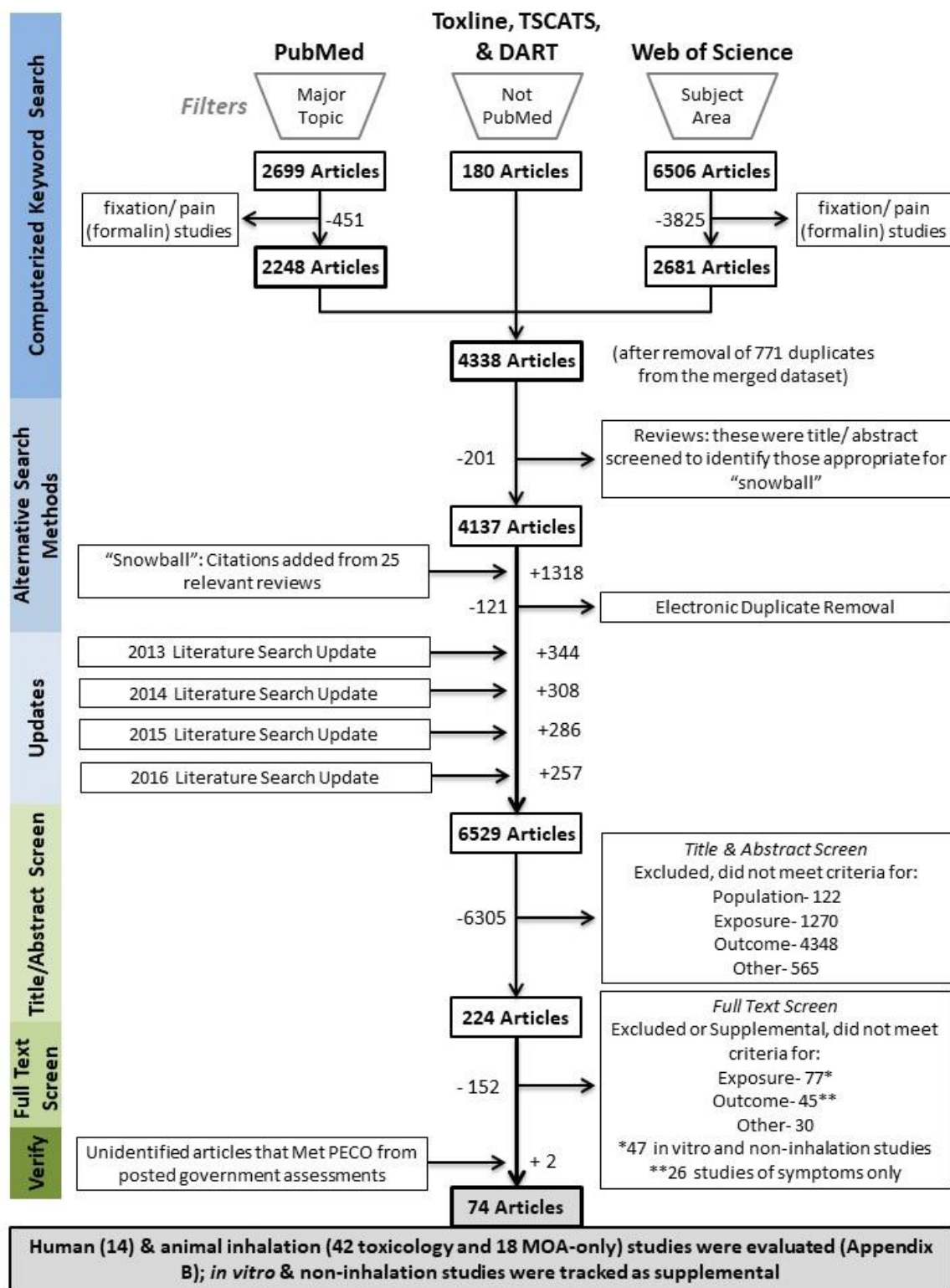


Figure B-13. Literature search documentation for sources of primary data pertaining to formaldehyde exposure and nervous system effects.

Literature Search Results from the Systematic Evidence Map Update through 2021

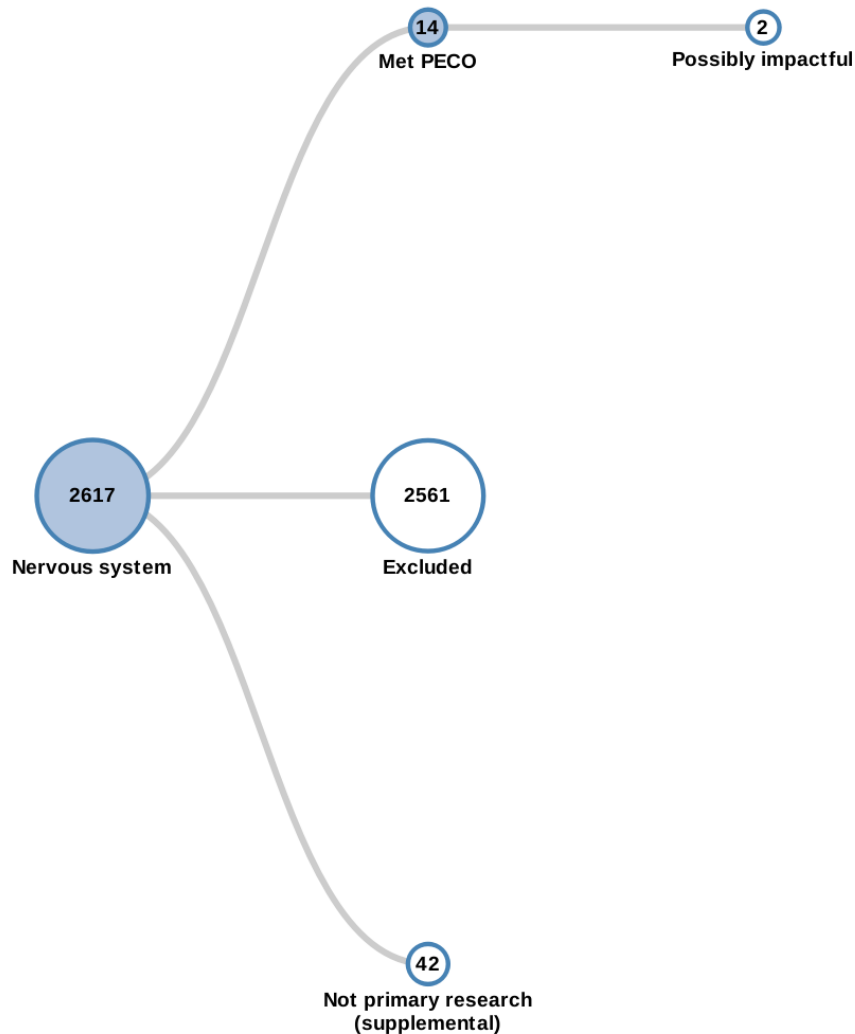


Figure B-14. Nervous system effects literature tree (interactive version [here](#)).

A total of 2,617 citations were retrieved for the assessment of nervous system effects and 14 studies met the PECO criteria.

Screening for Inclusion from the 2021 Systematic Evidence Map Update

Of the 14 studies that met PECO criteria, two human studies were deemed to be *possibly impactful* (see Table B-14). [Peters et al. \(2017\)](#) was identified in the literature search update and deemed to be *possibly impactful*, but already had been included in the 2017 draft. None of the identified animal or mechanistic studies were deemed *possibly impactful*. Thus, zero (0) additional studies were included.

Table B-14. Studies of nervous system effects

Reference	Study design	Exposure ^a	Endpoints	Impactful?	Rationale
Human Studies					
Bellavia et al. (2021)^b	General population Denmark case-control	Occupational history and job-exposure matrix, adults	Amyotrophic lateral sclerosis (ALS) incidence	Possibly impactful	Key endpoint of concern
Peters et al. (2017)	General population Sweden case-control	Occupational history and job-exposure matrix, adults	Amyotrophic lateral sclerosis (ALS) incidence	Possibly impactful	Already identified in 2017 draft
Animal Studies^c					
Askar and Halloull (2018)	Rat (Albino, strain not specified), male. Subchronic (12 wk; 6 hr/d, 5 d/wk)	Paraformaldehyde 0, 20 ppm (0, 24.6 mg/m ³) Inhalation	Cerebellar histopathology, cell counts, and cell morphology; evaluations of ROS and inflammatory markers	Not impactful	High levels
Cheng et al. (2016)	Mouse (Kunming), male Short-term (Up to 7 d; continuous)	Formalin 0, 0.08, 0.8 mg/m ³ Inhalation	Morris water maze	Not impactful	Formalin
Duan et al. (2018)	Mouse (Balb/c), male Short-term (18 d; 5 hr/d)	Formalin 0, 1 mg/m ³ Inhalation	Prefrontal cortex histology; brain ROS and inflammation markers, cytokines	Not impactful	Formalin; no saline plus formaldehyde control group
Ge et al. (2019)	Mouse (Kunming), male Short-term (21 d; continuous)	Formalin 0, 1 mg/m ³ Inhalation	Morris water maze, hippocampal morphology, brain ROS and cell signaling markers	Not impactful	Formalin

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Endpoints	Impactful?	Rationale
Huang et al. (2019)	Mouse (Kunming), male Short-term (14 d; 8 hr/d)	Formalin 0, 3 mg/m ³ Inhalation	Morris water maze; brain ROS and inflammatory markers; hippocampal histopathology and cell morphology	Not impactful	Formalin
Li et al. (2016)	Mouse (Kunming), male Short-term (7 d; 2 hr/d)	Formalin 0, 1, 2 ppm (0, 1.23, 2.46 mg/m ³) Inhalation	Open field activity; elevated plus maze test; forced swimming test; novel object recognition; counts of TH- and GR-immunoreactive neurons	Not impactful	Formalin; brief exposures
Li et al. (2020)	Mouse (Kunming), male Short-term (14 d; 8 hr/d)	Formalin 0, 0.5, 3 mg/m ³ Inhalation	Morris water maze; brain ROS and inflammatory markers; hippocampal histopathology and cell morphology	Not impactful	Formalin
	Mouse (Balb/c), male Short-term (7 d; 8 hr/d)		Brain neurotransmitters; ROS and inflammatory markers in hippocampus, brain stem, and cerebral cortex		
Mei et al. (2016)	Mouse (Balb/c), male Short-term (7 d; 8 hr/d) (in vitro experiments not PECO-relevant)	Unspecified test article 0, 3 mg/m ³ Inhalation	Morris water maze; qualitative hippocampal neuron staining; brain ROS and GSH	Not impactful	Formalin
Zhang et al. (2014b)	Rat (Sprague Dawley), male Short-term (14 d; 30-min, 2×/d)	Unspecified test article 0, 13.5 ppm (0, 16.6 mg/m ³) Inhalation	Buried food pellet behavioral testing; olfactory bulb synaptosomal and neuronal markers; olfactory sensory neuron maturation	Not impactful	Unknown test article; high levels; brief exposures
Mechanistic Studies					
Cao et al. (2015)	Mouse (Balb/c), male Short-term (7 d; 8 hr/d)	Unspecified test article 0, 0.5, 3 mg/m ³ Inhalation	Hippocampus, cortex, and brainstem ROS and inflammatory markers	Not impactful	Unknown test article

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Endpoints	Impactful?	Rationale
Fom et al. (2017)	<i>Drosophila melanogaster</i> (mutant strains: WT, p53 and p38b) Acute (6 or 24 hr)	Unspecified test article 0, 10, 100 µg/m ³ Inhalation	Behavioral (movement-based) quantification; microarray analyses (note survival test study design not extracted)	Not impactful	Non-mammalian; unknown test article
Li et al. (2015)	mouse (ICR), male, Acute or short-term (1 or 7 d; 6 hr/d)	Unspecified test article 0, 3 ppm (0, 3.69 mg/m ³) Inhalation	miRNA screening of olfactory bulb	Not impactful	Unknown test article

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

^aUse of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

^bAn additional study, ([Seals et al., 2017](#)), was identified from the reference list of ([Bellavia et al., 2021](#)). As this study was determined to be *possibly impactful*, it was incorporated into the Toxicological Review.

^cAnimal studies may include evaluation of mechanistic endpoints.

B.2.8. Developmental and Reproductive Effects

As detailed in Section 2.2.8 of the Toxicological Review, the review of developmental and reproductive effects included separate searches for human and animal studies, including in vivo studies of mechanistic endpoints. The human searches focused on time-to-pregnancy (TTP) as a measure of fecundability, reproductive parameters in males (e.g., semen parameters), spontaneous abortion, and birth outcomes (e.g., birthweight, malformations). Outcomes assessed in animal toxicology studies included developmental toxicity (prenatal survival, fetal and postnatal growth, and structural alterations and malformations), male reproductive toxicity (sperm count and morphology, testes and epididymal weight and histopathology, and functional measures), and female reproductive toxicity (hormone levels, ovarian and uterine weight and histopathology, and early embryo loss). Functional developmental outcomes (i.e., developmental neurotoxicity) were addressed in the section on potential nervous system effects (see also Appendix B.2.7). The search strings are documented in Table B-15, the literature search results through 2016 are documented in Figure B-15, and the literature search results through 2021 identified by the SEM are documented in Figure B-16, with the SEM screening decisions documented in the subsequent section.

Search Strings

Table B-15. Summary of search terms for developmental or reproductive toxicity

Database, search date	Terms
PubMed No date restriction	(formaldehyde [majr] OR paraformaldehyde OR formalin) AND ("reproductive toxicity" OR "reproductive toxicology" OR reproductive OR "developmental toxicity" OR "developmental toxicology" OR development OR developmental OR ontogen* OR "embryo toxicity" OR embryo OR embryon* OR embryog* OR embryot* OR "fetal loss" OR fetal OR fetus OR fetuses OR fetotoxi* OR miscarriage or miscarry OR "spontaneous abortion" OR "preimplantation loss" OR preimplantation OR "postimplantation loss" OR postimplantation OR implantation OR conception OR resorption OR fertility OR fertile OR infertility OR infertile OR pregnancy OR gestation OR neonatal OR neonate OR prenatal OR postnatal OR "menstrual cycle" OR "premature birth" OR "preterm birth" OR "low birth weight" OR "in utero" OR "fetal body weight" OR "fetal weight" OR pup OR "pup body weight" OR "pup weight" OR ovary OR ovaries OR ovu* OR sperm OR gamete OR "germ cells" OR "Sertoli cells" OR testes OR testis OR testic* OR uterus OR uteri* OR epididy* OR prostate OR "seminal vesicles" OR semen OR testosterone OR "luteinizing hormone" OR LH OR "follicle stimulating hormone" OR FSH OR estrogen OR estradiol OR "time to pregnancy" OR "time-to-pregnancy" OR TTP OR fecund*)
	NOT (fixative OR "formaldehyde fixation" OR "paraformaldehyde fixation" OR "formalin fixation" OR "formaldehyde fixed" or "paraformaldehyde fixed" OR "formalin fixed" OR "formaldehyde-fixed" or "paraformaldehyde-fixed" OR "formalin-fixed" OR formocresol OR dental OR dentistry OR immunogen OR vaccine OR vaccination OR metabolite)

Supplemental Information—Formaldehyde (Inhalation)

Database, search date	Terms
	[Note: for quality control, ≈1% (75) of the 7,589 excluded article titles were scanned in PubMed: 2 potentially relevant government reports were found, and 4 duplicates were excluded, resulting in 2,810 in the final database.]
Web of Science No date restriction Lemmatization “off”	SU=(Toxicology OR “Pharmacology & Pharmacy” OR “Public, Environmental & Occupational Health” OR “Cell Biology” OR “Reproductive Biology” OR “Biochemistry & Molecular Biology” OR Pathology OR “Obstetrics & Gynecology” OR “Environmental Sciences” OR “Anatomy & Morphology” OR Andrology OR “Veterinary Sciences” OR Physiology OR “Developmental Biology” OR “Research & Experimental Medicine” OR “Life Sciences Biomedicine Other Topics” OR “Veterinary Sciences”) AND TS=(formaldehyde OR paraformaldehyde OR formalin) AND TS=(“reproductive toxicity” OR “reproductive toxicology” OR reproductive OR “developmental toxicity” OR “developmental toxicology” OR development OR developmental OR ontogen* OR “embryo toxicity” OR embryo OR embryon* OR embryog* OR embryot* OR “fetal loss” OR fetal OR fetus OR fetuses OR fetotoxi* OR miscarriage or miscarry OR “spontaneous abortion” OR “preimplantation loss” OR preimplantation OR “postimplantation loss” OR postimplantation OR implantation OR conception OR resorption OR fertility OR fertile OR infertility OR infertile OR pregnancy OR gestation OR neonatal OR neonate OR prenatal OR postnatal OR “menstrual cycle” OR “premature birth” OR “preterm birth” OR “low birth weight” OR “in utero” OR “fetal body weight” OR “fetal weight” OR pup OR “pup body weight” OR “pup weight” OR ovary OR ovaries OR ovu* OR sperm OR gamete OR “germ cells” OR “Sertoli cells” OR testes OR testis OR testic* OR uterus OR uteri* OR epididy* OR prostate OR “seminal vesicles” OR semen OR testosterone OR “luteinizing hormone” OR LH OR “follicle stimulating hormone” OR FSH OR estrogen OR estradiol OR “time to pregnancy” OR “time-to-pregnancy” OR TTP OR fecund*)
	NOT (fixative OR “formaldehyde fixation” OR “paraformaldehyde fixation” OR “formalin fixation” OR “formaldehyde fixed” or “paraformaldehyde fixed” OR “formalin fixed” OR “formaldehyde-fixed” or “paraformaldehyde-fixed” OR “formalin-fixed” OR formocresol OR dental OR dentistry OR immunogen OR vaccine OR vaccination OR metabolite)
ToxNet (Toxline and DART) No date restriction	(formaldehyde OR paraformaldehyde OR formalin) AND (“reproductive toxicity” OR “reproductive toxicology” OR reproductive OR “developmental toxicity” OR “developmental toxicology” OR developmental) (including synonyms and CAS numbers, but excluding PubMed records)

Literature Search Results through 2016

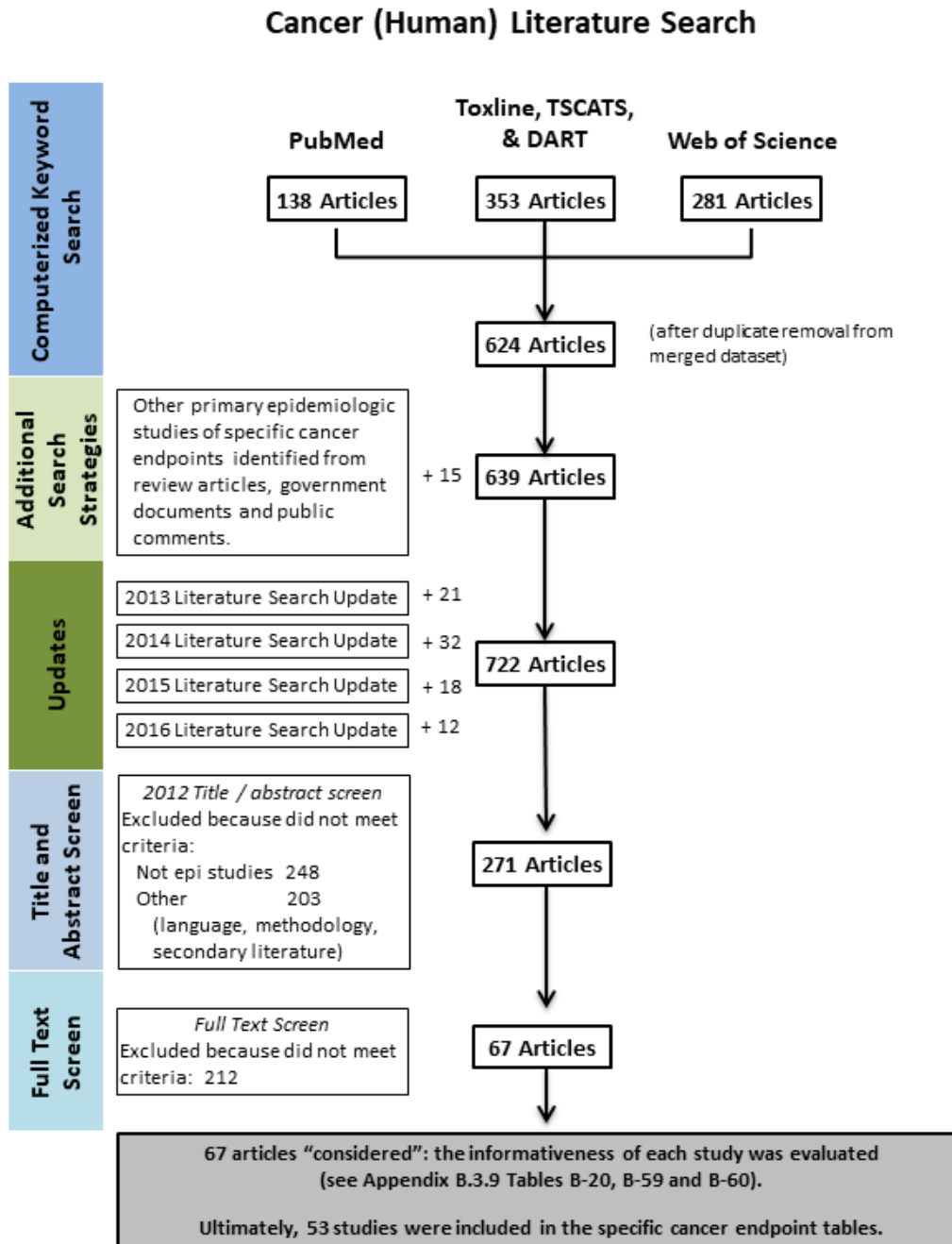


Figure B-15. Literature search documentation for sources of primary data pertaining to formaldehyde exposure and developmental and reproductive toxicity.

Literature Search Results from the Systematic Evidence Map Update through 2021

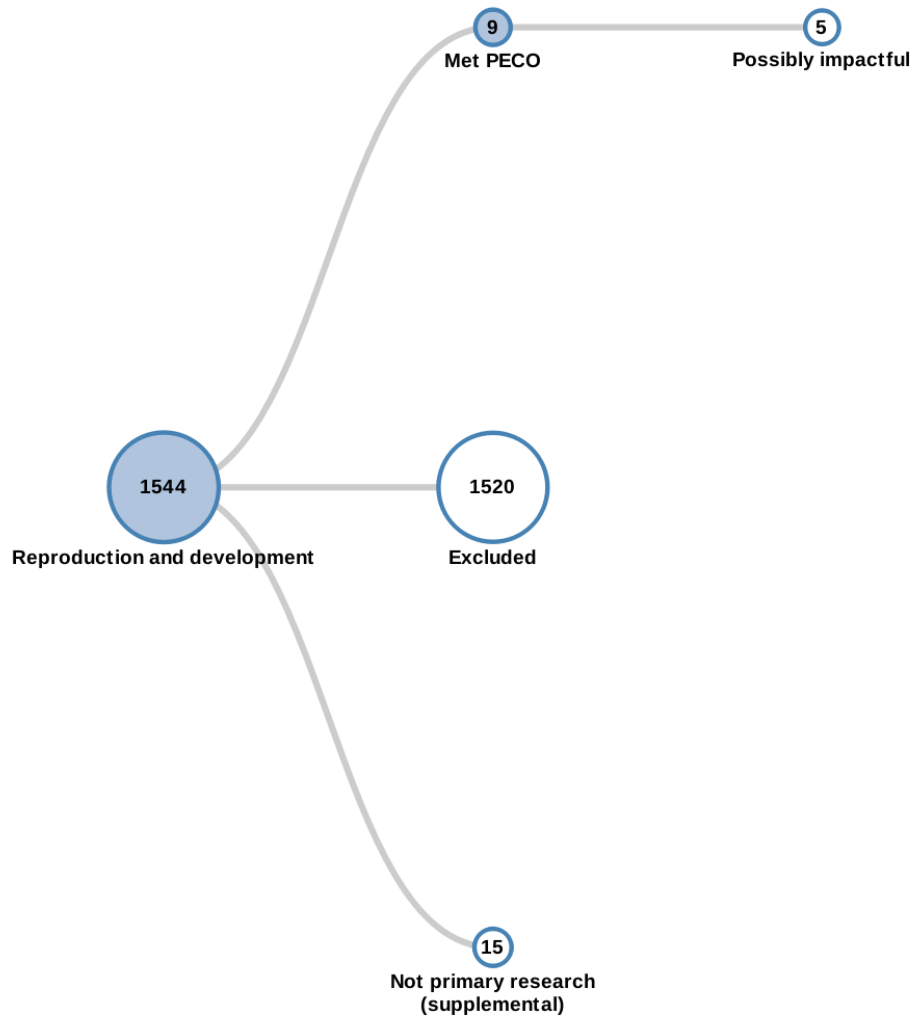


Figure B-16. Reproductive and developmental effects literature tree (interactive version [here](#)).

A total of 1,544 citations were retrieved for the assessment of reproductive and developmental effects and 9 studies met the PECO criteria.

Screening for Inclusion from the 2021 Systematic Evidence Map Update

Of the 9 studies that met PECO criteria, five were deemed to be *possibly impactful* (see Table B-16). There were four from the human literature and one from the animal literature. Neither of the identified mechanistic studies were deemed *possibly impactful*. [Wang et al. \(2015\)](#) was identified in the literature search update and deemed *possibly impactful*, but it already had been included in the 2017 draft.

Table B-16. Studies of reproductive and developmental effects

Reference	Study design	Exposure ^a	Endpoints	Impactful?	Rationale
Human Studies					
Amiri and Turner-Henson (2017)	General population southeastern U.S. cross-sectional	Air sampling, prenatal, exposure during pregnancy	Biparietal diameter, head circumference, abdominal circumference, femur length, ratio of abdominal circumference to femur length (AC/FL), estimated fetal weight	Possibly impactful	Key endpoints of concern for which there are few studies
Chang et al. (2017)	General population Seoul, South Korea birth cohort	Air sampling, prenatal, exposure during pregnancy	Birthweight, postnatal weight at 6, 12, 24, and 36 months.	Possibly impactful	Key endpoints of concern for which there are few studies
Franklin et al. (2019)	General population Australia birth cohort	Air sampling, prenatal, exposure during pregnancy	Gestational age, birth length, birth weight, head circumference	Possibly impactful	Key endpoints of concern for which there are few studies
Wang et al. (2015)	Occupational China cross-sectional	Air sampling and occupational history, adults, male plywood production workers	Semen volume, sperm concentration, total sperm count, sperm progressive motility and total sperm motility, curvilinear velocity, straight line velocity, linearity, time-average velocity, straightness, mean angular displacement, amplitude of lateral head displacement	Possibly impactful	Already identified in 2017 draft
Animal Studies^b					

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Endpoints	Impactful?	Rationale
Sapmaz et al. (2018)	Rat (Sprague Dawley), male Short-term (4 wk) or Subchronic (13 wk), 8 hr/d, 5 d/wk	Paraformaldehyde 0, 5 ppm (0, 6.15 mg/m ³) Inhalation	Testicular tubular atrophy, germinative epithelium height, seminiferous tubule diameter; markers of ROS in testicular tissue	Possibly impactful	Longer duration study; informative morphological endpoints
Ge et al. (2020b)	Rat (Sprague Dawley), male Subchronic (8 wk)	Formalin 0, 0.5, 2.46, 5 mg/m ³ Inhalation	Testicular seminiferous tubule histopathology and morphometry, SPO11 protein in testicular tissue	Not impactful	Formalin
Zang et al. (2017)	Mouse (C57BL/6), male Subchronic (60 d; 4 hr/d)	Formalin 0, 0.5, 5, 10 mg/m ³ Inhalation	Sexual behavior (mount latency, intromission latency, ejaculation latency, mount frequency, intromission frequency, copulatory efficacy); hormone measures (serum T and LH; testicular T); sperm number and motility; reproductive organ weights and histopathology	Not impactful	Formalin
Mechanistic Studies					
Fang et al. (2015)	Rat (Sprague Dawley), male Short-term (4 wk; 8 hr/d)	Unspecified test article 0, 0.5, 5, 10 mg/m ³ Inhalation	mTOR (mammalian target of rapamycin, a regulator of various cellular processes) mRNA expression, protein levels, and immunostaining in testes	Not impactful	Unspecified test article
Ibrahim et al. (2016)	Rat (Wistar), female (dam) Gestational (GD1-21; 1 hr/d, 5 d/wk)	Unspecified test article 0, 0.92 mg/m ³ Inhalation	Markers of ROS and inflammation in dam uterus at parturition; inflammation and immune parameters in offspring after PND30: BAL cell count and myeloperoxidase activity, lung cytokines and inflammatory markers; blood and bone marrow cell count	Not impactful	Unspecified test article

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

^aUse of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

^bAnimal studies may include evaluation of mechanistic endpoints.

B.2.9. Cancer

As detailed in Section 2.2.9 of the Toxicological Review, the review of the potential for carcinogenicity following formaldehyde exposure was performed separately for the following: (1) human studies of respiratory tract, lymphohematopoietic, or other cancers (including brain, lung, pancreatic, etc.); (2) experimental animal studies of respiratory tract (e.g., nasal) cancers; and (3) experimental animal studies of LHP cancers. Ultimately, studies on cancers of the respiratory tract or LHP system are discussed in the Toxicological Review, while human studies on other cancer types are documented in the Appendix B.3.9. The search strings are documented in Table B-17 (human studies), Table B-18 (animal studies of respiratory tract cancers), and Table B-19 (animal studies of LHP cancers). The corresponding literature search results through 2016 are documented in Figure B-17, Figure B-18, and Figure B-19, respectively. The corresponding literature search results through 2021 identified by the SEM are documented in Figure B-20, Figure B-21, and Figure B-22, respectively, with the SEM screening decisions documented in the subsequent sections.

Search Strings

Human Studies

Table B-17. Summary of search terms for carcinogenicity in humans

Database, search date	Terms
PubMed No date restriction	"formaldehyde"[Majr] AND ("neoplasms"[All Fields] OR "cancer"[All Fields] OR "leukaemia"[All Fields] OR "leukemia"[All Fields] OR "multiple myeloma"[All Fields] OR ("multiple"[All Fields] AND "myeloma"[All Fields]) OR "multiple myeloma"[All Fields] OR "myeloma"[All Fields] OR "lymphoma"[All Fields] OR "nasopharyngeal neoplasms"[All Fields] OR ("nasopharyngeal"[All Fields] AND "neoplasms"[All Fields]) OR "nasopharyngeal neoplasms"[All Fields] OR ("nasopharyngeal"[All Fields] AND "cancer"[All Fields]) OR "nasopharyngeal cancer"[All Fields] OR ("sinonasal" [All Fields] AND "neoplasms"[All Fields]) OR "neoplasms"[All Fields] OR "cancer"[All Fields] OR "oropharyngeal neoplasms"[All Fields] OR ("oropharyngeal"[All Fields] AND "neoplasms"[All Fields]) OR "oropharyngeal neoplasms"[All Fields] OR ("oropharyngeal"[All Fields] AND "neoplasms"[All Fields]) OR "laryngeal neoplasms"[All Fields] OR ("laryngeal"[All Fields] AND "neoplasms"[All Fields]) OR "laryngeal neoplasms"[All Fields] OR ("laryngeal"[All Fields] AND "cancer"[All Fields]) OR "laryngeal cancer"[All Fields]) AND (Epidemiol*[All Fields] OR "Case-control studies"[All Fields] OR "Cohort studies"[All Fields] OR "Follow-up studies"[All Fields] OR "Risk factors"[All Fields])
Web of Science No date restriction Lemmatization "off"	TS=formaldehyde AND (TS=neoplasms OR TS=cancer OR TS=leukaemia OR TS=leukemia OR TS=multiple myeloma OR (TS=multiple AND TS=myeloma) OR TS=multiple myeloma OR TS=myeloma OR TS=lymphoma OR TS=nasopharyngeal neoplasms OR (TS=nasopharyngeal AND TS=neoplasms) OR TS=nasopharyngeal neoplasms OR (TS=nasopharyngeal AND TS=cancer) OR TS=nasopharyngeal cancer OR (TS=sinonasal AND TS=neoplasms) OR TS=oropharyngeal neoplasms OR (TS=oropharyngeal AND TS=neoplasms) OR TS=oropharyngeal neoplasms OR (TS=oropharyngeal AND TS=neoplasms) OR TS=laryngeal neoplasms OR (TS=laryngeal AND TS=neoplasms) OR TS=laryngeal neoplasms OR (TS=laryngeal AND TS=cancer) OR TS=laryngeal cancer) AND (TS=Epidemiol* OR TS=Case-control studies OR TS=Cohort studies OR TS=Follow-up studies OR TS=Risk factors)
ToxNet (Toxline and DART) No date restriction	Formaldehyde AND (neoplasms OR neoplasms OR cancer OR leukaemia OR leukemia OR "multiple myeloma" OR (multiple AND myeloma) OR myeloma OR lymphoma OR "nasopharyngeal neoplasms" OR (nasopharyngeal AND neoplasms) OR "nasopharyngeal neoplasms" OR (nasopharyngeal AND cancer) OR

Supplemental Information—Formaldehyde (Inhalation)

Database, search date	Terms
English, not including PubMed	"nasopharyngeal cancer" OR (sinonasal AND neoplasms) OR "oropharyngeal neoplasms" OR (oropharyngeal AND neoplasms) OR "oropharyngeal neoplasms" OR (oropharyngeal AND neoplasms) OR "laryngeal neoplasms" OR (laryngeal AND neoplasms) OR "laryngeal neoplasms" OR (laryngeal AND cancer) OR "laryngeal cancer") AND (Epidemiol* OR "Case-control studies" OR "Cohort studies" OR "Follow-up studies" OR "Risk factors")

Animal Studies: Respiratory Tract Cancers

Table B-18. Summary of search terms for respiratory tract cancers in animals

Database, search date	Terms
PubMed 04/15/2013 No date restriction	Formaldehyde [majr] AND (animal OR rodent OR rat OR mouse OR hamster) AND (nasal OR nose OR buccal OR larynx OR lung OR mouth OR pharynx OR sinus OR trachea) AND (cancer OR dysplasia OR neoplasia OR tumor OR carcinoma OR polyp OR cytotoxicity OR neoplastic OR promoter OR pathology OR toxicity) NOT (formalin test OR formaldehyde fixation OR formalin fixed OR formaldehyde fixed OR formalin-induced OR formaldehyde-induced)
Web of Science 03/08/2013 No date restriction Lemmatization "off"	Formaldehyde (title) AND (animal OR rodent OR rat OR mouse OR hamster) AND (nasal OR nose OR buccal OR larynx OR lung OR mouth OR pharynx OR sinus OR trachea) AND (cancer OR dysplasia OR neoplasia OR tumor OR carcinoma OR polyp OR cytotoxicity OR neoplastic OR promoter OR pathology OR toxicity) NOT (formalin test OR formaldehyde fixation OR formalin fixed OR formaldehyde fixed OR formalin-induced OR formaldehyde-induced)

Animal Studies: Lymphohematopoietic Cancers

Table B-19. Summary of search terms for lymphohematopoietic cancers in animals

Database, search date	Terms
PubMed 04/15/2013 No date restriction	Formaldehyde [majr] AND (leukemia OR lymphoma OR hemolymphoreticular) AND (animal OR rodent OR monkey) NOT (formalin test OR formaldehyde fixation OR formalin fixed OR formaldehyde fixed OR formalin-induced OR formaldehyde-induced)
Web of Science 03/08/2013 No date restriction Lemmatization "off"	Formaldehyde (title) AND (leukemia OR lymphoma OR hemolymphoreticular) AND (animal OR rodent OR monkey) NOT (formalin test OR formaldehyde fixation OR formalin fixed OR formaldehyde fixed OR formalin-induced OR formaldehyde-induced) (topic)

Literature Search Results through 2016

Human Studies

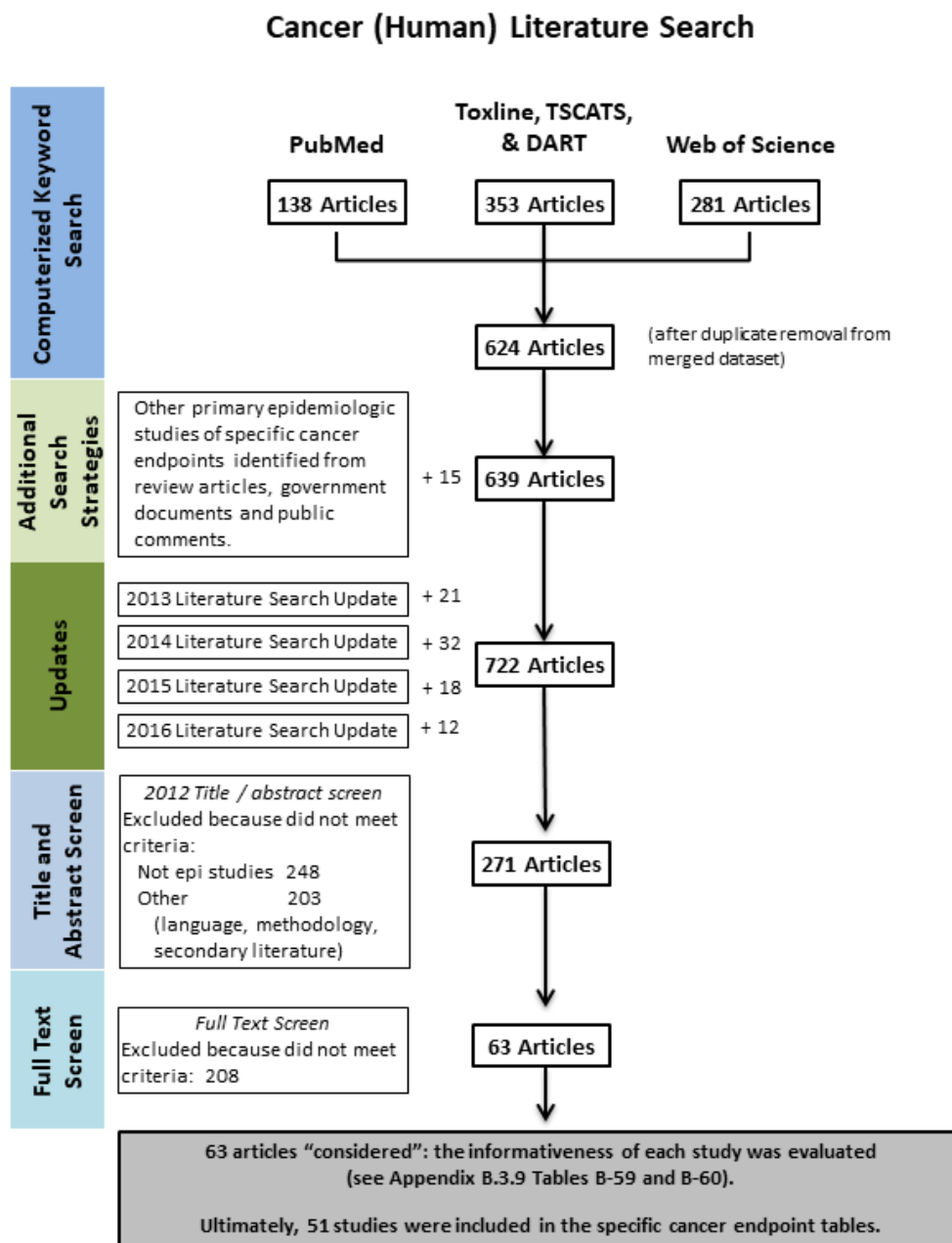


Figure B-17. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and upper respiratory or lymphohematopoietic cancers in humans.

Animal Studies: Respiratory Tract Cancers

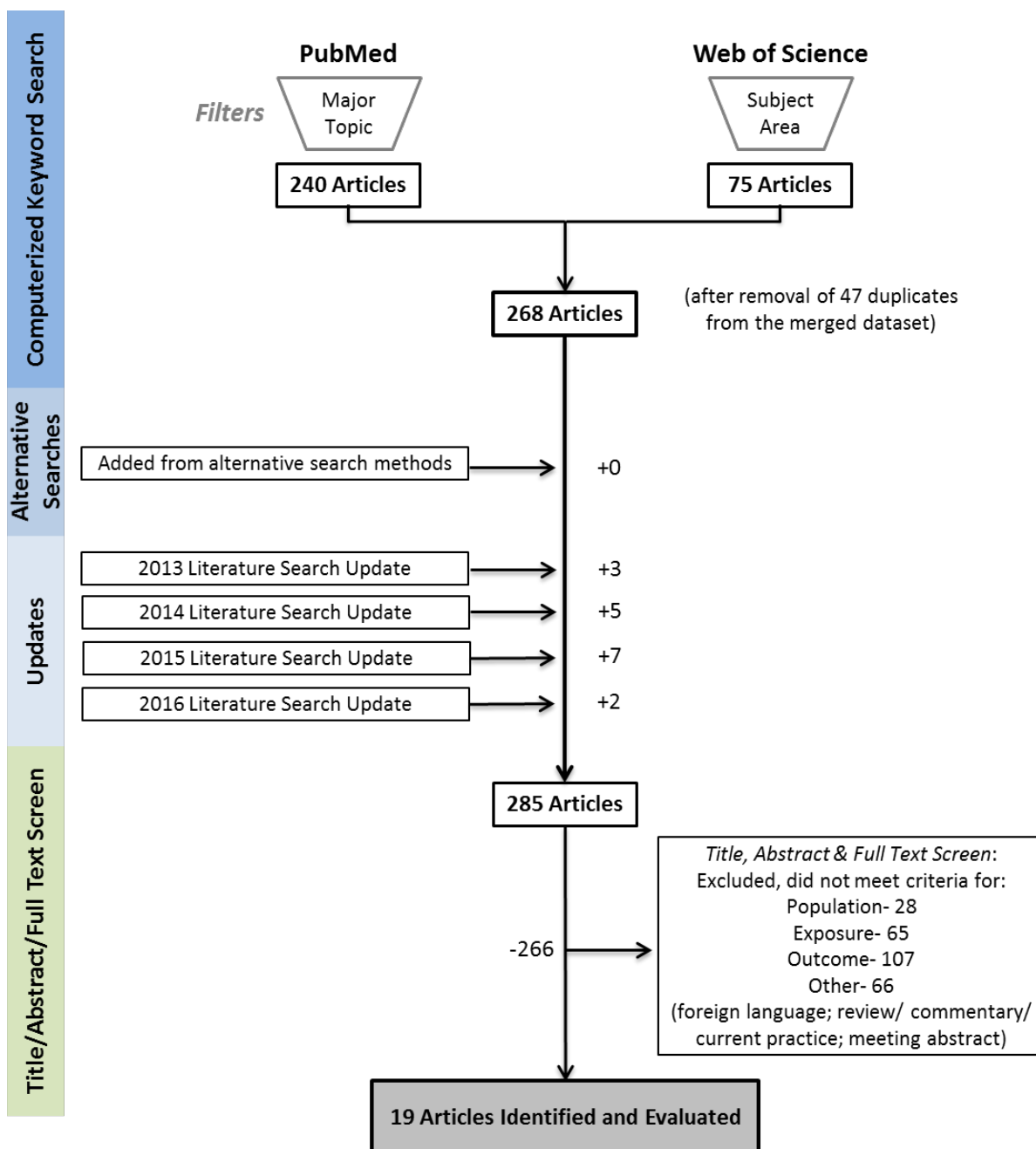


Figure B-18. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and upper respiratory tract (nasal) cancers in animals.

Animal Studies: Lymphohematopoietic Cancers

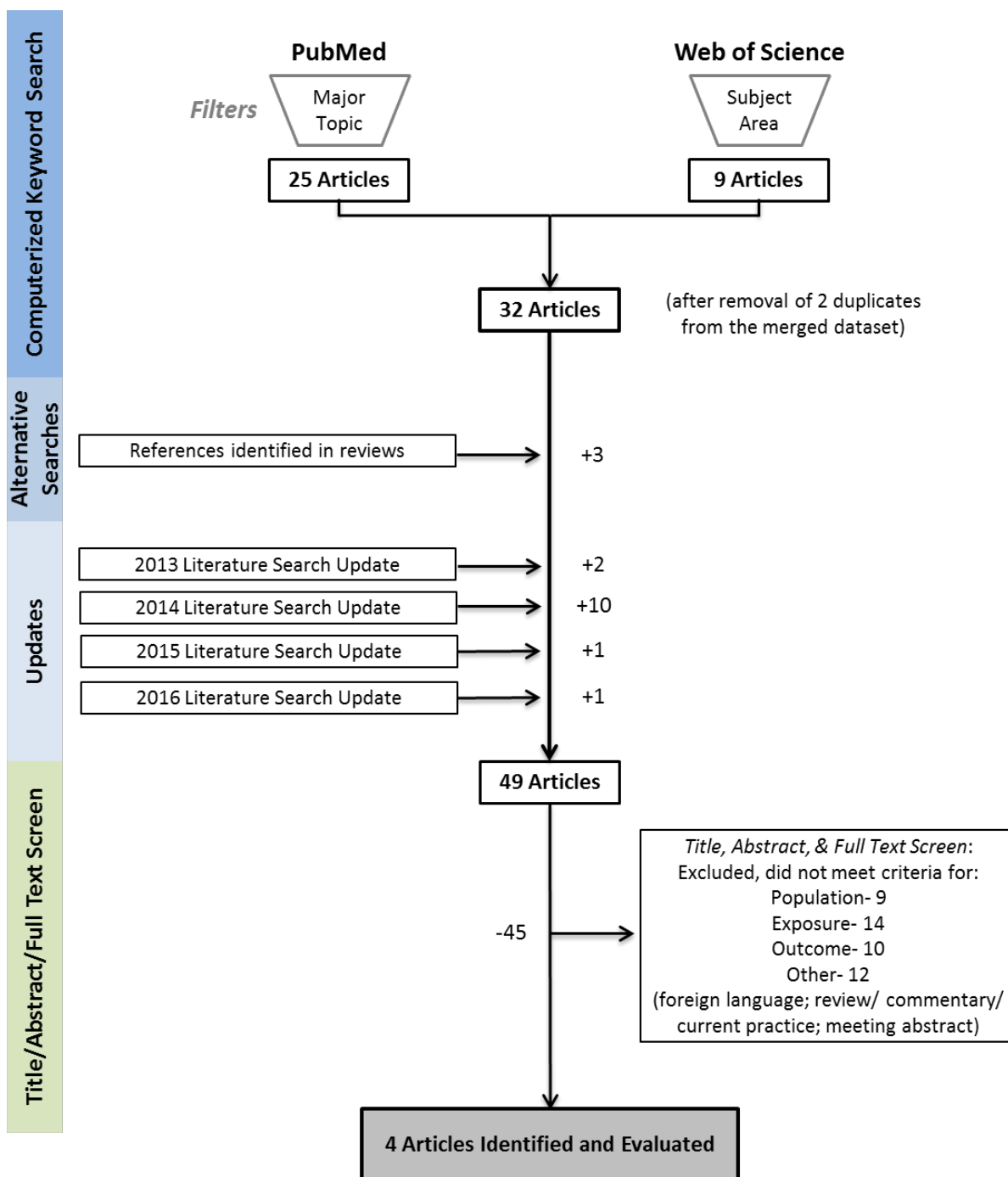


Figure B-19. Literature search documentation for sources of primary data pertaining to inhalation formaldehyde exposure and lymphohematopoietic (LHP) cancers in animals.

Literature Search Results from the Systematic Evidence Map Update through 2021

Human Studies

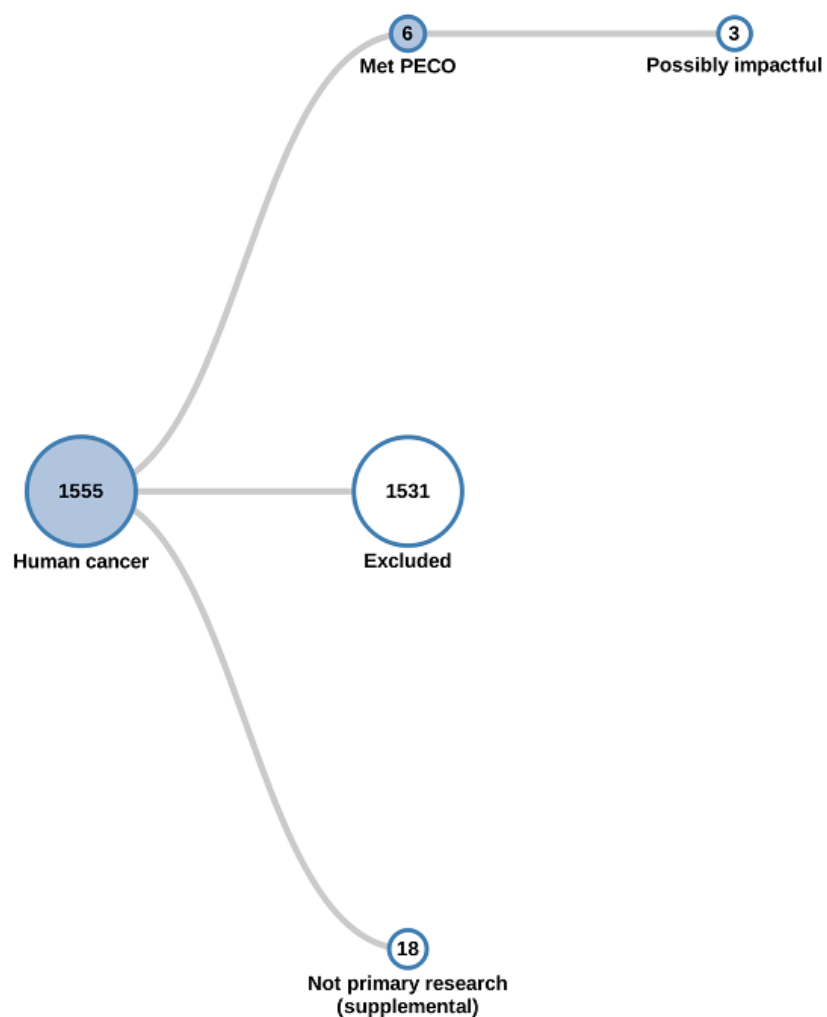


Figure B-20. Human cancer literature tree (interactive version [here](#)).

A total of 1,555 citations were retrieved for the assessment of cancer in humans and 6 studies met the PECO criteria.

Animal Studies: Respiratory Tract Cancers

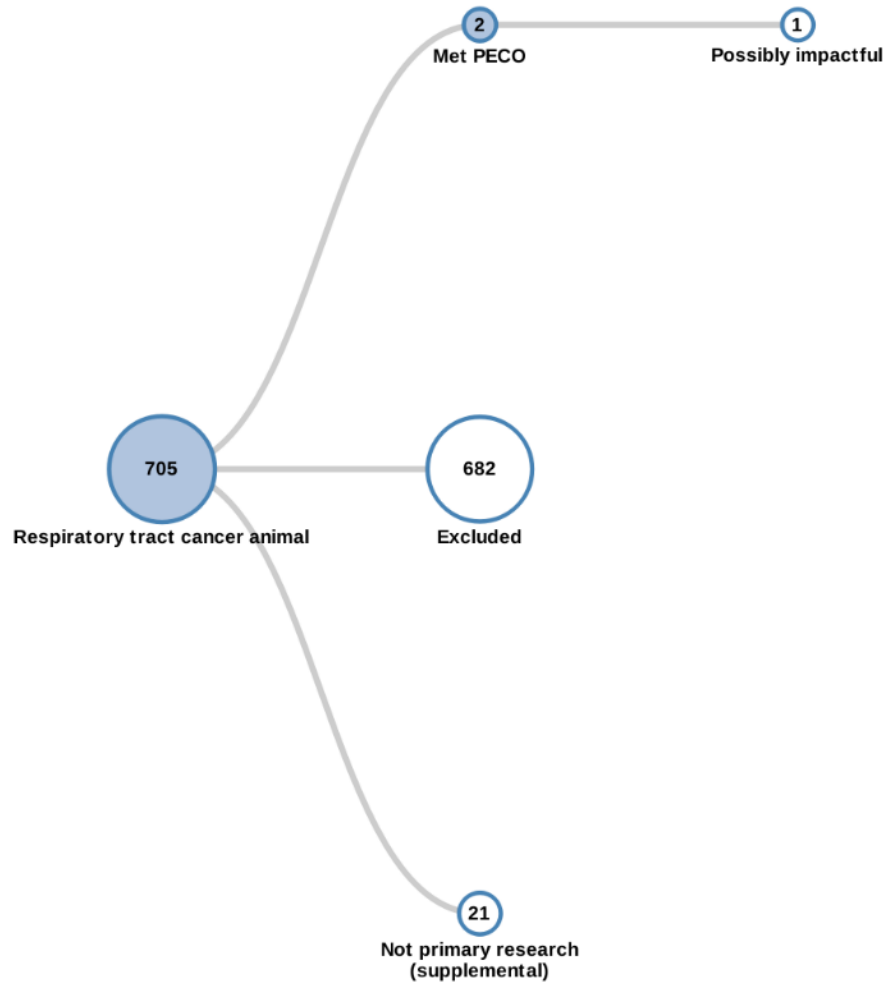


Figure B-21. Animal respiratory tract cancer literature tree (interactive version [here](#)).

A total of 705 citations were retrieved for the assessment of respiratory tract cancers in animals and 2 studies met the PECO criteria.

Animal Studies: Lymphohematopoietic Cancers

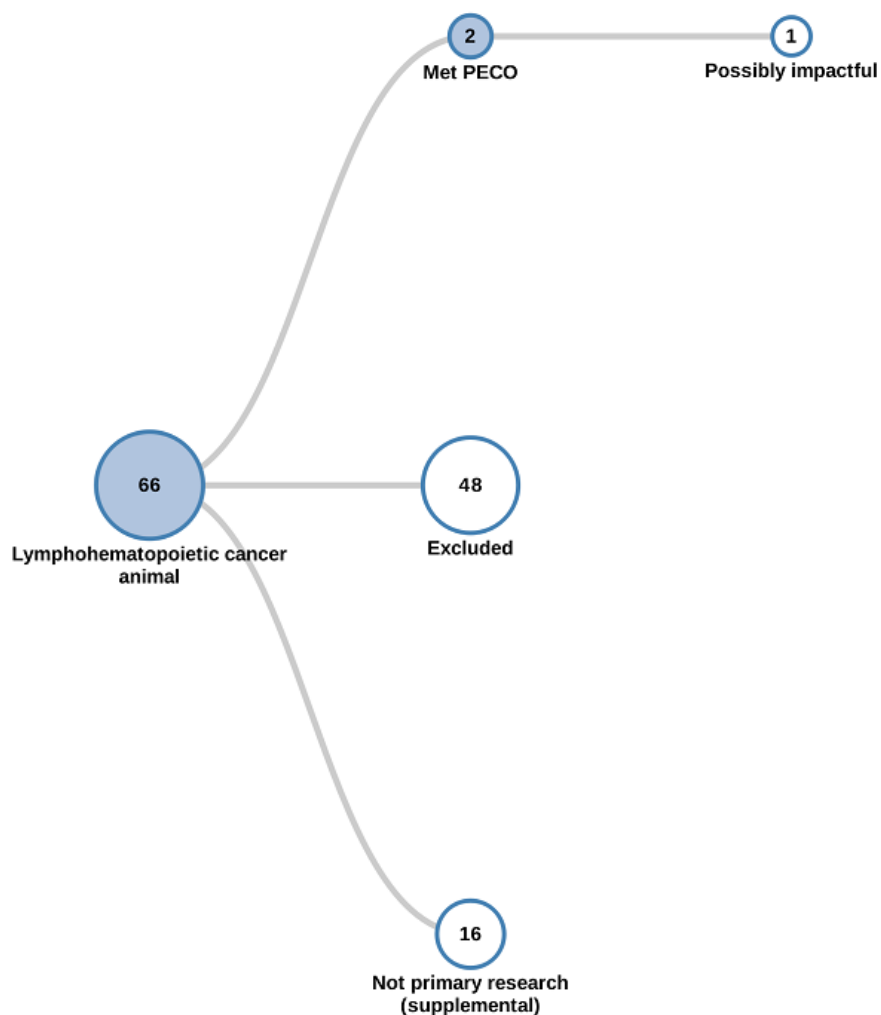


Figure B-22. Animal lymphohematopoietic cancer literature tree (interactive version [here](#)).

A total of 66 citations were retrieved for lymphohematopoietic cancers in animals and 2 studies met the PECO criteria.

Screening for Inclusion from the 2021 Systematic Evidence Map Update

Human Studies

Of the six studies that met the PECO criteria (see Table B-20), one *possibly impactful* study, [Checkoway et al. \(2015\)](#), had already been included in the 2017 draft as reanalysis of the primary NCI cohort, and one *not impactful* study, [Pira et al. \(2014\)](#), had already been included in the 2017 draft. The four new studies that met the PECO criteria increased the total number of studies that met PECO in the searches through 2017 (n = 63) to a total of 67 studies that met PECO.

Table B-20. Studies of site-specific cancer in humans

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
Humans					
Checkoway et al. (2015)	Occupational United States cohort	Air sampling, occupational history, and job-exposure matrix, adults, NCI cohort reanalysis	Cause-specific mortality [non-Hodgkin lymphoma mortality, chronic lymphocytic leukemia mortality, Hodgkin lymphoma mortality, multiple myeloma mortality, myeloid leukemia mortality, acute myeloid leukemia mortality, chronic myeloid leukemia mortality, all leukemia mortality, lymphohematopoietic cancer mortality]	Possibly impactful	Already identified in 2017 draft
Marsh et al. (2016)	Occupational United States cohort	Air sampling, occupational history, and job-exposure matrix, adults, NCI cohort NPC reanalysis	Nasopharyngeal cancer mortality	Possibly impactful	Additional analyses of key endpoint of concern
Möhner et al. (2019)	Occupational United States cohort	Occupation-based, adults, NCI cohort analysis	Mortality from nasopharyngeal cancer [oropharynx, nasopharynx, hypopharynx, pharynx, pharynx (unspecified)]	Possibly impactful	Additional analyses of key endpoints of concern

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
Pira et al. (2014)	Occupational Piedmont, Italy cohort	Occupational history, adults, laminated plastics workers	Cause-specific mortality [lymphoma, myeloma, leukemia, all lymphatic and hematopoietic tissue neoplasms]	Not impactful	Already identified in 2017 draft
Sernia et al. (2016)	Occupational Italy cohort	Current occupation, adults, university laboratory workers	NPC, leukemia/lymphoma	Not impactful	Inadequate exposure assessment; NPC well- established
Xie et al. (2017)	General population Hong Kong case-control	Occupational history and industrial code, self-report, adults	Nasopharyngeal carcinoma incidence	Not impactful	Inadequate exposure assessment; health effect well-established

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

Animal Studies: Respiratory Tract Cancers

Of the two studies meeting the PECO criteria (see Table B-21), one was deemed *possibly impactful*. This study, ([Morgan et al., 2017](#)) had already been included in the 2017 draft; thus, zero (0) additional studies were included.

Table B-21. Animal studies of respiratory tract cancers

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
Animal Studies					
Morgan et al. (2017)	Mouse (Trp53 haploinsufficient), Male Subchronic (8 wk; 6 hr/d, 5 d/wk), then held for 32 wk	Paraformaldehyde 0, 7.5 or 15 ppm (0, 9.2, 18.5 mg/m ³) Inhalation	Blood was collected for hematology, and major tissues and gross lesions were collected for histopathology (nasal and LHP cancer, and respiratory lesions)	Possibly impactful	Already included in 2017 draft
Soffritti et al. (2016)	Rat (SD), both sexes Chronic (continuous exposure from 6–104 wks of age)	Unspecified test article 0, 50 ppm Oral (drinking water)	Carcinogenicity study (presumed to include evaluation of nasal/URT tumors)	Not impactful	Oral exposure; high levels; formalin (note: would be screened as excluded, but inventoried due to rarity of chronic exposure duration studies of cancer)

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

Animal Studies: Lymphohematopoietic Cancers

Of the two studies that met the PECO criteria (see Table B-22), one was deemed *possibly impactful*. This study, ([Morgan et al. 2017](#)) had already been included in the 2017 draft; thus, zero (0) additional studies were included.

Table B-22. Animal studies of lymphohematopoietic cancer

Reference	Study design	Exposure	Endpoints	Impactful?	Rationale
Animal Studies					
Morgan et al. (2017)	Mouse (Trp53 haploinsufficient), Male Subchronic (8 wk; 6 hr/d, 5 d/wk), then held for 32 wk	Paraformaldehyde 0, 7.5 or 15 ppm (0, 9.2, 18.5 mg/m ³) Inhalation	All major tissues and gross lesions were collected for histopathology (including LHP tissues)	Possibly impactful	Already included in 2017 draft
Soffritti et al. (2016)	Rat (SD), both sexes Chronic (continuous exposure from 6–104 wks of age)	Unspecified test article 0, 50 ppm Oral (drinking water)	Carcinogenicity study (presumed to include evaluation of nasal/URT tumors)	Not impactful	Oral exposure; high levels; formalin (note: would be screened as excluded, but inventoried due to rarity of chronic exposure duration studies of cancer)

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

B.2.10. Mechanistic Information Relevant to Cancer

As detailed in Section 2.2.10 of the Toxicological Review, consolidated systematic approaches to identifying the literature examining mechanistic effects relevant to interpreting the potential for formaldehyde to cause either upper respiratory tract (URT) or lymphohematopoietic (LHP) cancers were not performed. Rather, the evidence bases of identified studies was compiled from searches and evaluations performed for genotoxicity (see description below), respiratory tract pathology (see Appendix B.2.5), and mechanistic information related to noncancer respiratory effects, including inflammation and immune changes (see Appendix B.2.6), augmented by studies cited in review articles retrieved through searches on specific cancer types as well as other national-level health assessments. For genotoxicity, a consistent set of search terms was applied within electronic databases (i.e., PubMed and Web of Science) to address the broader topic of mode of action for either respiratory tract or LHP cancers. The retrieved citations were screened for studies on genotoxic endpoints. These broad search terms (Tables B-23 and B-24 for respiratory tract and LHP cancer mechanisms, respectively) were used to ensure capture of newer literature (e.g., published after the available national-level health assessments). The literature search results through 2021 identified by the SEM are documented in Figure B-23 (respiratory tract cancer-related mechanisms) and Figure B-24 (LHP cancer-related mechanisms), with the SEM screening decisions documented in the subsequent sections.

Search Strings

Mechanistic Studies for Respiratory Tract Cancers, Focusing on Genotoxicity

Table B-23. Search terms for cancer mechanisms of respiratory cancers

Mechanisms of Respiratory Tract Cancers Focusing on Genotoxicity - Pubmed	
1	(Formaldehyde[tiab] OR formaldehyde[mh])
2	AND (nose[tiab] OR nasal[tiab] OR nasopharynx[tiab] OR nasopharyngeal[tiab] OR respiratory[tiab] OR bronchial[tiab] OR "upper respiratory"[tiab] OR mucociliary[tiab] OR mononuclear[tiab] OR "nasal mucosa"[tiab] OR "human bronchial"[tiab] OR "nasal cavity"[tiab] OR trachea[tiab] OR "oral mucosa"[tiab] OR lymphoblasts[tiab] OR "endothelial cells"[tiab] OR "respiratory tract"[tiab] OR olfactory[tiab] OR "nasal epithelia"[tiab] OR "nasal turbinates"[tiab] OR "nose"[mh] OR "nasopharynx"[mh] OR "trachea"[mh] OR "smell"[mh])
3	AND (tumor[tiab] OR carcinoma[tiab] OR cancer[tiab] OR neoplastic[tiab] OR cytotoxic[tiab] OR cytotoxicity[tiab] OR proliferation[tiab] OR "cell proliferation"[tiab] OR immunosuppression[tiab] OR immune[tiab] OR genotoxicity[tiab] OR genotoxic[tiab] OR mutation[tiab] OR mutagenic[tiab] OR epigenomic[tiab] OR epigenetic[tiab] OR microRNA[tiab] OR "micro RNA"[tiab] OR methylation[tiab] OR "chromosome aberration"[tiab] OR "chromosomal aberration"[tiab] OR micronuclei[tiab] OR MN[tiab] OR micronucleus[tiab] OR "sister chromatid exchange"[tiab] OR SCE[tiab] OR "single strand break"[tiab] OR SSB[tiab] OR glutathione[tiab] OR oxidation[tiab] OR "oxidative damage"[tiab] OR inflammation[tiab] OR "DNA-protein crosslink"[tiab] OR DPX[tiab] OR "DNA adduct"[tiab] OR clastogen[tiab] OR clastogenicity[tiab] OR promotion[tiab] OR promoter[tiab] OR "DNA repair"[tiab] OR "immune activation"[tiab] OR phagocyte[tiab] OR macrophages[tiab] OR cytogenetic[tiab] OR "regenerative cell proliferation"[tiab] OR

Supplemental Information—Formaldehyde (Inhalation)

Mechanisms of Respiratory Tract Cancers Focusing on Genotoxicity - Pubmed	
	mutagenesis[tiab] OR "DNA-protein crosslinks"[tiab] OR "respiratory cancer"[tiab] OR "nasal cancer"[tiab] OR "immune function"[tiab] OR "immune biomarkers"[tiab] OR "respiratory disease"[tiab] OR DPC[tiab] OR DPX[tiab] OR "DNA damage"[tiab] OR irritation[tiab] OR bronchitis[tiab] OR "regenerative hyperplasia"[tiab] OR toxicological[tiab] OR adenomas[tiab] OR rhinitis[tiab] OR dysplasia[tiab] OR metaplasia[tiab] OR inhalation[tiab] OR carcinogen[tiab] OR "chromosomal damages"[tiab] OR "nasal carcinoma"[tiab] OR toxicology[tiab] OR toxicity[tiab] OR "DNA-DNA cross-link"[tiab] OR "respiratory epithelium"[tiab] OR SCC[tiab] OR "pathological changes"[tiab] OR "histopathological nasal changes"[tiab] OR cilia[tiab] OR "nasal lesions"[tiab] OR "protein oxidation"[tiab] OR "cellular immunity"[tiab] OR autoantibodies[tiab] OR tumour[tiab] OR "cell damage"[tiab] OR "neoplasms"[mh] OR "carcinoma"[mh] OR "immunosuppression"[mh] OR "immune tolerance"[mh] OR "mutation"[mh] OR "epigenomics"[mh] OR "methylation"[mh] OR "glutathione"[mh] OR "inflammation"[mh] OR "phagocytes"[mh] OR "macrophages"[mh] OR "cytogenetics"[mh] OR "mutagenesis"[mh] OR "nose neoplasms"[mh] OR "bronchitis"[mh] OR "adenoma"[mh] OR "rhinitis"[mh] OR "metaplasia"[mh] OR "inhalation"[mh] OR "carcinogens"[mh] OR "toxicology"[mh] OR "toxicity"[Subheading] OR "cilia"[mh] OR "autoantibodies"[mh] OR "immune system phenomena"[mh] OR "mutagens"[mh] OR "Cytotoxicity, Immunologic"[mh] OR "Cell Proliferation"[mh] OR "MicroRNAs"[mh] OR "Chromosome Aberrations"[mh] OR "Sister Chromatid Exchange"[mh] OR "DNA Breaks, Single-Stranded"[mh] OR "DNA Adducts"[mh] OR "Promoter Regions, Genetic"[mh] OR "DNA Repair"[mh] OR "Respiratory Tract Diseases"[mh] OR "DNA Damage"[mh] OR "Respiratory Mucosa"[mh] OR "Immunity, Cellular"[mh])
4	NOT ("formalin test"[tiab] OR "formaldehyde fixation"[tiab] OR "formalin fixed"[tiab] OR "formaldehyde fixed"[tiab] OR formalin-induced[tiab] OR formaldehyde-induced[tiab])
Mechanisms of Respiratory Tract Cancers Focusing on Genotoxicity - WoS	
1	Formaldehyde (Title only)
2	AND (nose OR nasal OR nasopharynx OR nasopharyngeal OR respiratory OR bronchial OR upper-respiratory OR mucociliary OR mononuclear OR nasal-mucosa OR human-bronchial OR nasal-cavity OR trachea OR oral-mucosa OR lymphoblasts OR endothelial-cells OR respiratory-tract OR olfactory OR nasal-epithelia OR nasal-turbينات)
3	AND (tumor OR carcinoma OR cancer OR neoplastic OR cytotoxic OR cytotoxicity OR proliferation OR immunosuppression OR immune OR genotoxicity OR genotoxic OR mutation OR mutagenic OR epigenomic OR epigenetic OR microRNA OR micro-RNA OR methylation OR chromosome-aberration OR chromosomal-aberration OR micronuclei OR MN OR micronucleus OR sister-chromatid-exchange OR SCE OR single-strand-break OR SSB OR glutathione OR oxidation OR oxidative-damage OR inflammation OR DNA-protein-crosslink OR DPX OR DNA-adduct OR clastogen OR clastogenicity OR promotion OR promoter OR DNA-repair OR immune-activation-phagocyte OR macrophages OR cytogenetic OR regenerative-cell-proliferation OR mutagenesis OR DNA-protein-crosslinks OR respiratory-cancer OR nasal-cancer OR immune-function OR immune-biomarkers OR respiratory-disease OR DPC OR DPX OR DNA-damage OR irritation OR bronchitis OR regenerative-hyperplasia OR toxicological OR adenomas OR rhinitis OR dysplasia OR metaplasia OR inhalation OR carcinogen OR chromosomal-damages OR bronchitis OR nasal-carcinoma OR toxicology OR toxicity OR DNA-DNA-cross-link OR respiratory-epithelium OR SCC OR pathological-changes OR histopathological-nasal-changes OR cilia OR nasal-lesions OR protein-oxidation OR cellular-immunity OR autoantibodies OR tumour OR cell-damage)
4	NOT (formalin-test OR formaldehyde-fixation OR formalin-fixed OR formaldehyde-fixed OR formalin-induced OR formaldehyde-induced)

Mechanistic Studies for Lymphohematopoietic (LHP) Cancers, Focusing on Genotoxicity

Table B-24. Search terms for cancer mechanisms of LHP cancers

Mechanisms of LHP Cancers Focusing on Genotoxicity - Pubmed	
1	(Formaldehyde[tiab] OR formaldehyde[mh])
2	AND (blood[tiab] OR lymphocytes[tiab] OR "bone marrow"[tiab] OR hematopoietic[tiab] OR "hematopoietic stem cells"[tiab] OR leukocytes[tiab] OR "white blood cell"[tiab] OR "NK cell"[tiab] OR "natural killer cell"[tiab] OR b-lymphocyte[tiab] OR b-cell[tiab] OR t-lymphocyte[tiab] OR t-cell[tiab] OR leukemia[tiab] OR lymphoma[tiab] OR myeloid[tiab] OR serum[tiab] OR albumin[tiab] OR adduct[tiab] OR genotoxic[tiab] OR aneuploidy[tiab] OR pancytopenia[tiab] OR epigenomics[tiab] OR epigenetic[tiab] OR microRNA[tiab] OR "micro rna"[tiab] OR methylation[tiab] OR "chromosome aberration"[tiab] OR "chromosomal aberration"[tiab] OR micronucleus[tiab] OR "sister chromatid exchange"[tiab] OR glutathione[tiab] OR oxidation[tiab] OR "oxidative damage"[tiab] OR inflammation[tiab] OR dna-protein-crosslink[tiab] OR "dna adduct"[tiab] OR "immune activation"[tiab] OR "blood"[Subheading] OR "blood"[mh] OR "lymphocytes"[mh] OR "lymphocyte count"[mh] OR "bone marrow"[mh] OR "hematopoietic system"[mh] OR "hematopoietic stem cells"[mh] OR "leukocytes"[mh] OR "leukocyte count"[mh] OR "leukocytes"[mh] OR "killer cells, natural"[mh] OR "killer cells, natural"[mh] OR "b-lymphocytes"[mh] OR "b-lymphocytes"[mh] OR "t-lymphocytes"[mh] OR "t-lymphocytes"[mh] OR "leukemia"[mh] OR "lymphoma"[mh] OR "serum"[mh] OR "albumins"[mh] OR "aneuploidy"[mh] OR "pancytopenia"[mh] OR "epigenomics"[mh] OR "epigenomics"[mh] OR "micrornas"[mh] OR "micrornas"[mh] OR "methylation"[mh] OR "chromosome aberrations"[mh] OR "chromosome aberrations"[mh] OR "sister chromatid exchange"[mh] OR "glutathione"[mh] OR "inflammation"[mh] OR "dna adducts"[mh])
3	NOT ("formalin test"[tiab] OR "formaldehyde fixation"[tiab] OR "formalin fixed"[tiab] OR "formaldehyde fixed"[tiab] OR formalin-induced[tiab] OR formaldehyde-induced[tiab])
Mechanisms of LHP Cancers Focusing on Genotoxicity - WoS	
1	Formaldehyde (Title only)
2	AND (blood OR lymphocytes OR bone-marrow OR hematopoietic OR hematopoietic-stem-cells OR leukocytes OR white-blood-cell OR NK-cell OR natural-killer-cell OR b-lymphocyte OR b-cell OR t-lymphocyte OR t-cell OR leukemia OR lymphoma OR myeloid OR serum OR albumin OR adduct OR genotoxic OR aneuploidy OR pancytopenia OR epigenomics OR epigenetic OR microRNA OR micro-rna OR methylation OR chromosome-aberration OR chromosomal-aberration OR micronucleus OR sister-chromatid-exchange OR glutathione OR oxidation OR oxidative-damage OR inflammation OR dna-protein-crosslink OR dna-adduct OR immune-activation)
3	NOT (formalin-test OR formaldehyde-fixation OR formalin-fixed OR formaldehyde-fixed OR formalin-induced OR formaldehyde-induced)

Literature Search Results from the Systematic Evidence Map Update through 2021

Mechanistic Studies for Respiratory Tract Cancers, Focusing on Genotoxicity

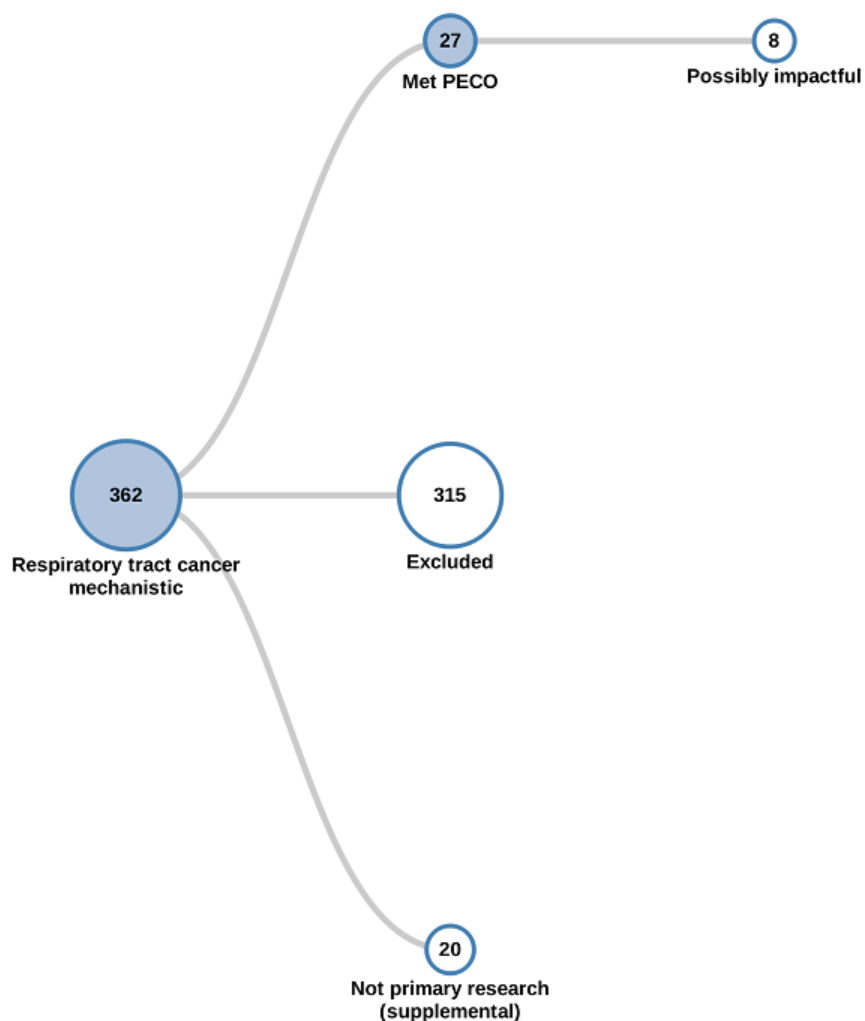


Figure B-23. Mechanistic respiratory tract cancer literature tree (interactive version [here](#)).

A total of 362 citations were retrieved for the assessment of mechanistic information informing respiratory tract cancers, focusing on genotoxicity, and 27 studies met the PECO criteria.

Mechanistic Studies for Lymphohematopoietic (LHP) Cancers, Focusing on Genotoxicity

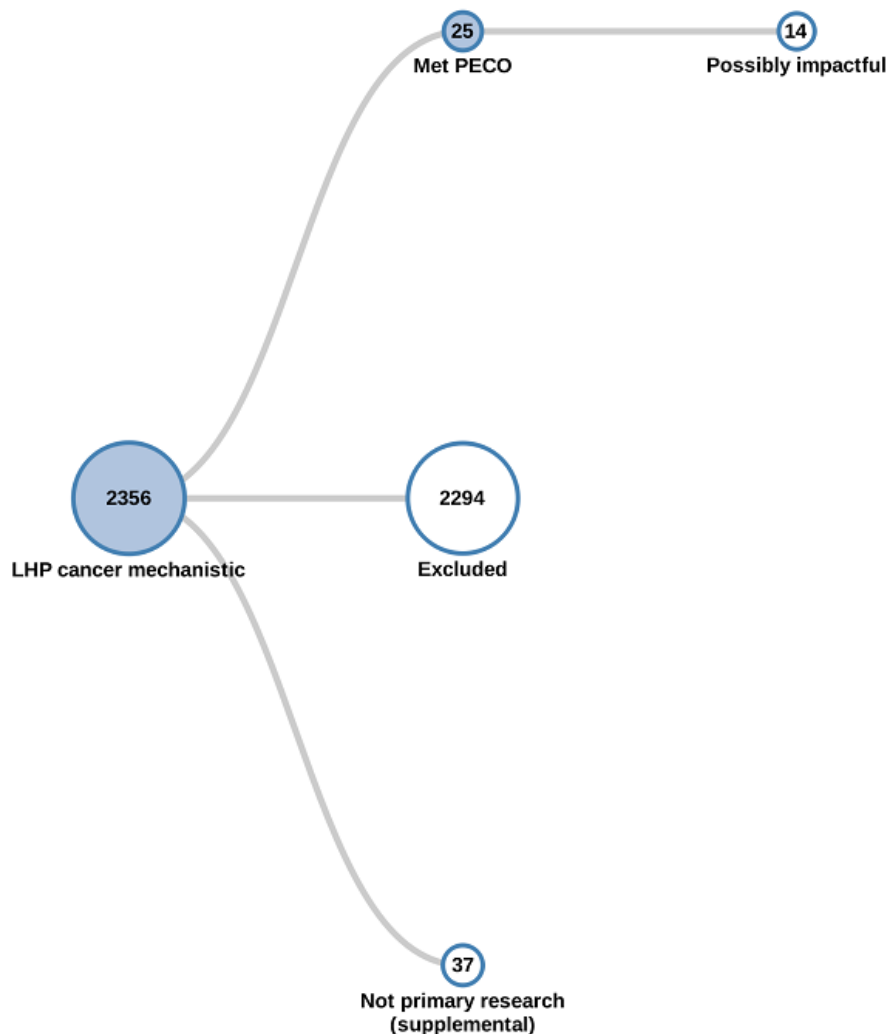


Figure B-24. Mechanistic lymphohematopoietic cancer literature tree (interactive version [here](#)).

A total of 2,356 citations were retrieved for the assessment of mechanistic information informing lymphohematopoietic cancers, focusing on genotoxicity, and 25 studies met the PECO criteria.

Screening for Inclusion from the 2021 Systematic Evidence Map Update

Mechanistic Studies for Respiratory Tract Cancers, Focusing on Genotoxicity

Of the 27 studies meeting PECO criteria, eight studies were deemed to be *possibly impactful* (note: one *possibly impactful* study is repeated under both the animal and in vitro/ex vivo sections). Table B-25 summarizes the SEM-identified studies of formaldehyde exposure in humans and animals, as well as in vitro or ex vivo experiments. Several studies relevant to endogenous formaldehyde, pharmacokinetic modeling and dosimetry also were included.

Table B-25. Mechanistic studies relating to respiratory tract cancers, focusing on genotoxicity

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
Human Studies					
Aglan and Mansour (2018)	Occupational Cairo, Egypt Cross-sectional	Air sampling Adult hairstylists	Buccal cell MN frequency	Possibly impactful	Specific markers; exposure levels relevant to human cancer interpretations
Costa et al. (2019)	Occupational Portugal Cross-sectional	Air sampling Adult anatomy-pathology laboratory workers	Buccal cell MN and nuclear budding, genotype analysis of selected polymorphisms	Possibly impactful	Specific markers; exposure levels relevant to human cancer interpretations
Peteffi et al. (2015)	Occupational Rio Grande do Sul, Brazil Cross-sectional	Air sampling Adult furniture workers	Micronucleus (MN) assay in buccal cells: nuclear buds, binucleated cells, Karyorrhexis	Possibly impactful	Specific markers; exposure levels relevant to human cancer interpretations
Bono et al. (2016)	Occupational Piedmont region, Italy Cross-sectional	Air sampling Adult plastic laminate workers	Malondialdehyde DNA adducts in swabbed nasal epithelial cells	Not impactful	Not key endpoint, adducts may or may not lead to more robust markers
Bruno et al. (2018)	Occupational Rome, Italy Cross-sectional	Air sampling Adult pathology laboratory workers	Counts of multinucleated ciliated cells, Karyorrhexis, Hyperchromatic SNS from middle portion of the inferior turbinate	Not impactful	Not key endpoint, nuclear abnormalities are non-specific markers

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
Animal Studies					
Leng et al. (2019)	Rat (Fischer 344), male Short-term (28 d; 6 hr/d)	Deuterated formaldehyde (no methanol) 0, 1, 30, 300 ppb (1.23, 36.9, 369 mg/m ³) [¹³ CD ₂]-HCHO Inhalation	DNA adducts in nose, lung (and other tissues)	Possibly impactful	Endpoints important to understanding dosimetry; low exposure levels
Zhao et al. (2020)	Mouse (BALB/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 3 mg/m ³ Inhalation	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies from nose and lung	Possibly impactful	Impactful endpoints (Note: formalin, but less of a concern in POE)
Bernardini et al. (2020)	Mouse (Swiss), male Short-term (4 wk; 4 hr/d, 5 d/wk)	unspecified test article 0, 0.5, 1, 5, 10 ppm (0, 0.62, 1.23, 6.15, 12.3 mg/m ³) Inhalation	MN, comet assay, and global methylation in lung	Not impactful	Unknown test article; no specific URT measures
Edrissi et al. (2017)	Rat (F344), male Short-term (7, 14, 21, or 28 d; 6 hr/d)	[¹³ C]-labeled formaldehyde 0, 2 ppm (0, 2.46 mg/m ³) Inhalation	FA-lysine adducts in nasal epithelium, lung, and trachea	Not impactful	Not key endpoint, adducts may or may not lead to more robust markers
In vitro/Ex vivo Studies					
Zhao et al. (2020)	Mouse (BALB/c), male Ex vivo primary lung and nose cells Acute (1 hr)	Formalin 0, 50, 100, 200, 400 µM In media	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies	Possibly impactful	Impactful endpoints (note: formalin; in vitro)
Anandarajan et al. (2020)	Yeast (<i>Schizosaccharomyces pombe</i>), deletion strains Short-term (3-5 d)	Formalin 0, 0.2, 0.5, 1.25, 1.5, 1.75 mM	Toxicogenomic profiling of pathways relating to formaldehyde detoxification and DNA repair—including	Not impactful	Non-mammalian; formalin; high dose

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
		(Note: included due to conserved DNA repair pathways between yeast and humans, and potential relevance to human susceptibility)	homologous recombination and nucleotide excision repair		
Chen et al. (2017)	Human immortalized bronchial epithelial cells Acute (up to 6 hr)	Unspecified test article 0, 0.5 mM In media	Inhibition of chromatin assembly, formaldehyde-histone adducts, gene expression	Not impactful	Unknown test article; in vitro; endpoint well-established in vitro
Gonzalez-Rivera et al. (2020)	Human immortalized bronchial epithelial cells Acute (2 hr)	Paraformaldehyde 0, 1 ppm (0, 1.23 mg/m ³) In vitro gaseous exposure	Cell phenotypic alterations; DNA damage	Not impactful	In vitro; endpoint well-established in vitro
Juarez et al. (2018)	Human immortalized, osteosarcoma, fibroblast, or epithelial colorectal adenocarcinoma cells Short-term (5 d; continuous)	Unspecified test article 0, 20, 40, 60, 80, 100 µM In media	genomic analysis (Note: included due to analyses across multiple cell lines which might reflect genomic signatures relevant to exposure of URT cells)	Not impactful	In vitro; indirect measure; no cell lines specific to URT
Kang et al. (2016)	Yeast (<i>Saccharomyces cerevisiae</i>), deletion strains 5 or 15 generations of exposure	Unspecified test article 0, 0.15, 0.3, 0.6 mM (Note: included due to conserved DNA repair pathways between yeast and humans, and potential relevance to human susceptibility)	Toxicogenomic profiling of pathways relating to RNA stability and DNA repair—including homologous recombination, single strand annealing, and post-replication repair	Not impactful	Non-mammalian; Unknown test article; high dose
Nazarpour-Noshadi et al. (2020)	Human immortalized lung epithelial cells Acute (24 hr; note: cytotoxicity up to 72 hr)	Unspecified test article 0, 25, 50, 100, 150, 200, and 300 µM In media	DNA damage (DNA ladder) and cytotoxicity/ apoptosis	Not impactful	Unknown test article; in vitro; endpoint well-established in vitro

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
Zhang et al. (2018a)	Human immortalized alveolar basal epithelial cells Acute (24 hr)	Freshly prepared formaldehyde solution 25 to 1,500 µM In media	DNA damage; chromosome damage; micronucleus frequency; cytotoxicity	Not impactful	In vitro; endpoint well-established in vitro
Zhang et al. (2020a)	Human immortalized bronchial epithelial cells Acute (3, 6, 12, 24 hr)	Formalin 0, 5, 10, 20, 40, 80 µM In media	DNA strand breaks; chromosome damage; DNA repair, ROS, and cell cycle markers	Not impactful	Formalin; in vitro; endpoint well-established in vitro
Zhang et al. (2020b)	Human Immortalized bronchial epithelial cells Acute (24 hr)	Formalin 0, 10, 40, 80 µM In media	DNA damage - comet assay; apoptosis; mitochondria-mediated apoptosis; reactive oxygen species levels	Not impactful	Formalin; in vitro; endpoint well-established in vitro
Modeling, Endogenous Formaldehyde, and Other Studies					
Campbell Jr et al. (2020)	Updated pharmacokinetic model developed here for formaldehyde dG adducts based on the previously developed models for formaldehyde DPX (Andersen et al., 2010); (Conolly et al., 2000).			Possibly impactful	Model potentially important to modeling dosimetry. (Note: discussed with regard to toxicokinetics, Section 3.1, and cancer dose-response, Section 5.2, not MOA analysis, Section 3.2.5)
Corley et al. (2015)	Excerpt from abstract: extended airway computational fluid dynamic (CFD) models of the rat and human were coupled with airway region-specific physiologically based pharmacokinetic (PBPK) tissue models to describe the kinetics of formaldehyde. Simulations of aldehyde no-observed-adverse-effect levels for nasal toxicity in the rat were conducted until breath-by-breath tissue concentration profiles reached steady state. Human oral breathing simulations were conducted using representative aldehyde yields from cigarette smoke.			Possibly impactful	Model potentially important to modeling dosimetry. (Note: discussed with regard to toxicokinetics, Section 3.1, and cancer dose-response, Section 5.2, not MOA analysis, Section 3.2.5)
Miller et al. (2017)	BBDR: Previously a computational fluid dynamics model was combined with a 2-stage clonal growth model to develop a MOA-based DR model. This paper reports changes that reflect a better understanding of populations of cells at risk of carcinogenic transformation in the pharynx, larynx and respiratory bronchiolar portions of the human respiratory tract and inclusion of basal cells in the pool of cells at risk.			Possibly impactful	Model potentially important to modeling dosimetry. (Note: discussed with regard to cancer dose-response, Section 5.2, not MOA analysis, Section 3.2.5)

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
Burgos-Barragan et al. (2017)	Mouse (C57BL/6 × 129SV hybrid background), WT or KO in ALDH2, FANCD2, or both (note: also included in vitro evaluations in human, chicken, and mouse cells)	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde detoxification processes in normal function)	Genotoxicity (DNA damage response markers) in vitro and in vivo (various tissues) when formaldehyde detoxification pathways are disrupted	Not impactful	No formaldehyde inhalation (note: included in the assessment as a reference study for discussion of potential sources of susceptibility relating to formaldehyde detoxification)
Starr and Swenberg (2016)	Update to prior non-primary research perspectives on how to calculate cancer risk			Not impactful	Non-primary research (note: discussed in the assessment given prior engagement on the topic and inclusion in other assessments)
Yang et al. (2020)	Excerpt from abstract: the deposition rates from the linear regressions of CH ₂ O, CH ₅ N, C ₂ H ₆ O, C ₂ H ₄ O ₂ , C ₃ H ₈ O, C ₆ H ₆ , C ₇ H ₈ , C ₈ H ₈ , and C ₈ H ₁₀ of 120 healthy volunteers were obtained with significantly different from the respective calculated deposition rates. In order to explore the effects of the breathing models and sampling time on the deposition rates of VOCs, volunteers were first asked to breathe successively with nasal-in-nasal-out, oral-in-nasal-out, and oral-in-oral-out breathing models before and after three meals for 3 d. In order to further validate the results, the deposition rates of the selected VOCs were calculated in 120 healthy volunteers using nasal-in-oral-out breathing model for unlimited time after the conventional lung function examination.			Not impactful	Not impactful to dosimetry modeling (note: briefly discussed in the assessment given use of human volunteers)
Yoo and Ito (2018a)	BBDR: PBPK-computational fluid dynamics hybrid analysis was integrated into the computer simulated person-based numerical simulation to estimate inhalation exposure and respiratory tissue dosimetry with the unsteady breathing cycle model.			Not impactful	Not impactful to dosimetry modeling (see below)
Yoo and Ito (2018b)	Excerpt from abstract: In this study, a CSP integrated with a virtual airway was developed and used to estimate inhalation exposure in an indoor environment. The virtual airway is a numerical respiratory tract model for CFD simulation that reproduces detailed geometry from the nasal/oral cavity to the bronchial tubes by way of the trachea. Physiologically based pharmacokinetic (PBPK)-CFD hybrid analysis is also integrated into the CSP. Through the coupled simulation of PBPK-CFD-CSP analysis, inhalation exposure under steady state conditions where formaldehyde was emitted from floor material was analyzed and respiratory tissue doses and their distributions of inhaled contaminants are discussed quantitatively.			Not impactful	Not impactful to dosimetry modeling [Note: these studies by Yoo and Ito (Yoo and Ito, 2018a, b), extended the (Corley et al., 2015) modeling by superposing on it the dynamics of formaldehyde

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
					exterior to the respiratory tract (i.e. within the room and surrounding the nose and mouth). Thus, they do not provide additional information unavailable in (Corley et al., 2015) of relevance to the assessment]

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

Abbreviations: MN = micronucleus (assay); ROS = reactive oxygen species; BBDR = biologically based dose-response (model).

^a Use of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

Mechanistic Studies for Lymphohematopoietic (LHP) Cancers, Focusing on Genotoxicity

Of the 25 studies meeting PECO criteria, 14 studies were deemed to be *possibly impactful* (see Table B-26). Studies relevant to pharmacokinetic modeling or dosimetry also were included. [Mundt et al. \(2017\)](#) was identified in the literature search update and included in the inventory table although it already had been included in the 2017 draft.

Table B-26. Mechanistic studies relating to lymphohematopoietic cancers, focusing on genotoxicity

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
Human Studies					
Aglan and Mansour (2018)	Occupational Cairo, Egypt Cross-sectional	Air sampling Adult hairstylists	PBL MN	Possibly impactful	Specific markers; exposure levels relevant to human cancer interpretations
Bassig et al. (2016)	Occupational Guangdong, China Cross-sectional,	Air sampling Adult formaldehyde factory workers	Frequency of monosomy 7 in isolated CFU-GM cells	Possibly impactful	Specific markers; exposure levels relevant to human cancer interpretations

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
Costa et al. (2015)	Occupational Northern and Central Portugal Cross-sectional	Air sampling Adult pathology workers	Chromosomal aberrations, comet assay, genotype analysis in blood cells	Possibly impactful	Specific markers; exposure levels relevant to human cancer interpretations
Costa et al. (2019)	Occupational Portugal Cross-sectional	Air sampling Adult anatomy-pathology laboratory workers	PBL MN and sister chromatid exchange; T-cell receptor mutations; genotype analysis of select polymorphisms	Possibly impactful	Specific markers; exposure levels relevant to human cancer interpretations
Mundt et al. (2017)	Occupational China Cross-sectional	Additional analysis of Zhang (2010) results Adult factory workers	Monosomy of chromosome 7 and 8, complete blood count	Possibly impactful	Already identified in 2017 draft
Petteff et al. (2015)	Occupational Rio Grande do Sul, Brazil Cross-sectional	Air sampling Adult furniture workers	Comet assay in PBLs [cell migration, frequency of damaged cells, damage index]	Possibly impactful	Markers of DNA damage; exposure levels relevant to human cancer interpretations
Wang et al. (2019)	Occupational Shanghai, China Cross-sectional	Air sampling Adult factory workers	Cytokinesis-blocked MN assay in PBLs	Possibly impactful	Specific markers; exposure levels relevant to human cancer interpretations
Zendehdel et al. (2017)	Occupational Tehran City, Iran Cross-sectional	Air sampling Adult melamine workers	Comet assay [tail moment, Olive moment in PBLs]	Possibly impactful	Markers of DNA damage; exposure levels relevant to human cancer interpretations
Barbosa et al. (2019)	Occupational Porto Alegre, Brazil Cross-sectional	Air sampling Adult beauty salon workers	Global DNA methylation (%) in PBLs	Not impactful	Note key endpoint, as not specific to genotoxicity
Zendehdel et al. (2018)	Occupational Tehran, Iran Cross-sectional	Air sampling Adult melamine workers	DNA damage (comet assay) in PBLs	Not impactful	Related to Zendehdel et al. (2017) , no additional results.
Animal Studies					

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
Leng et al. (2019)	Rat (Fischer 344), male Short-term (28 d; 6 hr/d)	Deuterated formaldehyde (no methanol) 0, 1, 30, 300 ppb (0, 1.23, 36.9, 369 µg/m ³) Inhalation	DNA adducts in blood, bone marrow (and other tissues)	Possibly impactful	Endpoints important to dosimetry; low exposure levels
Liu et al. (2017)	Mouse (ICR), male 20 wk (2 hr/d)	Unspecified test article 0, 1, 10 mg/m ³ Inhalation	Bone marrow cell MN; polychromatic erythrocytes (PCE)/normochromatic erythrocyte (NCE) ratio (immature/mature RBCs)	Possibly Impactful	Key endpoints of concern; longer duration study (note: presumed use of formalin limits interpretation)
Ma et al. (2020)	Mouse (Balb/c), male Subchronic (8 wk; 8 hr/d, 7 d/wk)	Formaldehyde in water (methanol free) 0, 2 mg/m ³ Inhalation	DNA damage (comet assay) in peripheral tissues (e.g., spleen; thymus); % of CD4+ T cells, CD8+ T cells, ratio of CD4+/CD8+ cells, and CD4 and CD8 cell phenotyping	Possibly impactful	Key endpoints of concern relevant to interpreting immune cell health and function
Aydemir et al. (2017)	Rat (Wistar albino), both sexes Subchronic (6 wk; 8 hr/d, 5 d/wk)	Formalin 0, 6 ppm (0, 7.38 mg/m ³) Inhalation (note: i.p. deemed not PECO relevant)	DNA damage (comet assay) and ROS markers in peripheral blood	Not impactful	Formalin; high level
Bernardini et al. (2020)	Mouse (Swiss), male Short-term (4 wk; 4 hr/d, 5 d/wk)	unspecified test article 0, 0.5, 1, 5, 10 ppm (0, 0.62, 1.23, 6.15, 12.3 mg/m ³) Inhalation	MN, comet assay, and global methylation in blood and bone marrow	Not impactful	Unknown test article
Edrissi et al. (2017)	Rat (F344), male Short-term (7, 14, 21, or 28 d; 6 hr/d)	[13C]-labeled formaldehyde 0, 2 ppm Inhalation	FA-lysine adducts in bone marrow and WBCs	Not impactful	Not key endpoint, adducts may or may not lead to more robust markers
Ge et al. (2020a)	Mouse (Balb/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 0.5, 3 mg/m ³ Inhalation	Myeloid progenitor cell (BFU-E and CFU-GM) colony counts and cytokines; bone marrow	Not impactful	Formalin; short-term (otherwise impactful endpoints)

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
			histology, ROS, and gene expression of cell cycle and DNA damage markers		
Wei et al. (2017b)	Mouse (BALB/c), male Short-term (2 wk; 8 hr/d, 5 d/wk)	Formalin 0, 3 mg/m ³ Inhalation	Bone marrow - myeloid progenitor formation assay, bone marrow cellularity	Not impactful	Formalin; short-term (otherwise impactful endpoints)
Wei et al. (2017a)	Mouse (BALB/c), male, Short-term (2 wk; 5 d/wk), followed by 7 d recovery	Formalin 0, 3 mg/m ³ Inhalation	Complete blood count, bone marrow histopathology, myeloid progenitor colony-forming cell assay, ROS and inflammatory markers, DNA-protein crosslinks	Not impactful	Formalin; short-term (otherwise impactful endpoints)
Zhao et al. (2020)	Mouse (Balb/c), male Short-term (2 wk; 8 hr/d, 5 d/wk) (note: ex vivo systemic tissues not PECO relevant)	Formalin 0, 3 mg/m ³	Formation of burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) cellular colonies in bone marrow and spleen	Not impactful	Formalin; short-term (otherwise impactful endpoints)
Modeling, Endogenous Formaldehyde, and Other Studies					
Burgos-Barragan et al. (2017)	Mouse (C57BL/6 × 129SV hybrid background), WT or KO in ALDH2, FANCD2, or both (note: also includes in vitro evaluations in human, chicken, and mouse cells)	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde detoxification in normal processes)	Colony Forming Units (CFU) from bone marrow stem cells and progenitor cells	Possibly impactful	No formaldehyde inhalation but included as a reference study for discussion of potential sources of susceptibility relating to formaldehyde detoxification; cell production from bone marrow is a key endpoint of concern
Dingler et al. (2020)	Mouse (C57BL/6 background), ALDH2 and ALDH5 WT, single, and double KO, both sexes (note: also includes primary cultures of	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde	Genotoxicity in peripheral blood cells and bone marrow (MN assay, SCE); bone marrow stem cell and progenitor cell quantification, lineage characterization, and B cell	Possibly impactful	No formaldehyde inhalation but included as a reference study for discussion of potential sources of susceptibility relating to formaldehyde

Supplemental Information—Formaldehyde (Inhalation)

Reference	Study design	Exposure ^a	Mechanistic endpoints	Impactful?	Rationale
	human PBLs, fibroblasts, and buccal cells not deemed PECO-relevant)	detoxification processes in normal function)	maturation; thymic development and cell lineage characterization; complete blood cell count, cell cycle profiling		detoxification; cell production from bone marrow is a key endpoint of concern
García-Calderón et al. (2018)	Mouse (C57BL/6 background), WT or KO in ALDH5 or FANCD2 (note: also includes in vitro evaluations not deemed PECO-relevant)	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde detoxification in normal processes)	Bone marrow HSPC lineage, function, and genotoxicity; complete blood cell count	Possibly impactful	No formaldehyde inhalation but included as a reference study for discussion of potential sources of susceptibility relating to formaldehyde detoxification; cell production from bone marrow is a key endpoint of concern
Nakamura et al. (2020)	Mouse (C57BL/6 background), ALDH2 and ALDH5 WT, single, and double KO, both sexes Observed GD0 to PND25	No formaldehyde inhalation exposures (note: included since it evaluates essentiality of formaldehyde detoxification processes in normal function)	Postnatal survival and gross organ observations (e.g., spleen, liver, lung thymus)	Not impactful	No formaldehyde inhalation and not key endpoint of concern (note: included as a reference study for discussion of potential sources of susceptibility relating to formaldehyde detoxification)
Starr and Swenberg (2016)	Update to prior non-primary research perspectives on how to calculate cancer risk			Not impactful	Non-primary research (note: discussed in the assessment given prior engagement on the topic and inclusion in other assessments)

Abbreviations: PBL = peripheral blood leukocytes; MN = micronucleus; WBC = white blood cell.

Rows for studies judged as “not impactful” are shaded gray; unshaded rows highlight studies incorporated into the assessment.

^a Use of methanol-stabilized formalin was inferred in some studies based on study-specific description (e.g., 37% stock solution).

B.3. STUDY EVALUATION

General approaches used in the identification and evaluation of individual studies are summarized in Section 2.3 of the Toxicological Review, with additional details outlined under each of the evaluated hazards. Because formaldehyde exposure-related issues were a significant concern in this assessment, a separate description of the considerations for judging exposure assessments in observational epidemiology studies is included in the Toxicological Review and documented as part of the health outcome-specific evaluations in the Appendix sections below. All experimental studies considered for use in hazard identification, including controlled exposure studies in both humans and animals, were separately evaluated to assess the quality of the inhalation exposure protocols (documented in Section B.3.1 below).

B.3.1. Exposure Quality Evaluations

Typical human exposures to formaldehyde can be complex and difficult to translate to experimental systems. Experimental exposure to formaldehyde by inhalation is typically achieved through volatilization of formalin or depolymerization of paraformaldehyde. Methanol, present in aqueous formaldehyde solutions to inhibit polymerization, is a potential confounder of associations between observed health outcomes and formaldehyde exposure via formalin. Thus, the test article used to generate the formaldehyde atmosphere in experimental studies was critically evaluated, including consideration of whether a methanol-only control group was used.² Based on the assumption that most labs used commercially available formalin for convenience, the test article is assumed to be formalin (and is documented as such) if the test article was not reported. Inhalation study uncertainties or limitations are shaded for easy recognition. A study's exposure quality may be upgraded if a study author provides key missing data. Each study was subjectively ranked as having **Good**, **Adequate**, or **Deficient** exposure characterization based upon the number and severity of the uncertainties and limitations identified, defined as follows:

- **Good Exposure Characterization:** There are no notable uncertainties or limitations regarding exposure methodology.

²While one study used a sprayer in a heated vessel to generate formaldehyde from a formalin solution containing a known concentration of methanol ([Kamata et al., 1997](#)), presumably resulting in the release of formaldehyde and methanol in proportions that would be conserved from liquid to gas (i.e., allowing air methanol levels to be relatively accurately estimated based on air formaldehyde levels), the remaining formalin studies generally evaporated formalin from solution. Notably, the liquid:air partitioning of methanol and formaldehyde is influenced by the proportions of these agents in aqueous solutions ([Albert et al., 2000](#)). Thus, as chamber methanol levels were not analytically measured in the other identified studies, a methanol control group may not eliminate uncertainty. Unfortunately, a calculation for estimating methanol levels released (e.g., by evaporation) from formalin solutions at different levels of inhaled formaldehyde was not identified.

Supplemental Information—Formaldehyde (Inhalation)

- **Adequate Exposure Characterization:** There are minor uncertainties or limitations regarding exposure methodology.
- **Deficient Exposure Characterization:** There are serious uncertainties or limitations regarding exposure methodology.

The details of the evaluation approach methods are provided in Section 2.3.1 of the Toxicological Review. Within the tables below documenting the results of the exposure quality evaluations, studies are arranged alphabetically within each ‘Good’, ‘Adequate’, or ‘Deficient’ rating grouping. The results of these evaluations are included as one domain evaluated for experimental studies in humans or animals to classify confidence (see Appendix B.3.2 to B.3.10 below).

Table B-27. Inhalation exposure quality: formaldehyde (Note: exposure deficiencies are shaded)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Good Exposure Characterization: there are no notable uncertainties or limitations regarding exposure methodology						
Adams et al. (1987) Mouse	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Ahmed et al. (2007) Mouse	Paraformaldehyde	NR	HPLC	Reported	NA	Dynamic whole-body
Albert et al. (1982) Rat See Sellakumar et al. (1985)	Paraformaldehyde	—	—	—	—	—
Andersen et al. (2010) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Appelman et al. (1988) Rat	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Babiuk et al. (1985) Rat	Paraformaldehyde (and 7 other aldehydes)	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Bach et al. (1990) Human [Exposure parameters are inferred from coauthor using same climate chamber in Anderson and Mølhave, Andersen and Mølhave (1983)]	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic “climate chamber”
Barrow (1983) Mouse and Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry and colorimetric method	Reported	NA	Dynamic head-only
Battelle (1981) See (Kerns et al., 1983)	Paraformaldehyde	—	—	—	—	—

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Berglund and Nordin (1992) Human	Freshly prepared formalin from paraformaldehyde (no methanol)	Evaporation	IR spectrophotometry; sodium bisulfite method; acetyl acetone method	Reported	NA	Dynamic olfactometer
Berglund et al. (2012) Human	Freshly prepared formalin from paraformaldehyde (no methanol)	Evaporation	IR spectrophotometry; acetyl acetone method	Reported	NA	Dynamic olfactometer
Casanova et al. (1994) Rat	Paraformaldehyde, [¹⁴ C]-paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Cassee et al. (1996b); Cassee et al. (1996a) Rat	Freshly prepared formalin from paraformaldehyde (no methanol) and/or acetaldehyde, acrolein	Evaporation	Formaldehyde analyzer	Reported	NA	Dynamic nose-only
Cassee and Feron (1994) Rat	Freshly prepared formalin from paraformaldehyde (no methanol). Exposures were to PFA only, ozone only, or to both chemicals	Evaporation	IR spectrophotometry	Reported	NA	Dynamic nose-only
Chang et al. (1981) Rat and mouse	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry and colorimetric method	Reported	NA	Dynamic head-only
Chang et al. (1983) Rat and mouse	Paraformaldehyde and [¹⁴ C]-paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body and head-only
<i>See also</i> Kerns et al. (1983); (Battelle, 1982)	Paraformaldehyde	—	—	—	NA	—
Coon et al. (1970) Rat, guinea pig, rabbit, dog, monkey	Freshly prepared formalin (paraformaldehyde added to hot distilled water; 1.35% solution)	Spray nozzle and evaporation of solution	IR analyzer equipped with a catalytic oxidizer	Reported	NA	Dynamic whole-body
Dalbey (1982) Hamster	Paraformaldehyde	Thermal depolymerization	Colorimetric analysis	Within 5% of target	NA	Dynamic whole-body

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Dallas et al. (1989) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Day et al. (1984) Human	UFFI off-gas products	Broken-up UFFI foam was dampened with water, then gases collected in 4500 L polyethylene balloons.	Chromotropic acid	Reported	NA	Dynamic whole-body
Dean et al. (1984) Mouse	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Dinsdale et al. (1993) Rat <i>Experiment 2</i> <i>(See also Experiment 1-Inadequate)</i>	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Feron et al. (1988) Rat	Paraformaldehyde	Thermal depolymerization	Colorimetric	Reported	NA	Dynamic whole-body
Fujimaki et al. (2004b) Mouse	Paraformaldehyde	NR	HPLC	Reported	NA	Dynamic whole-body
Green et al. (1987) Human	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Green et al. (1989) Human	Paraformaldehyde	Thermal depolymerization	Colorimetric monitor	Reported	NA	Dynamic whole-body
Groten et al. (1997) Rat	Paraformaldehyde alone or in combination with dichloromethane, aspirin, di(2-ethylhexyl)-phthalate, cadmium chloride, stannous chloride, butyl hydroxyanisol, loperamide, and spermine	Vaporization of freshly made formalin	Colorometric method	Reported (sampled in the animals' breathing zone)	NA	Dynamic whole-body

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Hayashi et al. (2004) Mouse	Paraformaldehyde	Thermal depolymerization	HPLC	Reported	NA	Dynamic whole-body
Holmstrom et al. (1989b) Rat	Paraformaldehyde with and without wood dust	Thermal depolymerization	Formaldehyde meter	Reported	NA	Dynamic whole-body
Jakab (1992) Mouse	Paraformaldehyde; exposure was to formaldehyde gas with or without carbon black aerosol	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Kamata et al. (1997) Rat	Formalin with 10% methanol A methanol control group was used	Sprayed into a bottle heated to 70°C	Acetylacetone	Reported for formaldehyde and methanol	NA	Dynamic nose-only
Swenberg et al. (1980a); Kerns et al. (1983); Battelle (1982) Rat and mouse	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Kulle et al. (1987) Human	Paraformaldehyde (reference provided)	Thermal depolymerization	Toxic gas monitor, chromotropic acid	Reported	NA	Dynamic whole-body
Kulle (1993) Human	Paraformaldehyde (reference provided)	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Kuper et al. (2011) Rat	Probably freshly prepared formalin (10.21% formaldehyde)	NR	IR spectrophotometry	Reported	NA	Dynamic whole-body
Larsen et al. (2013) Mouse	Polyacetal (a formaldehyde polymer) in permeation tubes	Permeation tube in a Kin-Tek gas standard generator	HPLC	Reported	NA	Dynamic head-only
Martin (1990) Rat	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Monteiro-Riviere and Popp (1986) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Monticello et al. (1991) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Monticello et al. (1996) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Monticello and Morgan (1997) Rat Based on Monticello et al. (1996)	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Morgan et al. (1986a) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	±5% of nominal	NA	Dynamic head-only
Morgan et al. (1986c) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic whole-body
Müller et al. (2012) Human	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor, HPLC	Reported	NA	Dynamic whole-body
Mueller et al. (2013) Human	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor HPLC	Reported	NA	Dynamic whole-body
Ozen et al. (2002) Rat	Paraformaldehyde	Thermal depolymerization	Gas chromatography and formaldehyde monitor	Reported	NA	Dynamic whole-body
Reuzel et al. (1990) Rat	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	Reported	NA	Dynamic whole-body
Riedel et al. (1996) Guinea pig	Formaldehyde gas	Pressurized bottles	Photometric	Reported (in animals' breathing zone)	NA	Dynamic whole-body
Roemer et al. (1993) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Within 10% of nominal	NA	Dynamic head-only

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Rusch et al. (1983) Rat, monkey, hamster	Freshly prepared formalin (unstabilized 5% solution with 0.03% methanol)	Air was bubbled through formalin	Chromotropic acid	Reported	NA	Dynamic whole-body
Saldiva et al. (1985) Rat	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Sauder et al. (1986) Human	Paraformaldehyde (reference provided)	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Sauder et al. (1987) Human	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Sellakumar et al. (1985) and Albert et al. (1982) Rat	Paraformaldehyde; exposure to formaldehyde and/or HCl. Co-exposure to formaldehyde and HCl forms bis(chloromethyl)-ether (BCME), a carcinogenic reaction product.	A slurry of PFA in paraffin oil (kerosene) was generated by thermal depolymerization. HCl was from a compressed gas tank.	PFA: Chromotropic acid HCl: titration with NaOH BCME: gas chromatography/mass spectrometry	Reported [NOTE: HCl is a powerful catalyst for the polymerization of formaldehyde into oligomers (Bevington and Norrish, 2012). Unlike formaldehyde gas, oligomer particles may be respirable]	NA	Dynamic whole-body
Sheppard et al. (1984) Human	Freshly prepared formalin from paraformaldehyde (methanol-free)	Air was bubbled through formalin	IR spectrophotometry	Reported	NA	Respiratory valve mouthpiece
Songur et al. (2003) Rat	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	Reported	NA	Dynamic whole-body
Songur et al. (2008) Rat	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	Reported	NA	Dynamic whole-body
Sorg et al. (2001a) Rat [Cited exposure parameters from Sorg et al. (1998)]	Paraformaldehyde	Thermal depolymerization	Photoacoustic multi-gas monitor	Reported	NA	Dynamic whole-body

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Swenberg et al. (1980b) See Kerns et al. (1983)	Paraformaldehyde	—	—	—	NA	—
Swiecichowski et al. (1993) Guinea pig	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Tobe et al. (1985b) ; Tobe et al. (1985a) [Study report] Rat	Formalin (w/10% methanol) A methanol control group was used	Sprayed into a heated glass bath	Acetylacetone	Reported for formaldehyde and methanol	NA	Dynamic whole-body
Tsukahara et al. (2006) Mouse	Paraformaldehyde	NR	HPLC	Reported	NA	Dynamic whole-body
Usanmaz et al. (2002) Mouse	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	Reported	NA	Dynamic Not described
Vosoughi et al. (2013) Mouse	Paraformaldehyde	Thermal depolymerization	Photoionization detector	Reported	NA	Dynamic
Wood and Coleman (1995) Mouse	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported. Animals were able to stop irritating formaldehyde exposure	NA	Dynamic whole-body
Woutersen et al. (1987) Rat	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Reported	NA	Dynamic whole-body
Woutersen et al. (1989) Rat	Paraformaldehyde	Thermal depolymerization	Colorimetric	Reported	NA	Dynamic whole-body
Zeller et al. (2011) Human	Paraformaldehyde	Thermal depolymerization	HPLC and formaldehyde monitor	Reported	NA	Dynamic whole body
Zitting et al. (1982) Rat	Polyacetal plastic (Delrin®)	Oxidative thermodegradation (250°C) to formaldehyde, formic acid, and acrolein	Visible absorption spectrometry (NIOSH, 1972)	Reported	NA	Dynamic whole-body

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Zwart et al. (1988) Rat	Paraformaldehyde	Thermal depolymerization (Woutersen et al., 1987)	Colorimetric	Reported	NA	Dynamic whole-body (reference provided)
Adequate Exposure Characterization: there are minor uncertainties or limitations regarding exposure methodology.						
Andersen (1979) ; also described in Andersen and Mølhave (1983) Human	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Within 20% of target	NA	Dynamic whole-body
Andersen et al. (2008) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry, HPLC	Reported (≈30% variation in atmospheres)	NA	Dynamic whole-body
Andersen and Molhave (1983) [book chapter] Human	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	Within 20% of target	NA	Dynamic “climate chamber”
Apfelbach and Weiler (1991) Rat	Paraformaldehyde	Thermal depolymerization	HPLC	NR	NA	NR Exposures in plexiglas holding cages
Aslan et al. (2006) Rat	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	NR “Desired concentrations were prepared”	NA	Dynamic whole-body
Bender et al. (1983) Human	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	NR	NA	Dynamic smog chamber with 7 sets of ports
Boja et al. (1985) Rat	Paraformaldehyde	Thermal depolymerization	Gas chromatography	NR	NA	Dynamic whole-body
Chang and Barrow (1984) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry and colorimetric method	NR	NA	Dynamic head-only

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Fujimaki et al. (2004b) Mouse [Exposure parameters in Fujimaki et al. (2004a)]	Paraformaldehyde	NR (Secondary source not found)	Formaldehyde monitor	NR	NA	Dynamic whole-body
Holmstrom et al. (1989a) Rat	Paraformaldehyde	Thermal depolymerization	NR	Reported	NA	Dynamic whole-body
Horton et al. (1963) Mouse	Paraformaldehyde	Thermal depolymerization	Method of Goldman and Yagoda (reference provided)	NR	NA	Dynamic whole-body
Ito et al. (1996) Rat	Formalin w/13% methanol A methanol control group was used	Formalin was placed in 50°C diffusion tubes	4-amino-3-hydrazino-5-mercapto-1,2,4-triazole method; analytical method for methanol NR	Reported NR for methanol	NA	Dynamic (not described)
Kulle and Cooper (1975) Rat	Paraformaldehyde	Thermal depolymerization	Chromotropic acid	NR	NA	Dynamic olfactometer
Lang et al. (2008) Human	Paraformaldehyde (and ethyl acetate as a masking agent)	Thermal depolymerization	Dinitrophenylhydrazine and HPLC analysis Formaldehyde monitor	Reported	NA	“Quasi static conditions”
Meng et al. (2010) Rat	Paraformaldehyde	Thermal depolymerization	IR Spectrophotometry	NR	NA	Dynamic (not described)
Moeller et al. (2011) Monkey	[¹³ CD ₂]-formaldehyde	NR	NR	Reported	NA	Dynamic whole-body
Monticello et al. (1989) Monkey	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	NR	NA	Dynamic whole-body
Morgan et al. (1984) Frog	Paraformaldehyde An ex vivo study of frog palates exposed to formaldehyde gas	Thermal depolymerization	IR spectrophotometry and colorimetric assay	Within 20% of nominal	NA	This is not an inhalation chamber study

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Nielsen et al. (1999) Mouse	Paraformaldehyde	Thermal depolymerization	NR	NR	NA	Dynamic whole-body
Morgan et al. (2017) Mouse	Paraformaldehyde	Thermal depolymerization	Formaldehyde meter	NR	NA	Dynamic whole-body
Ozen et al. (2003a) Rat	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	NR	NA	Dynamic whole-body
Ozen et al. (2003b) Rat	Paraformaldehyde	Thermal depolymerization	Gas chromatography and formaldehyde monitor	NR	NA	Dynamic whole-body
Ozen et al. (2005) Rat	Paraformaldehyde	Thermal depolymerization	Formaldehyde monitor	NR	NA	Dynamic whole-body
Sari et al. (2004) Mouse	Paraformaldehyde	NR (Secondary source not found)	“a chemical method” and Formtector XP-308	Reported	NA	Dynamic whole-body
Sari et al. (2005) Mouse Cited exposure parameters from Sari et al. (2004)	Paraformaldehyde (Mice were exposed intranasally to 500 ppm toluene/mouse 6 hr/d for 3 da prior to formaldehyde exposure)	NR (Secondary source not found)	“measured chemically” and Formtector XP-308	Reported	NA	Dynamic whole-body
Sari et al. (2005) Mouse	Paraformaldehyde	NR (Secondary source not found)	“measured chemically” and Formtector XP-308	Reported	NA	Dynamic whole-body
Sarsilmaz et al. (1999) Rat	Paraformaldehyde	Thermal depolymerization (reference provided)	Formaldehyde monitor	NR	NA	Dynamic whole-body
Sarsilmaz et al. (2007) Rat [Assumed to be the same cohort as Aslan et al. (2006)]	Paraformaldehyde	Thermal depolymerization (reference provided)	Formaldehyde monitor	NR “Desired concentrations were prepared”	NA	Dynamic “prism-shaped glass covers”

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Schachter et al. (1986a) Human	Paraformaldehyde (apparent co-exposure to 2-propanol)	Thermal depolymerization over boiling 2-propanol	Chromotropic acid	Reported	NA	Dynamic whole-body
Schachter et al. (1987) Human	Paraformaldehyde (apparent co-exposure to 2-propanol)	Thermal depolymerization over boiling 2-propanol	Chromotropic acid	Reported	NA	Dynamic whole-body
Sorg et al. (1998) Rat	Paraformaldehyde	Thermal depolymerization	HPLC	Reported 44% decline in concentration over the course of the experiment	NA	Dynamic whole-body
Sorg et al. (2001b) Rat <i>Experiment 2 and 3</i> <i>(See also Experiment 1-Inadequate)</i>	Paraformaldehyde	Thermal depolymerization	HPLC (Sorg et al., 1998)	NR	NA	Dynamic whole-body
Sorg et al. (2004) Rat	Paraformaldehyde with co-exposure to orange oil (a known irritant)	Thermal depolymerization	Photoacoustic multi-gas monitor	Reported	NA	NR
Sorg and Hochstatter (1999) Rat <i>Experiment 2</i> <i>(See also Experiment 1-Inadequate)</i>	Paraformaldehyde	Thermal depolymerization	HPLC (Sorg et al., 1998)	NR	NA	Dynamic whole-body
Wilmer et al. (1987) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	NR	NA	Dynamic whole-body
Wilmer et al. (1989) Rat	Paraformaldehyde	Thermal depolymerization	IR spectrophotometry	NR	NA	Dynamic Whole-body
Witek et al. (1986) Human	Paraformaldehyde (apparent co-exposure to 2-propanol)	Thermal depolymerization over boiling 2-propanol (82.5°C)	Chromotropic acid	Reported	NA	Dynamic whole-body

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Witek et al. (1987) Human	Paraformaldehyde (apparent co-exposure to 2-propanol)	Thermal depolymerization over boiling 2-propanol (82.5°C)	Chromotropic acid	Reported	NA	Dynamic whole-body
Deficient Exposure Characterization: there are serious uncertainties or limitations regarding exposure methodology.						
Al-Saraj (2009) Rabbit	10% Formalin No methanol control [Pretreatment with Ivermectin which can cause cleft palate and clubbed forelimbs in rabbits]	Evaporation	Colorimetric method (based on a reference) Methanol not measured	Reported (12 ppm)	NA	Dynamic whole-body
Amdur (1960) Guinea pig	Formalin (37%)	Sintered glass bubbler	Colorimetric method and chromotropic acid	Reported	NaCl particles measured	Dynamic whole-body
Arican et al. (2009) Rat	Paraformaldehyde	Thermal depolymerization	NR	NR	NA	Dynamic whole-body
Bansal et al. (2011) Rabbit	10% Formalin 40% Formalin No methanol control	Evaporation from open containers	NR	NR Target and nominal concentrations also NR	NA	Open containers of formalin were placed below cages
Biagini et al. (1989) Monkey	Formalin w/10-15% methanol No methanol control [Anesthesia with ketamine and xylazine, which cause bronchodilation, could affect pulmonary function measurements.]	Injected into a GC injector and heated to 220-230°C	Formaldehyde monitor Methanol not measured	Reported	NA	Dynamic whole-body
Bian et al. (2012) Rat	Formalin No methanol control	Evaporation	Formaldehyde meter Methanol not measured	10.0 ± 1.0 mL/m ³	NA	Dynamic whole-body
Bhalla et al. (1991) Rat	Paraformaldehyde	Thermal depolymerization	NR	NR	NA	Dynamic nose-only

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Bokina et al. (1976) Rabbit	NR No methanol control	NR	NR	NR	NA	NR
Buckley et al. (1984) Mouse	Formalin (co-exposure to methanol) No methanol control	NR	IR spectrophotometry Methanol not measured	Reported	NA	Dynamic whole-body
Casset et al. (2006) Human	Formalin (35% aqueous medicinal solution of formaldehyde; co-exposure to methanol) No methanol control	Evaporated from a Pyrex boiler at 85°C	HPLC Methanol not measured	<10% of target	NA	Dynamic whole-body with subjects wearing masks
Chonglei et al. (2012) Mouse	Mice were simultaneously exposed to formaldehyde, benzene, toluene, and xylene vapors. The test article for formaldehyde was NR	NR	Digital electrochemical analyzer and gas chromatography	NR	NA	Dynamic whole-body (airflow not reported)
Cometto-Muñiz and Hernández (1990) Human	NR No methanol control	NR	Chromotropic acid	Reported	NA	Dynamic olfactometer
Day et al. (1984) Human	Solution of formalin in methanol. No methanol control	Atomized and then evaporated on a hot plate.	Chromotropic acid Methanol not measured	Reported	NA	Dynamic whole-body
de Ceaurriz et al. (1981) Mouse	NR No methanol control	NR	Colorimetric method Methanol not measured	NR	NA	Dynamic whole-body
Dinsdale et al. (1993) Rat <i>Experiment 1</i> <i>(See also Experiment 2 - Good)</i>	Formalin (co-exposure to methanol) No methanol control	Jet atomizer (Exp 1)	IR spectrophotometry Methanol not measured	Reported	NA	Dynamic whole-body
Ezratty et al. (2007) Human	Formalin (co-exposure to methanol) No methanol control	Thermal depolymerization	Semiconductor gas sensor Methanol not measured	NR	NA	Dynamic whole-body

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Falk et al. (1994) Human	Formalin (co-exposure to methanol) No methanol control.	Evaporation from a heated glass surface	Liquid chromatography	Reported for treated and negative control groups	NA	Dynamic Whole-body
Gieroba et al. (1994) Rabbit	38% Formalin No methanol control	Evaporation	None	NR	NA	A tube delivered FA vapor to rabbits' nares
Gofmekler (1968) Rat	NR No methanol control	NR	NR Methanol not measured	NR	NA	NR
Gofmekler and Bonashevskaya (1969) Rat	NR No methanol control	NR	NR Methanol not measured	NR	NA	NR
Golalipour et al. (2007) Rat	NR but exposure would have been to formalin with co-exposure to methanol No methanol control	NR, but formaldehyde and methanol would have off-gassed from necropsy tubs of formalin	Formaldehyde Draeger tubes Methanol not measured	Reported	NA	Not a chamber study; rats exposed in dissection room
Guseva (1973b) Rat	NR No methanol control	NR Rats were simultaneously exposed by inhalation and drinking water	Fuchsin sulfurous acid method Methanol not measured	NR	NA	Dynamic (not described)
Han et al. (2015) Rat	NR No methanol control	NR	NR Methanol not measured	NR	NA	Static
Harving et al. (1990) Human	Alkaline solution of formalin; co-exposure to methanol No methanol control	Thermal depolymerization	Acetylacetone Methanol not measured	Reported	NA	Dynamic whole-body
Silva Ibrahim et al. (2015) Rat	Formalin (purity NR) A vehicle control group was exposed to water No methanol control	Ultrasonic nebulizer	NR	NR	0.5-1 µm MMAD NR	Dynamic whole-body

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Ionescu et al. (1978) Rabbit	NR (probably aerosolized formalin) No methanol control	NR	NR Methanol not measured	NR (target and nominal concentrations also NR)	NA	Static
Jaeger and Gearhart (1982) Mouse and Rat	Formalin No methanol control	Aerosolization and evaporation	IR spectrophotometry and colorimetric method Methanol not measured	Reported	NA	Dynamic whole-body (Mason jar)
Kamata et al. (1996b) Rat	Formalin (with 10% methanol) No methanol control	Sprayed into a bottle heated to 70°C	Acetylacetone Methanol not measured	Reported	NA	Dynamic whole-body
Kamata et al. (1996a) Rat	Formalin with 10% methanol No methanol control	Sprayed into a bottle heated to 70°C	Acetylacetone Methanol not measured	Reported	NA	Dynamic nose-only
Kane and Alarie (1977) Mouse	Formalin No methanol control	Evaporation	Colorimetric method Methanol not measured	Reported	NA	Dynamic head-only
Katsnelson et al. (2013) Rat	NR No methanol control	NR	NR Methanol not measured	Reported	NA	Dynamic whole-body
Kimura et al. (2010) Rat	37% Formalin with 15% methanol No methanol control	Dynamic gas generator (evaporation)	4-amino-3-hydrazino-5-mercapto-1,2,4-triazole method Methanol not measured	NR	NA	Dynamic whole-body
Kim et al. (2013b) Mouse	NR No methanol control	NR	HPLC	NR	NA	NR
Kitaev et al. (1984) Rat	NR No methanol control	NR	Gravimetric (not described) Methanol not measured	NR	NA	Dynamic (not described)
Krakowiak et al. (1998) Human	10% Formalin No methanol control	Evaporation	Chromotropic acid Methanol not measured	Reported	NA	Dynamic whole-body
Kum et al. (2007a); Kum et al. (2007b) Rat	Formalin No methanol control	NR	Gas detection pump (reference provided) Methanol not measured	NR	NA	Dynamic whole-body

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Lee et al. (1984) Guinea pig	4% Formalin w/1% methanol 37% formalin w/10% methanol No methanol control	Aerosol generated by a nebulizer	Formaldehyde: chromotropic acid Methanol: IR spectrophotometry	NR for formaldehyde or methanol	NR	Dynamic whole-body
Liao et al. (2010) Rat	Formalin No methanol control	NR	Formaldehyde meter Methanol not measured	NR	NA	Static
Lino dos Santos Franco et al. (2006) Rat	Formalin (diluted to 1%; with 0.32% methanol) A methanol control group was used.	Ultrasonic nebulizer	NR for formaldehyde or methanol	NR for formaldehyde or methanol (nominal concentration NR)	NR	Dynamic whole-body
Lino dos Santos Franco et al. (2009) Rat	Formalin No methanol control	Ultrasonic nebulizer	NR	NR Methanol not measured	NR	Dynamic (probably whole-body)
Lino-Dos-Santos-Franco et al. (2011b) Rat	Formalin (diluted to 1%; with 0.32% methanol) No methanol control	Ultrasonic nebulizer	NR	NR Methanol not measured	NR	NR
Liu et al. (2009a) Rat	Formalin (37%) No methanol control	Evaporation from the inner walls of the static chamber	Formaldehyde monitor	Reported	NA	Static
Liu et al. (2010) Rat	Formalin (37%) No methanol control	Evaporation from the inner walls of the static chamber	Formaldehyde monitor	Reported	NA	Static
LICM (2006) Mouse	Wood baseboard (not described); co-exposure to unidentified chemicals	NR	NR	NR	NA	Dynamic Not described
Maiellaro et al. (2014) Rat	Formalin (source and purity NR) The vehicle control was exposed to water	Ultrasonic nebulizer	NR Methanol not measured	NR Note: one exposure level tested	Reported	Dynamic

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Malek et al. (2003c) Malek et al. (2003a) Malek et al. (2003b) Rat	Formalin No methanol control	Evaporation from a dish in the chamber	Formaldehyde Draeger tubes Methanol not measured	Reported	NA	Static with holes
Malek et al. (2004) Mouse	Formalin No methanol control	Evaporation from a dish in the chamber	Formaldehyde Draeger tubes Methanol not measured	Reported	NA	Static with holes
Maronpot et al. (1986) Mouse	Formalin (9.2%w/v) No methanol control	Nebulization and evaporation	Chromotropic acid	Reported	NA	Dynamic whole-body
Matsuoka et al. (2010) Mouse	Formalin No methanol control	Evaporation	Cosmos® smell sensor	NR	NA	Dynamic whole-body
Monfared (2012) Mouse	NR No methanol control	NR	NR	NR	NA	Dynamic whole-body
Morgan (1983) Rat	Paraformaldehyde (reference provided)	Thermal depolymerization	NR	NR	NA	Dynamic whole-body
Nalivaiko et al. (2003) Rabbit	Paraformaldehyde	Thermal depolymerization	None	NR	NA	A tube delivered formaldehyde vapor to rabbits' nares
Ohtsuka et al. (1997) Rat	NR No methanol control	Aerosol generated by an atomizer	NR Methanol not measured	NR	NR	Dynamic whole-body "test room"
Ohtsuka et al. (2003) Rat	1% Formalin No methanol control	Aerosol generated by an atomizer	NR Methanol not measured	NR	NR	Dynamic whole-body "test room"
Pazdrak et al. (1993) Human	NR No methanol control	NR	IR spectrophotometry	Reported	NA	Dynamic whole-body
Pitten et al. (2000) Rat	Formalin No methanol control	Evaporation from a dish in the chamber	Acetylacetone method and photometric evaluation Methanol not measured	Reported	NA	Static
Pross et al. (1987) Human	Formalin No methanol control	Evaporation of formalin aerosol	Formalin: chromotropic acid Methanol not measured	NR	NA	Dynamic whole-body

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Pross et al. (1987) Human	Milled UFFI particles (4 µm) contaminated with heavy microbial growth	UFFI aerosol generation not described	UFFI aerosol: gravimetric filters and an aerodynamic particle sizer	NR	NA	Dynamic whole-body
Pross et al. (1987) Human	UFFI off-gas products.	UFFI off-gas generated by passing air through beds of fractured UFFI wetted with water	NR	NR	NA	Dynamic whole-body
Pushkina et al. (1968) Rat	NR No methanol control	NR	NR Methanol not measured	NR	NA	NR
Sadakane et al. (2002) Mouse	Formalin (0.5% solution in saline No methanol control	Aerosol generated by an ultrasonic nebulizer	NR Methanol not measured	NR	NR	NR
Saillenfait et al. (1989) Rat	Formalin w/10% methanol No methanol control	Air was bubbled through formalin	IR spectrophotometry Methanol not measured	Reported	NA	Dynamic
Sandikci et al. (2007b) Rat	NR No methanol control	NR	NR (reference provided) Methanol not measured	NR	NA	Dynamic whole-body
Sandikci et al. (2009) Rat	NR No methanol control	NR	Formaldehyde Draeger tubes	NR	NA	Dynamic whole-body
Sanotskii et al. (1976) Rat	NR No methanol control	NR	Colorimetry (not described) Methanol not measured	NR	NA	Dynamic (not described)
Schreiber et al. (1979) Hamster	NR No methanol control	NR	NR	NR	NA	NR
Schuck et al. (1966) Human	Formaldehyde and other photooxidation products	Formaldehyde was generated during propylene photooxidation and ethylene photooxidations in a reaction chamber exposed to high intensity UV light (3,000 Å)	Chromotropic acid	Mean concentrations provided in a graph	NA	Reaction chamber with welding masks attached for eye exposure

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Senichenkova (1991b) Rat	NR No methanol control	NR	Gravimetric (not described) Methanol not measured	NR	NA	Dynamic (not described)
Senichenkova and Chebotar (1996b) Rat	NR No methanol control	NR	Gravimetric (not described) Methanol not measured	NR	NA	Dynamic (not described)
Sheveleva (1971) Rat	NR No methanol control	NR	NR (reference provided); Methanol not measured	Reported	NA	Dynamic whole-body
Sorg et al. (1996) Rat	Formalin No methanol control	Air was bubbled through formalin	NR Methanol not measured	Reported	NA	Dynamic whole-body
Sorg et al. (2001b) Rat <i>Experiment 1</i> <i>(See also Experiments 2 and 3- Adequate)</i>	Formalin No methanol control	Evaporation of formalin	NR Methanol not measured	NR	NA	Dynamic whole-body
(Sorg et al., 2002) Rat	Formalin No methanol control	Evaporation	None	NR	NA	Cotton swabs containing various formalin dilutions were placed in a maze
Sorg and Hochstatter (1999) Rat <i>Experiment 1</i> <i>(See also Experiment 2- Adequate)</i>	Formalin No methanol control	Air was bubbled through formalin	NR	NR	NA	Dynamic whole-body
Speit et al. (2011b) Rat	Formalin No methanol control	Evaporation	NR Methanol not measured	Reported	NA	Dynamic whole-body
Swenberg et al. (1983b) [book chapter] Rat and Mouse	[¹⁴ C]- formaldehyde	NR	NR	NR	NA	NR

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Swenberg et al. (1986) [book chapter] Rat and Mouse	NR No methanol control	NR	NR	NR	NA	NR
Tani et al. (1986) Rabbit	37% Formalin No methanol control	Evaporation	4-amino-3-hydrazino-5-mercapto-1,2,4-triazole method Methanol not measured	NR	NA	Direct exposure to the upper and lower respiratory tract via two T-tubes
Tepper et al. (1995) Mouse	Carpet containing volatile organic compounds, pesticide residues, and microbiological flora	Heating of carpet	Gas chromatography High resolution mass spectrometry	Reported for formaldehyde and 9 other specific organic chemicals	NR	Dynamic head-only
Tarkowski and Gorski (1995) Mouse	NR No methanol control	NR	NR Methanol not measured	NR	NA	NR
Wang et al. (2012) Rat	NR No methanol control	NR	NR Methanol not measured	NR	NA	Static (not otherwise described)
Weber-Tschopp et al. (1977) Human	Formalin (35%) No methanol control	A syringe delivered formalin to a heated (120°C) Pyrex glass tube	Chromotropic acid Methanol not measured	Reported	NA	Dynamic whole-body
Xing Sy (2007) Mouse	NR No methanol control	NR	NR	NR	NA	NR
Yang et al. (2001) Human	Plywood (5 layers) which off-gassed formaldehyde and traces of C ₆ –C ₁₁ aldehydes.	The plywood was cut into 50- × 10-cm planks and placed in a small chamber to facilitate off-gassing.	Formaldehyde monitor	Reported for formaldehyde, but location of measures NR; concentrations of other gases NR	NA	Eyes were exposed via modified swim goggles
Yorgancilar et al. (2012) Rat	NR No methanol control	NR	NR	NR	--	NR

Supplemental Information—Formaldehyde (Inhalation)

Study/species	Test article characterization and controls	Generation method	Analytical method	Analytical concentrations	Particle size	Chamber description
Yu and Blessing (1997) Rabbit	38% Formalin No methanol control	Evaporation	None	NR	NA	A tube delivered formaldehyde vapor to rabbits' nares
Yu and Blessing (1999) Rabbit	NR No methanol control	NR	None	NR	NA	formaldehyde vapor puffed in front of the rabbits's nares
Zhang et al. (2013) Mouse	Formalin (10%) No methanol control	NR	NR	NR	NA	Dynamic nose-only
Zhang et al. (2014b) Rat	Formalin No methanol control	Evaporation	NR	Reported but questionable	NA	Static
Zhou et al. (2006) Rat	NR No methanol control	NR	Formtector Methanol not measured	NR	NA	NR
Zhou et al. (2011a) Rat	NR No methanol control	NR	NR Methanol not measured	NR	NA	Static
Zhou et al. (2011b) Rat	NR No methanol control	NR	NR Methanol not measured	NR	NA	Static

HPLC – high performance liquid chromatography; IR – infrared; MMAD (σ_g) – mass median aerodynamic diameter (geometric standard deviation); NA – Not applicable; NR – not reported; PFA – paraformaldehyde.

B.3.2. Sensory Irritation

The details of the study evaluation approach methods are provided in Section 2.3.2 of the Toxicological Review. Within Tables B-28 (residential studies), B-29 (school-based studies) B-30 (controlled exposure studies), B-31 (anatomy course studies), and B-32 (occupational studies) below documenting the results of the study evaluations for sensory irritation, studies are arranged alphabetically. Gray shading is indicative of aspects of the study design or analysis that decreased EPA's confidence rating.

Residential Studies

Table B-28. Evaluation of studies examining sensory irritation in humans: residential studies

Reference, setting and design	Consideration of participant selection and compar-ability	Exposure measure and range	Outcome measure	Consideration of likely con-founding	Analysis and completeness of results	Size	Confidence
Bracken et al. (1985) (Ontario) Residential (prevalence)	Exposed homes randomly selected from a group currently being monitored for formaldehyde and previously at homeowner request. Participant selection likely based on exposure and outcome status.	Area samples; average of 3 hr samples; 5 per home. UFFI Mean 0.07, max 0.13 mg/m ³ ; non-UFFI Mean 0.06, max 0.12 mg/m ³ ; Lab Mean 0.15, max 7.2 mg/m ³ . Details of sampling protocol not provided. Most samples may have been below LOD (NIOSH, 1977, chromotropic)	Self-report, ATS question-naire. Response was not blinded to presence of UFFI.	Exposed: Homes with UFFI, Referent: non-UFFI homes from university community; age and smoking prevalence similar.	Symptom prevalence estimated from graphs in Figures 1 and 2 in publication. Compared prevalence by exposure group, t-test	N = 54 exposed; N = 26 referent	Not Informative Participant selection likely based on exposure and outcome status; Small exposure contrast (formaldehyde concentration similar in comparison groups); potential for exposure status to affect outcome classification
Dally et al. (1981) (Wisconsin) Residential (prevalence)	Survey of homes reported to State Division of Health because of symptoms;	Area samples; average of 30–60 min samples in multiple locations. LOD 0.12 mg/m ³ Mobile homes,	Self-report, questionnaire. Responses blind to formaldehyde measurements.	No comparison group; smoking status was not associated with formaldehyde	Symptom prevalence among exposed	N = 256	Not Informative No comparison group; Participant selection potentially

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and compar-ability	Exposure measure and range	Outcome measure	Consideration of likely con-founding	Analysis and completeness of results	Size	Confidence
	Participant selection potentially based on exposure and outcome status.	Median 0.58, range <0.12 to 4.53 mg/m ³ . Conventional, Median 0.12, range <0.12 to 1.34 mg/m ³ .		concentration; no adjusted results provided			based on exposure and outcome status, limited statistical analyses
Hanrahan et al. (1984) (Wisconsin) Residential (prevalence)	Recruited from a randomly selected list of mobile homes in Wisconsin; response rate 31%. Formaldehyde concentrations, age, and gender were comparable to a subsample of nonrespondents who did not return the health questionnaire, and participants blinded to formaldehyde concentration. Differential participation in the study based on symptom severity is unknown but cannot be ruled out.	Area samples; average of 1-hr samples from 2 rooms. Median 0.2 mg/m ³ , range <0.12 to 0.98 mg/m ³ ; Adequate contrast. Details of sampling provided: closed residence with no point formaldehyde emissions; sampling and analytic protocols referenced (NIOSH chromotropic acid method); Quality control conducted; lowest measurement was 0.12 mg/m ³ (LOD was not stated, but even lower concentrations were measured in outdoor air. Temperature and relative humidity were recorded.	Self-report, questionnaire, no description. Response blind to formaldehyde measurements. Symptoms ascertainment period (since moving into homes) did not correspond to exposure measurement period.	Logistic regression adjusting for age, gender, and smoking status. Gas appliances were turned off and smoking was not permitted during the sampling period.	Logistic regression, provided graph of predicted mean prevalence normalized to mean age, and upper and lower 95% CI by concentration from regression model. QC analysis showed no sample contamination. Smoking was found to not increase exposure concentration.	N = 61	Medium Low participation rate, but exposure and demographic characteristics were comparable among respondents and nonrespondents to the health questionnaire. Differential participation in the study based on symptom severity is unknown but cannot be ruled out. Uncertainty regarding correspondence of one hour exposure measurement with period for symptom ascertainment (years); Questionnaire not described but outcomes reported without knowledge of formaldehyde levels.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and compar-ability	Exposure measure and range	Outcome measure	Consideration of likely con-founding	Analysis and completeness of results	Size	Confidence
Liu et al. (1991) (California) Residential (prevalence)	Recruited from a randomly selected, age-stratified list of mobile homes in California; response rate 44%. Uncertainty regarding differential or nondifferential selection based on knowledge of exposure or symptoms.	Area samples using passive monitors; 7-d average in 2 rooms in 2 seasons. Mean summer 0.089 ppm, winter 0.088 ppm; TWA concentration estimated using average concentration multiplied by # hours spent in the home per day during the week of sampling. Validity study (Sexton et al., 1986) reported LOD of 0.01 ± 0.30 ppm; range, LOD - 0.57 mg/m ³	Self-report, mailed questionnaire, no description. Responses blind to formaldehyde measurements. Appropriate time frame relative to exposure measurements.	Logistic regression adjusting for age, gender, smoking status, status of chronic respiratory disease/allergy.	Logistic regression, beta coefficients for change in symptom prevalence per concentration change were not provided. Prevalence estimated from graph of prevalence by category of formaldehyde TWA exposure in publication.	N = 836 homes, 1,096–1,394 individuals	Medium Questionnaire not described but outcomes reported without knowledge of formaldehyde levels. Low participation rate but uncertain whether possible selection bias, if any, was nondifferential or differential.
Lovreglio et al. (2009) (prevalence)	Selection of 59 homes in city. Uncertainty regarding participant recruitment process or participation rate.	24 hr samples in kitchen in 59 homes; reported mean, median, range.	Self-report, questionnaire (onset of symptoms while in kitchen). No description of questionnaire but responses likely not related to exposure status.	Formaldehyde and acetaldehyde concentrations were correlated ($p = 0.001$). Formaldehyde concentrations varied by smoking status. Data analyses not described, no adjustment or stratification.	No data provided, qualitative results only.	N = 182 subjects living in 59 homes	Not Informative Results of data analysis were not provided; confounding by smoking or co-exposure was not addressed. Questionnaire was not described but responses likely not related to exposure status. Uncertainty regarding participant recruitment process

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and compar-ability	Exposure measure and range	Outcome measure	Consideration of likely con-founding	Analysis and completeness of results	Size	Confidence
							or participation rate.
Main and Hogan (1983) (prevalence)	21 exposed individuals working in 2 mobile trailers for 34 mo. 18 referent staff members who did not work in the trailers. Uncertainty regarding participant recruitment process or participation rate.	Three 1-hr area samples using impingers taken on 4 occasions (August, September, December, April) always on a Monday. At least 1 sample was taken from each office in both trailers. Concentration range: 0.15–1.97 mg/m ³ . Details of sampling provided: closed residence with no point formaldehyde emissions; sampling and analytic protocols referenced; referent group assumed to have no exposure.	Self-report, ATS questionnaire, symptom history at work. Responses among exposed likely not blinded to exposure status.	Groups were comparable for smoking history, race, and SES. Exposed were older (mean age 38) with more men (76%) compared to referents (mean age 30, 50% males). Potential dissimilarity of administrative employees and police officers (healthier); direction of bias possibly away from null; more exposure to ETS among referent; possible direction toward null.	Symptom prevalence at work compared between exposed and referent, chi-square; small sample size.	Exposed N = 21, Referent N = 18	Low Potential dissimilarity between comparison groups; more exposure to ETS among referent; small sample size with increased potential for unreliable results. Uncertainty regarding participant recruitment process or participation rate. Responses among exposed likely not blinded to exposure status.
Norsted et al. (1985) (Texas) Residential (prevalence)	Homes selected on request of residents; Potential selection based on exposure and outcome status.	Sampling protocols not described	Self-report; symptom reports not blind to exposure status	No comparison group; no adjusted results provided	Total # participants in homes unknown.	N = 443 mobile homes	Not Informative Potential selection based on exposure and outcome status.; Reporting deficiencies, no comparisons

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and compar-ability	Exposure measure and range	Outcome measure	Consideration of likely con-founding	Analysis and completeness of results	Size	Confidence
Olsen and Dossing (1982) (Denmark) Day care center workers in mobile homes (prevalence)	Recruited from all newly built mobile day care centers in 2 boroughs (n = 7) and 3 referent centers selected at random; response rates 94% exposed, 76% referent. Responses similar in exposed and referent to 3 questions not expected to be related to formaldehyde.	Area samples; average of 2-hr samples in 2–4 locations, on 1 occasion. Exposed mean 0.43, range 0.24 to 0.55 mg/m ³ ; referent mean 0.08, range 0.05 to 0.11 mg/m ³ ; Details of sampling provided: closed residence with no point formaldehyde emissions; sampling and analytic protocols referenced	Self-report, questionnaire; linear analogue scale for severity, experience within one month; questionnaire described, and citation provided. Less certainty regarding characterization of average formaldehyde concentrations corresponding to outcome measurements in last month.	Referent selected from stationary child-care facilities in same residential area. Age and smoking prevalence similar in exposed and referent.	Prevalence and severity presented in graphs; comparisons between exposed and referent groups.	Exposed N = 66; Referent N = 26	Medium Some uncertainties regarding temporal concordance of exposure (< 1 d) and symptom assessments (within last month), but not expected to be substantial. Sample size in referent group small.
Ritchie and Lehnen (1987) ;(Minnesota) Residential (prevalence)	Selection into survey at request of family physician; potential selection based on exposure and outcome status.	Area samples; average of 30-min samples in 2 rooms. Bedroom mean: Mobile homes 0.43 mg/m ³ , Conventional 0.15 mg/m ³ , range 0.012 (LOD) to 6.79 mg/m ³ . Details of sampling provided: closed residence with no point formaldehyde emissions; sampling & analytic protocols referenced see also	Self-report, interview; symptoms same day as exposure measurements, no description of questionnaire, but respondents did not know the formaldehyde measurement for their homes	Prevalence stratified by age, gender, and smoking status.	Presented graphs of prevalence by exposure (3 categories); tables of prevalence (SE) by type of home, exposure category, and smoking status	N = 2,000 residents; 891 homes	Low (↑) Potential selection based on exposure and outcome status. No description of questionnaire, but respondents did not know the formaldehyde measurement for their home

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and compar-ability	Exposure measure and range	Outcome measure	Consideration of likely con-founding	Analysis and completeness of results	Size	Confidence
		Ritchie and Lehnen (1985)					
Salonen et al. (2009) (Finland) (prevalence)	Building selected because of complaints and symptom reports of occupants; potential selection based on exposure and outcome status	Area sampling in 20 of 176 buildings selected from database of Finnish Institute of Occupational Health, 2001–2006, N = 1–12 per building; during work hours 9–4 pm for 1–2 hrs. LOD 0.5 ppb Mean 0.011 mg/m ³ ; Max 0.044 mg/m ³ .	Self-report, standardized questionnaire; Indoor Air Questionnaire (citation provided). Responses solicited in reference to identified air quality problems.	No comparison buildings evaluated. Compared concentrations to recommended indoor limit (RIL)	Presented ratio of average concentration divided by recommended indoor limit (based on RD50 for respiration rate in mouse bioassay and adjustment to 24 hrs based on Haber's Law.	N = 20 buildings	Not Informative Potential selection based on exposure and outcome status; outcome responses were not blinded; no comparison group
Thun et al. (1982) (prevalence)	No information to evaluate	No formaldehyde measurements	Self-report, no description of questionnaire; new symptoms over a 1 year period.	Exposed: Homes with UFFI, Referent: homes without UFFI. No information to compare exposed and referent	Data were not provided, qualitative results with <i>p</i> -values	N = 1,396 exposed, 1,395 referent	Not Informative Inadequate reporting detail; no formaldehyde measurements
Zhai et al. (2013) Jan 2008–Dec 2009 (China) (prevalence)	Provided criteria for selection of homes in defined area (2008–2009); evaluated 186 homes in Shenyang, China; homes were decorated in last 4 years and occupied within the last 3 years.	Cited Code for indoor environmental pollution control of civil building engineering (GB50325-2001); sampling duration not reported. Samplers in breathing zone in bedroom, living room and kitchen; N = 558 in 186 homes; exposure groups polluted	Respiratory symptoms via questionnaire by interview (ATS, 1978); randomly selected one adult from each house, plus 82 children (assisted by parents); timing of questionnaire administration in relation to air	Regression analyses of combined respiratory symptoms were adjusted; Prevalence ratios for specific symptoms/ disorders unadjusted for other variables, characteristics in 2 groups not described;	Compared symptom prevalence for children and adults by exposure category (reported <i>p</i> -values); multivariate logistic regression of respiratory system symptoms (all) in children and adults, adjusting for age, gender, smoking in	Polluted homes N = 119; Non-polluted homes N = 67	Analysis of combined respiratory symptoms Medium Sampling duration not reported; timing of questionnaire administration in relation to air monitoring uncertain.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and compar-ability	Exposure measure and range	Outcome measure	Consideration of likely con-founding	Analysis and completeness of results	Size	Confidence
		homes: > 0.08 mg/m ³ , mean 0.09–0.13 mg/m ³ in 3 rooms; nonpolluted ≤0.08 mg/m ³ , mean 0.04–0.047 mg/m ³ .	monitoring uncertain.		family, occupation, education, ventilation frequency, domestic pets, house facing, family history of allergy, height, weight.		Symptom prevalence ratios Medium Sampling duration not reported; timing of questionnaire administration in relation to air monitoring uncertain. Although potential confounders were not considered in symptom-specific analysis, the magnitude of the differences is unlikely to be explained by confounders.

School-based Studies**Table B-29. Evaluations of studies examining sensory irritation in humans: school-based studies**

Reference setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Wantke et al. (1996a) (Austria) Schools (panel, intervention)	Children at school where symptoms were reported; evaluated all children attending 3 forms.	Area samples; Sample number and duration not described; s.d. not reported. Concentration in 3 grades: Before move: 0.053, 0.085, 0.092 mg/m ³ ; After move: 0.036, 0.028, 0.032 mg/m ³	Symptoms assessed before and 3 mos after a move to a different school building. Symptoms reported by parents in a standardized questionnaire. Participants and investigators not blinded.	Comparison to self before and after removal from exposure	Symptom prevalence before and after move; McNemar test of difference	N = 62	<i>Not Informative</i> Participants and investigators not blinded; Reporting deficiencies

Controlled Exposure Studies in Humans

In addition to including shading (as described above for epidemiology studies), the controlled exposure studies in humans include italicized aspects of the study design or analysis highlight less significant limitations that had a minor impact on confidence classifications.

Table B-30. Evaluations of studies examining sensory irritation in humans: controlled human exposure studies

Reference and Confidence	Exposure assessment (details on exposure quality evaluation in Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size
Andersen (1979); Andersen and Molhave (1983)^a Confidence: <i>Medium</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported as within 20% of target; 0.24, 0.4, 0.81, 1.61 mg/m ³ <i>Adequate</i>	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, <i>blinding not described</i> . 31.2% smokers.	Within person comparison	Provided prevalence	<i>N</i> = 16
Bender et al. (1983) Confidence: <i>Low</i>	Paraformaldehyde, dynamic chamber, analytical concentrations not reported; 0, 0.43, 0.69, 0.86, 1.11, 1.23 mg/m ³ <i>Adequate</i>	Self-report response (eye only), time to 1st response	Order of exposure assignment not described, blinding not described	Within person comparison	Provided prevalence	<i>N</i> = 7
Berglund et al. (2012) Confidence: <i>Medium</i>	Paraformaldehyde, analytical concentrations reported; series of 18, 0.0078–1.23 mg/m ³ ; <i>Good</i>	Nasal irritation (< 3 sec sniffs); Self-report, forced choice response Emphasis on threshold for detection, <i>less direct relevance to symptom severity</i> .	Exposure concentrations randomly presented; <i>blinding not described</i> .	Within person comparison	Graph of detection prevalence by In concentration	<i>N</i> = 31
Cometto-Muñiz and Hernández (1990) <i>Not informative</i>	Test article NR, analytical concentrations reported; 1.23, 4.31, 8.49, 20.5 mg/m ³ <i>Deficient</i>	“Pungency” symptoms (includes irritant and non-specific symptoms) from 2.5 sec sniffs; self-report	“Irregular order” of exposure presentation (but not random), blinding not described, healthy nonsmokers	Within person comparison	Graph of perceived intensity (alone or in combination with ammonia)	<i>N</i> = 28
Day et al. (1984) <i>Not informative</i>	Marginal; no clean air exposure, 1.23 mg/m ³ <i>Deficient</i>	Self-report, questionnaire	Nonrandom exposure assignment, blinding not described	No comparisons	Provided prevalence	<i>N</i> = 18

Supplemental Information—Formaldehyde (Inhalation)

Reference and Confidence	Exposure assessment (details on exposure quality evaluation in Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size
Green et al. (1987) Confidence: <i>High</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0, 3.69 mg/m ³ <i>Good</i>	Self-report, questionnaire including nonirritant sham symptoms to assess bias; symptom severity scores	Random assignment to order of exposure, <i>single blinded</i> .	Within person comparison	Provided prevalence & statistical analyses	<i>N</i> = 22
Green et al. (1989) Confidence: <i>High</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0, 3.69 mg/m ³ <i>Good</i>	Self-report, questionnaire including nonirritant sham symptoms to assess bias; symptom severity scores	Random assignment to order of exposure, double blinded.	Within person comparison	Provided score data and statistical analyses graphically	<i>N</i> = 24
Krakowiak et al. (1998) <i>Not informative</i>	Formalin, no methanol control; analytic concentrations reported; 0.5 mg/m ³ <i>Deficient</i>	Self-report, diary; symptom scores	Nonrandom exposure assignment, single blinded.	Within person comparison	Provided average symptom scores	2 groups. <i>N</i> = 10 in each
Kulle et al. (1987) ; Kulle (1993) ^a Confidence: <i>Medium</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported; I: 0, 0.62, 1.23, 2.46, II: 0, 1.23, 3.69 mg/m ³ <i>Good</i>	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, <i>blinding not described</i> .	Within person comparison	<i>Regression coefficients not provided, only p- values</i>	I: <i>N</i> = 10; II: <i>N</i> = 9
Lang et al. (2008) ^a Confidence: <i>High</i>	Paraformaldehyde, “quasi- static” chamber conditions, analytical concentrations reported; 0, 0.19, 0.37, 0.62, peaks to 1.23 mg/m ³ <i>Adequate</i>	Self-report, questionnaire; objective measures	Random assignment to order of exposure, double blinded.	Within person comparison	Graphs/tables and statistical analyses	<i>N</i> = 21

Supplemental Information—Formaldehyde (Inhalation)

Reference and Confidence	Exposure assessment (details on exposure quality evaluation in Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size
Mueller et al. (2013)^a Confidence: <i>High</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported; clean air, 0.37 + 4 peaks of 0.74 mg/m ³ , 0.49 + 4 peaks of 0.98 mg/m ³ , 0.62 mg/m ³ and 0.86 mg/m ³ <i>Good</i>	Self-report, questionnaire; objective measures	Exposure concentrations randomly presented; blinding not described.	Within person comparison	Graphs of difference between pre- and end of test values	<i>N</i> = 41
Sauder et al. (1986) <i>Not informative</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0, 3.69 mg/m ³ <i>Good</i>	Self-report, questionnaire; symptom scores	Nonrandom exposure assignment, blinding not described.	Within person comparison	Provided average symptom scores & statistical analyses	<i>N</i> = 9
Witek et al. (1986); Schachter et al. (1986a) Confidence: <i>Medium</i>	Paraformaldehyde over boiling 2-propanol, dynamic chamber, analytical concentrations reported <i>Adequate</i>	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, double blinded.	Within person comparison	Provided prevalence and score	<i>N</i> = 15
Schachter et al. (1987) Confidence: <i>Medium</i>	Paraformaldehyde over boiling 2-propanol, dynamic chamber, analytical concentrations reported.; 0, 2.46 mg/m ³ <i>Adequate</i>	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, double blinded. Participants had routine occupational formaldehyde exposure, <i>N</i> = 2 smokers.	Within person comparison	Provided prevalence and scores	<i>N</i> = 15
Schuck et al. (1966) <i>Not informative</i>	Propylene and ethylene photooxidation with UV light; eye exposure only; analytic concentration reported graphically; 0.12–1.23 mg/m ³ <i>Deficient</i>	Self-report, questionnaire; objective measures	Nonrandom exposure assignment, blinding not described	Within person comparison	Graphs	<i>N</i> = 12

Supplemental Information—Formaldehyde (Inhalation)

Reference and Confidence	Exposure assessment (details on exposure quality evaluation in Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size
Witek et al. (1986); Witek et al. (1987) Confidence: <i>Medium</i>	Paraformaldehyde over boiling 2-propanol, dynamic chamber, analytical concentrations reported; 0, 2.46 mg/m ³ <i>Adequate</i>	Self-report, questionnaire; symptom scores	Random assignment to order of exposure, double blinded.	Within person comparison	Provided prevalence and score	<i>N</i> = 15
Yang et al. (2001) <i>Not informative</i>	Plywood exposure; 2.03, 3.68, 5.3 mg/m ³ ; eye exposure only; Analytical concentrations reported for formaldehyde but not for other off gassed compounds <i>Deficient</i>	Objective measure	Random assignment to order of exposure, double blinded. 25% smokers.	Within person comparison	Graph of eye blink frequency and table of <i>p</i> -values	<i>N</i> = 8

^aAt the request of EPA's Office of Chemical Safety and Pollution Prevention, EPA's Human Studies Review Board review of four intentional exposure studies for potential use in assessing acute formaldehyde inhalation determined the ethical conduct of this study to be adequate.

Anatomy Course Studies

Table B-31. Evaluation of studies examining sensory irritation in humans: anatomy courses

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Akbar-Khanzadeh et al. (1994) (Ohio) Anatomy students (cross-sectional)	Medical students and instructors in anatomy lab; referents were nonmedical students and instructors. Of 54 volunteers, 5 referents dropped out and 3 smokers excluded.	TWA personal breathing zone samples obtained on all exposed subjects (9 d), and 1 unexposed (6 d). Exposed mean 1.53, range 0.086 to 3.62 mg/m ³ . Referent mean 0.12, range 0.09 to 0.17 mg/m ³ .	Self-report, Medical Research Council standardized questionnaire	No comparisons reported.	Provided symptom prevalence during exposure, no comparison to baseline or to unexposed; no statistical data analysis	34 exposed; 12 referent	<i>Not Informative</i> No within person comparison to baseline or the referent; Reporting deficiencies
Chia et al. (1992) (Singapore) Anatomy students (cross-sectional)	Medical students in 1 st year lab course (92% participation); referent group = 3 rd or 4 th year medical students (participation rate not reported)	Area samples at dissecting tables, n=6, collected on two occasions. Personal samples, n=14 students, duration 2.5 hours; mean 0.91, SD = 0.22 mg/m ³ , range 0.50 to 1.48 mg/m ³ , LOD = 0.062 mg/m ³ . Assumed no formaldehyde exposure in referent based on activities (ward rounds and classroom).	Self-report, modified MRC standardized questionnaire; symptoms during previous 4 wks of course (recall accuracy reduced?)	Comparison to referent matched on age, sex, and ethnicity; Questions about dissimilarity of 1 st and 4 th year students	Symptom prevalence in exposed compared to referent; Referent activities very different	Exposed N = 150; referent N = 189	<i>Low</i> Questions about dissimilarity of 1 st and 4 th year students and potential for recall bias during previous 4 weeks of course. No measurements of formaldehyde among referent group.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Fleisher (1987) Anatomy students (cross-sectional)	44% of 204 surveyed in gross anatomy course; of those less than 50% responded to both questionnaires. Possible greater motivation to participate among those with symptoms.	Area samples in 6 labs, 1 d during semester (approximately 3 hrs); Dräger tubes, 3 labs, LOD 1.23 mg/m ³ , NIOSH method, 3 labs, LOD 0.02 mg/m ³ . Personal breathing zone for 2 instructors. 0.64, 0.18 mg/m ³ ; probable nondifferential misclassification due to sampling method with low sensitivity (3 labs) and low frequency of sampling. Adequate differentiation between exposure groups	Self-report, questionnaire; data collection 1 mo after end of course; symptoms all or some of the time, rarely or never. (temporal gap reduced recall accuracy?)	Within person comparison: symptoms during lab with exposure compared to lab with no exposure to formaldehyde.	Compared mean symptom scores, paired <i>t</i> -test	N = 38	<i>Low</i> Low response to both questionnaires and selection potential; with potential for symptoms to contribute to motivation to participate. temporal gap in symptom response reduced recall accuracy potential
Kriebel et al. (1993) (Massachusetts) Anatomy students (panel)	96% participation	Personal samples in the breathing zone, 1–1.5 hrs; multiple days. Range 0.60–1.14 mg/m ³ , geometric mean = 0.9, SD 1.5 mg/m ³	Self-report; questionnaire before, during and immediately after lab each day	Within person comparison: symptoms during and after lab compared to prelab symptoms.	Symptom prevalence before, during and after lab. Mean prelab and cross-lab change over 10 weeks evaluated using multivariate linear regression	N=24	<i>High</i>
Kriebel et al. (2001) (Massachusetts) Anatomy students (panel)	94.4% participation; attendance declined from n=37 to n=10 over 13 wks (better	Individual TWA using zone-exposure matrix based on continuous monitoring in 6 homogenous sampling zones (LOD = 0.06	Self-report, questionnaire; symptom intensity 10-point scale	Within person comparison: symptoms before and after lab	Generalized estimating equation regression accounting for lack of independence of repeated measures	N=38	<i>Medium</i> Decline in attendance over time potentially based on exposure and outcome status.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	attendance by healthy individuals?)	mg/m ³). 12 min work-zone concentrations calculated using sampling data and recorded work; locations. Mean 1.35, SD 0.69 mg/m ³ ; 12 min peak 13.42 mg/m ³			in individuals; symptom intensity, % change per ppm or ppm-weeks		
Mori et al. (2016) (Japan) Medical students, 1 st and 2 nd year	Students (2 nd year) enrolled in afternoon gross anatomy classes, April–July 2013, mean age 22.9 years; compared to nonexposed 1 st year students, mean age 21.2 years. 75% males	Area sample, 5 locations during class on same day questionnaires were completed. Mean (SD) 0.1 (0.02) ppm	Questionnaire, 16 subjective symptoms, frequency never, sometimes, or often; administered April 2013 before, May 2013 during, and January 2014, 6 months after completion of course.	Presented characteristics by exposure group; adjusted for age, sex and allergy status in regression models.	Prevalence of symptoms compared, Cochran's Q test and McNemar's test; Regression of presence or absence of symptoms in relation to exposure group on day of survey, controlling for doctor-diagnosed allergies, sex and age	123 exposed (98.4%); 114 unexposed (91.9%)	<i>High</i>
Saowakon et al. (2015) (Thailand) Medical students and academic staff	Students and faculty in gross anatomy dissection labs; Selection, recruitment and participation was not reported. Ages 19–21 years, nonsmokers with no history of chronic respiratory	Personal samplers (n=36 students, 4 instructors); area samples, all NIOSH-2016 method; 3-hr samples over duration of class, 3 classes, January, August, and October Students: Mean (SD) ppm Class 1: 0.193 (0.120)	Questionnaire, 20 symptoms, completed before start of dissection and after chest and abdominal opening (classes 2 & 3); Severity scale, 0–4.		Reported each symptom as percentage of score for all symptoms averaged over all classes; no comparisons	N=36 students; n=4 instructors	<i>Not Informative</i> No within person comparison to baseline or the referent; reporting deficiencies

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	disease or symptomatic illness	Class 2: 0.271 (0.159) Class 3: 0.828 (0.182)					
Takahashi et al. (2007) (Japan) Medical students (panel)	Selection, recruitment and participation was not reported.	Exposure measurements on day 1. Area samples in 8 locations in lab, > 10 min; Personal samples (breathing zone) on 18/143 students. Mean 3.0, SD = 0.60 mg/m ³ , range 2.2 to 4.6 mg/m ³ .	Self-report, questionnaire after 1 st day and at end of 2-mo course. Questionnaire not described. Lack of temporal correspondence between exposure measurements and symptom measurements at end of course.	Within person comparison: symptoms after 1 st day and at end of course	Symptom prevalence after first day and after lab at end of course; McNemar exact test (estimated from Figure 1 in publication).	N = 143	<i>Medium</i> Large gap between symptom ascertainment at end of course and exposure measurements. Selection, recruitment and participation was not reported.
Takigawa et al. (2005) (Japan) Anatomy students (intervention)	Volunteers; 76% completed questionnaires both before and during lab	Area samples in 9 locations in lab, > 10 min. Personal samples on 24 of 78 in phase I (2001) (duration 42–962 minutes); median 3.3 mg/m ³ , range 2.2 to 8.9 mg/m ³ , and on 46 of 79 in phase II (2004) (duration 100–540 min); median 0.88 mg/m ³ , range 0.40 to 3.4 mg/m ³ .	Self-report, questionnaire before and during each course; frequency (4-point scale); score change during session	Groups similar in age and % male/female; prevalence of smoking not reported.	Symptom change index, 25 symptoms, by phase of intervention; Mann-Whitney test.	N = 78	<i>Medium</i> Uncertainty regarding impact of smoking on sensitivity of response to intervention

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Uba et al. (1989) (California) Anatomy students (panel)	78.6% completed both questionnaires	Personal sampling (impingers) in the breathing zone over 7 mos; multiple days; TWA concentration; range 0.06 to 1.14 mg/m ³	Self-report; American Thoracic Society questionnaire; symptoms after lab on one day in November (at approx. 8–10 wks); symptoms before 1 st day and after last day (Sept 1984–Apr 1985)	Within person comparison: persistent symptoms beginning and end of course (7 mos); also symptoms during lab session compared to lab with no exposure to formaldehyde.	Numbers with symptoms in exposed and unexposed labs; McNemar's test paired samples, OR, <i>p</i> -value.	N = 81	<i>High</i>
Wantke et al. (1996b) (Austria) Anatomy students (panel)	Volunteers; participation 37.5% (45 of 120 students)	Area samples; Continuous daily measurements for formaldehyde at 2 locations during 3-hr lab, 5 d/wk for 4 wks. Mean 0.15, range 0.07 to 0.27 mg/m ³	Self-report, standardized questionnaire at beginning (symptoms during 3 mos before lab) and at end of course (symptoms over last 4 wks). Potential for differential recall of symptoms.	Within person comparison	Symptom prevalence before and during lab; McNemar exact test; multiple measurements during course would be ideal	N = 45	<i>Low</i> Low participation, Potential for differential recall of symptoms.
Wantke et al. (2000) Austria Anatomy students (panel)	Uncertainty regarding selection and recruitment details; Study repeated in subset. 27 of the 45 students in Wantke et al. (1996b)	Area samples; Continuous daily measurements for formaldehyde and phenol at 2 locations during lab, exposures for 43 d. Mean 0.27, range 0.13 to 0.41 mg/m ³	Self-report, questionnaire at beginning, 5 wks and 10 wks, Daily symptom cards during class.	Within person comparison; symptoms at beginning and during lab at middle and end of 10-wk course	Symptom prevalence before, middle and at end of 10 wk course; McNemar exact test.	N = 27	<i>Medium</i> Uncertainty regarding selection and recruitment details.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Wei et al. (2007) Anatomy students (cross-sectional)	Volunteer, all students present on the day that sampling was conducted.	Area samples near dissection tables, 30 min samples, N = 12. Measurements before, beginning, middle and completion of 3-mo gross anatomy class. Geometric mean: before 0.03, beginning 0.89, middle 0.76, end 0.24 mg/m ³	Self-report, questionnaire on sampling days after 2 hrs of lab. Symptom questionnaire was not completed outside of class so difference may have been influenced by perception relative to symptoms in class (possibly resulting in overestimation of risk)	Within person comparison	Frequency of symptoms during class; prevalence and severity scores during class compared to “usual life situation”; Walsh test (inadequate comparison)	N = 79–94	<i>Not Informative</i> Comparison to “usual life” unreliable

Occupational Studies

Table B-32. Evaluations of studies examining sensory irritation in humans: occupational studies

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Alexandersson et al. (1982) (prevalence)	All exposed workers employed >1 year; evaluated employees present at work on study day (both exposed and referent); Selection for healthy survivors	TWA personal sampling for formaldehyde, terpenes & dust, N= 31; 1 working d, 6–7 hrs 0.05–1.62 mg/m ³ ; no measurements for referent group; Although no	Self-report, British Medical Research Council questionnaire; symptoms at work, same day as exposure assessment	Symptom prevalence in exposed compared to referent. Exposed: employees of carpentry works; referents were not exposed to formaldehyde or other irritants in same factory;	Symptom prevalence at work compared between exposed and referent, chi-square	N = 47 exposed; N = 20 referent	<i>Medium</i> Potential for healthy survivor bias with attenuation in measure of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		measurements in referent, high concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent		Similar % age, height, sex, & weight. Prevalence smoking 48% in exposed, 40% in referent.			
Alexandersson and Hedenstierna (1989) (prevalence, [Note: follow-up of Alexandersson et al. (1982)])	Evaluated employees who participated in previous study, 4 year follow-up (Alexandersson et al., 1982); 13 exposed and 2 referents lost-to-follow-up; 13 exposed transferred to unexposed jobs possible survivor bias	TWA using personal sampling, 3–4 15 min samples/person; 2 working d; Mean 0.5 mg/m ³ ; Mean peak 0.69 mg/m ³ limited sampling period; although no measurements in referent, high concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent	Self-report, British Medical Research Council questionnaire	Symptom prevalence in exposed compared to referent. Exposed: employees of carpentry works; referents were not exposed to formaldehyde or other irritants in same factory; Similar % age, height, sex, & weight. Prevalence smoking 50% in exposed, 33% in referent. Moderate concern for confounding by smoking (direction of bias unclear).	Change in symptom prevalence at work 1980–1984, chi-square	N = 21 exposed; N = 18 referent	<i>Low</i> Evidence of healthy survivor bias; confounding by smoking
Alexandersson and	Selection for healthy; evaluated employees present at work on	TWA using personal sampling, 3–4 15	Self-report, standardized questionnaire;	Symptom prevalence among workers exposed	Symptom prevalence at work compared between exposed	N = 38 exposed; N	<i>Low</i> Potential for healthy survivor bias with

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Hedenstierna (1988) Supplemental: Alexandersson (1988) (prevalence)	study day (both exposed and referent)	min samples/person; 1 working d, no concentration reported for referent 0.12–1.32 mg/m ³ Although no measurements in referent, high concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent	outcome assessed same day as exposure	to acid-hardening lacquers; referents were "nonexposed" employees at same factory. All male, exposed slightly younger, 50% smokers; referent: 33% smokers. Sampled for dust and solvents: authors considered all exposures to be very low and not confounders. Moderate concern for confounding by smoking (direction of bias unclear).	and referent, chi-square; no adjustment	= 18 referent	attenuation in measure of association. Confounding and no adjustment in analyses
Herbert et al. (1994) (prevalence)	Participation >90% in exposed, >80% in referent; Healthy survivor effect likely similar among exposed and referent groups	TWA continuous sample in breathing zone; 5 sites, 2 d 0.09–0.33 mg/m ³ referent not reported; sampled for dust. Although no measurements in referent, formaldehyde exposure not expected for oil/gas field workers, adequate	Self-report, Respiratory symptoms ascertained via interview using standardized questionnaire	Possible respiratory irritants in comparison group (oil sands workers); higher prevalence of smokers (52% vs 28%) and shorter duration of employment among exposed, (5 versus 10 years)	Symptom prevalence compared by exposure group, chi-square; unadjusted analyses	N = 99 exposed; N = 165 referent	<i>Low</i> Different prevalence smoking and duration of employment between exposed and referent; no adjustment in analyses

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		exposure contrast likely for comparison of exposed and referent.					
Holmström and Wilhelmsson (1988); Wilhelmsson and Holmstrom (1992) (prevalence)	100% participation; healthy survivor bias probable; source populations for exposed and referent (government clerks) were different, raising possible unmeasured confounding	Area samples in one group, 1979–1984, personal samples (1–2 hrs) in 1985 in all groups. Sampling data in referent. 0.05–0.5 mg/m ³ Adequate exposure contrast for comparison of exposed and referent	Self-report, questionnaire	Groups similar for age and smoking, 87% and 93% male in exposed, 56% male in referent (gender related differences in perception of irritation?) No exposure to solvents, concentrations for other chemicals all <1% of OEL (phenol, ammonia, epichlorhydrin, methanol and ethanol).	Compared symptoms prevalence across exposure groups, chi-square; unadjusted analyses	N = 70 Group 1, N = 100 Group 2; N = 36 referent	<i>Low</i> (↓) Potential for healthy survivor bias with attenuation in measure of association.; groups selected from different source populations; Questionnaire not described. Potential confounding and no adjustment in analyses
Holmström et al. (1991) (prevalence)	Details of recruitment and participation not described. Healthy survivor bias probable; source populations for exposed and referent were different, raising possible unmeasured confounding	Personal exposure measurements stable through year, average 0.2–0.3 mg/m ³ , peaks seldom > 0.5 mg/m ³ Formaldehyde Concentration, mean	Self-report, questionnaire	MDF group slightly older (44.1 year) compared to wood (39.3 year) and referent (39.9 year); % male varied, smoking less prevalent in referent	Exposed groups each compared to referent; prevalence rate difference, 95% confidence intervals; no adjustment	MDF: N = 16 Wood: N = 29 Referent: N = 36	<i>Low</i> (↓) Uncertainty regarding selection and recruitment details; Potential for healthy survivor bias with attenuation in measure of association.; groups selected from different source populations; Potential confounding and no adjustment in analyses

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		MDF 0.26 mg/m ³ , wood dust 0.25 mg/m ³ , referent 0.09 mg/m ³ ; adequate exposure contrast for comparison of exposed and referent					
Holness and Nethercott (1989) (prevalence)	Minimal concern for selection bias. Recruitment source was list provided by funeral home association, 86.6% of eligible participated. Participation rate among referents was not given.	2 area samples (impingers), during embalming, 30 to 180 min. Gave concentration for referent. 0.1–1.0 mg/m ³ Adequate exposure contrast for comparison of exposed and referent	Self-report, American Thoracic Society questionnaire; before and after embalming	Symptom prevalence compared between exposed (apprentice funeral service workers) and unexposed (service volunteers and paid students), probable unmeasured confounders. Groups similar for age, height, and smoking status. Source of formaldehyde exposure was formalin (also contained methanol)	Comparisons between exposed and referent, logistic regression adjusted for # pack-years smoked. Provided data and results of statistical analyses	N = 84 exposed; N = 38 referent	<i>Medium</i> Differences in source populations for exposed and referent groups lead to uncertainty in comparability, with no consideration of potential confounding.
Horvath et al. (1988) (Wisconsin) Occupational	71% participation in exposed; 88% participation in referent. Age and sex	8-hr TWA using Personal and area sampling on day of exam.	Self-report, American Thoracic Society questionnaire;	Symptom prevalence in exposed workers at a particleboard	Symptom prevalence during work in exposed and referent compared;	N = 109 exposed; N = 254 referent	<i>Medium</i> Uncertain whether

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(prevalence)	distribution in participants similar to entire workforce in their respective companies. Evaluated and ruled out survivor bias using reasons for leaving employment among 54 former employees; evaluated characteristics of 30/45 nonparticipants who were younger and higher % male, with similar % smokers and mobile home residency.	Exposed mean 1.04, range 0.32 to 4.48 mg/m ³ . Referent mean 0.06, range 0.04–0.15 mg/m ³ ; adequate exposure contrast for comparison of exposed and referent	assessed same day as exposure assessment; before and after shift	manufacturing plant compared to referent workers at 2 food production plants. Higher proportion male in exposed and slightly older average age (expect any potential bias toward null for symptoms). Smoking and mobile home residency similar. Particulate exposure in exposed and referent (different sources), other chemical exposures were not detectable or below PEL.	prevalence at end of shift using multiple regression. Uncertain whether analyses adjusted for age or gender.		analyses adjusted for age or gender, although groups were compared and differences were not large.
Kilburn et al. (1985a) (prevalence)	76 female histology technicians in 23 hospitals & 2 labs (exposed), 97% of eligible, mean (SD) age 40.8 (11.6) years; 56 women in referent (secretaries and clerks in same institutions) matched with 40 of the	Environmental samples for formaldehyde, xylene, toluene, and chloroform by regional NIOSH laboratory in 10 of 25 labs; 1–4 hours sampling time; self-report of	Self-report, questionnaire, composite experience for previous months or years (reduced accuracy of recall)	Incomplete matching: Among 76 exposed, group of 40 matched to referent on age, cigarette smoking, and ethnicity; multiple chemical exposures; evaluated effects among participants	Prevalence by hours formaldehyde exposure and xylene exposure; results of statistical analyses not reported.	N = 76 exposed; N = 56 referent	<i>Low</i> Extended time period for symptom reporting may have reduced accuracy of recall; incomplete matching; results of statistical analyses were not reported.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	technicians for age, cigarette smoking, and ethnicity, mean (SD) age 39.5 (10.5) years.	duration of exposure (hrs/d) 0.25–2.34 mg/m ³ ; adequate exposure contrast for comparison of exposed and referent		with >4 hrs formaldehyde exposure/d stratified by 2 levels for xylene.			
Löfstedt et al. (2011b) (prevalence)	>90 % participation in exposed and referent; healthy worker survival? Higher proportion of referents had ever had asthma or allergic symptoms in childhood	Individual samples over a single 8-hr shift 0.013–0.19 mg/m ³ , geometric mean 0.037 mg/m ³ ; subjects categorized into low and high formaldehyde using LOD; also sampled MCA, ICA and dust	Self-report, questionnaire; existence of symptoms during prior week (reduced recall accuracy?)	Referent from the same industry (not workers in core production or die casting), comparable for age; smoking prevalence, prevalence female, and work duration higher in referent. Symptom prevalence compared between groups. Co-exposures measured but not adjusted for in analysis. Independent effect of formaldehyde could not be determined	Logistic regression models, symptoms by referent, low and high formaldehyde groups; no adjustment for other irritants (isocyanic acid, methyl isocyanate, dust) which were strongly associated with symptoms. Also restricted analyses excluding asthma or allergies, females, or smokers with similar results	N = 43 of 48 exposed; N = 69 of 84 referents	<i>Low</i> (↑) Could not distinguish effect of formaldehyde from those of other irritants that were strongly associated with symptoms; Questionnaire not described. Potential for information bias (reduced recall accuracy); higher proportion of asthma or allergy among referents raises potential healthy worker survival
Neghab et al. (2011) (prevalence)	100% participation; healthy worker survival?	Area samples (40 minutes, N = 7) in 7 workshops and 1 in office area.	Self-report, interview & American Thoracic Society	Referent from the same industry and comparable for socioeconomic	Symptom prevalence compared by exposure group, chi-square	N = 70 exposed N = 24 referents	<i>Medium</i> Potential for healthy survivor bias with

Reference, setting and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		Mean 0.96 mg/m ³ ; SD 0.49 mg/m ³ ; adequate exposure contrast for comparison of exposed and referent	questionnaire; symptoms at work	status, age, smoking prevalence (25%). Symptom prevalence compared between groups.			attenuation in measure of association.

B.3.3. Pulmonary Function

The details of the study evaluation approach methods are provided in Section 2.3.3 of the Toxicological Review. Within Tables B-33 (including epidemiological laboratory student studies, residential- and school-based studies, and occupational studies) and B-34 (controlled exposure studies) below documenting the results of the study evaluations for pulmonary function, studies are arranged alphabetically. Gray shading is indicative of aspects of the study design or analysis that decreased EPA's confidence rating.

Epidemiology Studies

Table B-33. Evaluation of formaldehyde - pulmonary function epidemiology studies

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Laboratory Students Studies							
Akbar-Khanzadeh et al. (1994) (Cross-sectional)	Medical students and instructors in anatomy lab; referents were nonmedical students and instructors. Of 54	TWA personal breathing zone samples obtained on all exposed subjects, 9 d, and 1 unexposed. 6 d	Cross-lab change using pre- and postlab spirometry using ATS criteria on 1 d per student; all had at least 6 wks of formaldehyde	Age (26 vs. 32 year), height and weight similar between exposed and unexposed; 21% with history of asthma in exposed	Mean (SD) absolute value at baseline and mean % difference across lab compared within and	N = 34 exposed; 12 referents	Cross-lab change <i>Medium</i> Small sample size in referent. Potential confounding by history of asthma in between group analyses.

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	volunteers, 5 referents dropped out and 3 smokers excluded.	Range 0.086–3.62 mg/m ³ Also sampled methanol (mean 110 ppm) and phenol (not detected)	exposure at time of spirometry	and none in referent; nonsmokers	between groups; <i>t</i> -test		
Akbar-Khanzadeh and Mlynek (1997) (Cross-sectional)	Medical students enrolled in anatomy laboratories matched by age to referent class occurring at the same time (physiotherapy students)	Personal (breathing zone) (<i>n</i> = 44) and area (<i>n</i> = 76) formaldehyde samples Range 0.34–5.47 mg/m ³	% predicted; prelab and postlab spirometric variables; 4 students assessed each time	Variables expressed as a percentage of reference values accounting for height, weight, age, sex, and race; all nonsmokers. Matching incomplete for males. Potential confounding by time related changes in individual response	Mean cross-lab change analyzed within and between groups using regression model and <i>t</i> -test	N = 50 exposed; 36 referents	Cross-lab change <i>Low</i> (↓) Potential confounding by time related changes in individual response and for difference in gender composition in between group comparison.
Binawara et al. (2010) (Cross-sectional)	Excluded individuals with symptoms, stress, type-1 allergy, respiratory disease, and smokers First-year medical students in anatomy lab	No formaldehyde measurements	Pre- and postlab spirometry, % predicted, day of course not reported	Within person change	Percent predicted prelab compared to postlab means (SD), <i>t</i> -test; no comparison group	N = 80	Cross-lab change <i>Low</i> No comparison group
Chia et al. (1992) (Cross-sectional)	Subjects selected randomly; all agreed to participate	Area samples at dissecting tables, <i>n</i> = 6, collected on two occasions. Personal samples,	Spirometric measures (published methods); once before and after	Within person change; before and after dissection means adjusted for	Means, absolute values adjusted for age and height, stratified by gender; and <i>p</i> -	N = 22	Cross-lab change <i>Low</i> No comparison group; Small sample size

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		n = 14 students, duration 2.5 hrs Range 0.50–1.48 mg/m ³	dissection, 1 st d after 2-wk vacation.	age and height, stratified by sex.	values; no SE; no comparison group		
Khalig and Tripathi (2009) (Cross-sectional)	Participants randomly selected; excluded students with respiratory illness or previous exposure to formalin; all nonsmokers	No formaldehyde measurements. Formaldehyde exposure assumed for dissection classes	Pre- and postlab spirometry; 3 tests using best value, measured on 1 st day of exposure and 24 hrs after	Within person change	Mean absolute value (SD) compared pre- and postlab, t-test; no comparison group	N = 20	Cross-lab change <i>Low</i> No comparison group; Small sample size
Kriebel et al. (2001) (panel study)	51 gross anatomy students (out of 54 total) during a 12-wk class meeting once per week. 94% participation; attendance declined from n = 38 to n = 10 over 13 wks (better attendance by healthy individuals?)	Work-exposure matrix from sampling in 6 work zones, multiple days, and reported time spent in each zone Average 1.35 mg/m ³ , 10-min peak 13.42 mg/m ³	Spirometric measures (ATS methods) before and at end of 13 wks. PEF, prelab and across-lab change every weekly lab session	Within person change; multiple measurements; 2 smokers and 7 ex-smokers, PEF in smokers no different from nonsmokers	PEF as fraction of value before 1 st lab session analyzed longitudinally; Individual prelab and cross-lab change data analyzed together in relation to recent, average and cumulative formaldehyde in single generalized estimating equations model. GEE adjusted for cold on lab day. Cross-lab change: no comparison group.	N = 38 of 51 with pre- and postlab measures for ≥1 week	Longitudinal <i>Medium</i> (↓) Attrition and declining concentration over course—bias to healthy individuals and toward null Cross-lab change <i>Low</i> No comparison group
	24 clinical anatomy students (out of 25)	Personal samples in the breathing	PEF repeated measures Wright	Within person change; multiple	Mean absolute value (SD) prelab		Longitudinal <i>Medium</i>

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Kriebel et al. (1993) (panel study)	total) during a 10-wk anatomy class meeting once a week. 96% participation	zone, 1–1.5 hrs of 3-hr lab; multiple days Range 0.60–1.14 mg/m ³ Pentachloro-phenol measured but not detected.	flow meter; measured 1–3 times during each weekly lab	measurements; one smoker	(longitudinal) and cross-lab change in pulmonary function analyzed in separate models using random effects models including asthma, asthma*week, eye and nose or throat symptoms. Provided data and results of statistical analyses; Also showed absolute value (SD) and cross-lab change (SD) at weeks 1 and 2 and 9 and 10 Cross-lab change: no comparison group	N = 20 in analysis out of 24	Small sample size Cross-lab change <i>Low</i> No comparison group
Mohammad `pour and Maleki (2011) (cross-sectional)	30 veterinary students, male and female, aged 18–20 year, nonsmokers; selection of participants not described	No formaldehyde measurements	Pre- and postlab spirometry	Within person change; nonsmokers, age comparable	Mean absolute value (SD) compared pre- and postlab, ANOVA; tested interaction between sexes and exposure	N = 15 females; N = 15 males	<i>Not Informative</i> No comparison group, small sample size increased potential for unreliable results. Exposure levels uncertain and likely variable in this occupational group
Saowakon et al. (2015) (Thailand)	Students and faculty in gross anatomy dissection labs; selection, recruitment, and	Personal samplers (n = 36 students, 4 instructors); area samples, all	Siblemed 120 portable spirometer, completed before start of dissection	Within person change; all nonsmokers	Average change over one 3-hr lab session in the exposed group (Within person	N = 36 students; N = 4 instructors	Cross-lab change <i>Low</i> No comparison group

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Medical students and academic staff	participation were not reported. Ages 19–21 years, nonsmokers with no history of chronic respiratory disease or symptomatic illness	NIOSH-2016 method; 3-hr samples over duration of class, 3 classes, January, August, and October Students: Mean (SD) ppm Class 1: 0.193 (0.120) Class 2: 0.271 (0.159) Class 3: 0.828 (0.182)	and after end of dissection lab, maximum of two readings		change), paired <i>t</i> -test. Uncertainty whether each participant was assessed more than once. No comparison to referent group		
Uba et al. (1989) (panel study)	96 medical students (72.5% participation) during a 7 mo anatomy class meeting twice a week	Personal sampling monitors (impingers) in the breathing zone; multiple days and during 3 different months TWA Range 0.06–1.14 mg/m ³	Spirometric measures (ATS methods); Absolute value (SD) pre- and postlab and cross-shift change before Day 0 (before exposure), at 2 wks and 7 mos	Within person change; all nonsmokers	Cross-shift change in pulmonary function analyzed using repeated measures ANOVA, adjusted for sex; change at 2 wks and 7 mos compared to the baseline day (longitudinal). Compared mean values measured at noon on baseline day, 2 wks and 7 mos. (cross-lab change)	N = 96	Longitudinal <i>High</i> Cross-lab change <i>High</i>
Residential Studies and School-Based Studies							

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Bentayeb et al. (2015) ; (Cross-sectional), 2009–2011	Elderly (20 randomly selected per home) permanently living in randomly selected nursing homes (8 per city) in selected city in 7 countries. Exclusion criteria stated (neurological or psychiatric disorders)	Measurements in common room, uncertainty regarding average individual exposure; 1 wk samples; also measured particulates, NO ₂ , ozone, temperature, humidity and CO ₂ ; range of 1 wk averages 0.001–0.021 mg/m ³ , median 0.006 mg/m ³ ; categorical (low and high) based on median concentration in each nursing home	Assessed by same team in all countries; medical visit and standardized questionnaire (European Community Respiratory Health Survey); spirometry (ATS/ European Respiratory Society guidelines), % predicted	Adjusted for sex, age, country, BMI, highest school level, smoking, and season. Confounding by co-exposures was not assessed.	General estimating equations analysis, accounting for correlations within nursing homes; adjusted OR (95% CI); stratification by presence or ventilation. Presented risk above versus below the median formaldehyde level in each nursing home (medians were not reported).	N = 600	<i>Medium</i> Confounding by co-exposures was not assessed; median concentrations which defined low and high exposure categories were not reported.
Broder et al. (1988b, 1988c) ; Broder et al. (1988a) (Cross-sectional)	Identification of exposed through households with UFFI registered with state consumer agency; referents selected randomly from houses on adjacent streets; concern for possible over-reporting of symptoms but not	Area samples on 2 successive days in hallway, all bedrooms and yard. Median conc. in rooms were similar, Inside: referent 0.035 ppm, range 0.006–0.112 ppm [0.043 mg/m ³ , range 0.007–0.138	Spirometry protocol described	Adjustment for important confounders in data analysis.	Regression models of spirometry values between and within each exposure group, analysis adjusted for total hrs spent in house/wk, outside temperature, gender, age, height, smoking, and race;	N = 1,726 exposed; N = 720 referent	Within group analyses <i>Medium</i> Results not presented for formaldehyde Between group analysis <i>Low</i> Small exposure contrast between exposure groups

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	for pulmonary function	mg/m ³]. 90% 0.061; UFFI 0.043 ppm, range 0.007–0.227 [0.053 mg/m ³ , range 0.009–0.279 mg/m ³], 90% 0.073 ppm Outside: referent 0.005 ppm, UFFI 0.005 ppm. Exposure contrast between exposure groups small.			presented only statistically significant regression coefficients; no data shown for formaldehyde associations		
Franklin et al. (2000) (Cross-sectional)	Recruitment through local schools; response rate of participants was not described. Participation not expected to be influenced by outcome or exposure	3–4 d passive samples in bedroom and main living area Median (IQR) 0.019 (0.011, 0.035) mg/m ³ (communication by author)	Spirometry protocol (ATS), measurements in clinic	Children with current or history of upper or lower respiratory tract disease were excluded. % predicted based on age, sex, and height. Mean eNOS levels by exposure category adjusted for age and atopic status	Mean absolute value (SD) and % predicted (SD) by exposure group (<50 and ≥50 ppb); only 10 homes in high exposure group (data provided by author); no demographic info except for age	N = 224	<i>Medium</i> Limited exposure contrast; few subjects in high exposure group
Krzyzanowski et al. (1990) , adults & children (cross-sectional)	A stratified random sample of 202 households of municipal employees;	Two 1-week household samples, multiple locations Mean 0.032	PEF, Wright flow meter measured 4 times daily for 2 wks	Potential confounding analyzed in analysis	Random effects model accounting for repeated measures, adjusted for asthma, acute respiratory illness,	N = 202; repeated measures	<i>High</i>

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	eligibility criteria described	mg/m ³ ; maximum 0.172 mg/m ³			smoking, SES, NO ₂ , time of day; separate analyses for 15 years and younger, and over 15 years of age.		
Marks et al. (2010)	Schools and classrooms were selected using a 2-stage process, all students in selected classrooms (grades 4, 5, or 6) were recruited. Participation: 418 subjects (77%) of 543 students in selected classes.	One area sample in each classroom 2 d/wk for 6 wks	Spirometry protocol described	Randomized double blind intervention study of unflued and flued gas heaters, NO ₂ and formaldehyde levels varied together in same direction	Analysis of effects in relation to heater use (flued vs unflued), correlated co-exposures. No formaldehyde-specific analyses	N = 400	<i>Not Informative</i> No quantitative analyses specifically for formaldehyde
Norback et al. (1995) (Cross-sectional)	Recruited from 154 randomly selected members of general population; 57% participated. Possibly not representative sample because study design selected 50% subjects with asthma symptoms (may respond differently to formaldehyde exposure)	Formaldehyde (one 2-hr sample) in the bedroom at pillow height. Also measured guanine in bedroom (house dust mites), and room temperature, air humidity, VOCs, respirable dust, and CO ₂ in living room and bedroom. Limited sampling period in closed	Spirometry and peak flow protocol described; FEV ₁ (percent predicted accounting for age, sex, and height). PEF measured twice per day for 7 d; constructed variable for PEF variability (assessed in asthma section)	Analysis did not account for high prevalence of asthma symptoms in study group; VOC concentrations were correlated and effects could not be separated from those of formaldehyde (No data presented)	FEV ₁ was percent predicted accounting for age, sex, and height; Kendall's rank correlation test	N = 88	<i>Low</i> Exposure: Most exposed to concentration <LOQ Study population selected for high prevalence of asthma symptoms; Correlated co-exposures: VOCs

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		residence with no point formaldehyde emissions; sampling and analytic protocols referenced (Andersson et al., 1981) LOQ 0.1 mg/m ³ ; Formaldehyde and Range <0.005–0.110 µg/m ³ (most <LOQ)					
Wallner et al. (2012)	433 children (aged 6–10 years) with spirometry of 596 eligible (72.7%) in 2 classrooms each at 9 of 19 schools that volunteered to participate in study. 72.7% participation	Measurements of 252 chemicals in 9 home classrooms (exposed 6–7 hrs/d); 24 hr samples, 2 samples per classroom, 2 seasons; all students in class assigned the median chemical concentration; median 29.8 µg/m ³ (range 6.5–136.5 µg/m ³ , all values > LOQ	Spirometry protocol described; percent of reference	Reference values based on gender, age, height, and weight of children; regression analysis controlled for SES (education and occupation of parents, urban/rural, # smokers at home. No adjustment for other chemicals in classroom. Do not expect correlation between formaldehyde and PBDE congeners or phthalates in dust	Associations with lung function analyzed for 34 chemicals; no adjustment for multiple comparisons; multiple regression model, % change per 1 SD increase in formaldehyde (value of SD not reported).	N = 433	<i>Medium</i> No adjustment for co-exposures in classroom that were also associated with pulmonary function, but correlation not anticipated
Occupational Studies							

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Alexandersson et al. (1982)	All exposed workers employed >1 year, recruitment from workers present on study day (healthy worker effect). Referents selected from plant employees not exposed to irritants; participation rate not reported. Cross-shift change not evaluated in referent	TWA personal sampling; 1 working d. Range in exposed 0.05–1.62 mg/m ³ ; referent not reported; although no measurements in referent, high concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent	Spirometric measures (ATS methods); measured on Monday morning and after work in exposed; referents tested either in the morning or afternoon	Preshift variables compared to reference equations	Preshift values compared to predicted based on age, height, and gender evaluated within exposed and referent groups. SD not reported; - Cross-shift change: compared mean values before and after shift in exposed (paired t-test) No comparison group	N = 47 exposed; N = 20 referent	Pre-shift <i>Medium</i> (↓) Concern for selection for healthy. <i>P</i>-values were reported Cross-shift <i>Low</i> No comparison group
Alexandersson et al. (1982); Alexandersson and Hedenstierna (1989)	Possible selection for healthy during 4-year follow-up; 13 exposed and 2 referents lost-to-follow-up; 13 exposed transferred to unexposed jobs	TWA using personal sampling among all exposed; 3–4 measurements of 15 min periods during 2 working d. Range in 1980 exposed 0.05–1.62 mg/m ³ ; referent not reported; Range in 1985 not reported. Sampled for dust. Although no measurements in referent, high	Spirometric measures (ATS methods); measured on Monday morning across shift in exposed; referents tested either in the morning or afternoon	Values compared to predicted normal based on age, gender, and height; analyses stratified by smoking status. Dust levels considered to be low.	Mean absolute value (SD) before work compared to predicted normal based on age, gender, and height in 1980 and 1984, and mean difference from predicted (SD) in 1984 by smoking status; 5-year change corrected for age-dependent change; stratified by smoking. Mean change across shift (SD) stratified by	N = 21 exposed; N = 18 referent	Pre-shift <i>Medium</i> (↓) Potential for healthy survivor bias with attenuation in measure of association; small sample Cross-shift <i>Low</i> No comparison group

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent.			smoking, no comparison group (low) Cross-shift change: Mean change across shift (SD) stratified by smoking, no comparison group.		
Alexandersson and Hedenstierna (1988) ; Alexandersson (1988)	Selection for healthy; evaluated employees present at work on study day	TWA using personal sampling, 3–4 15 min samples/person; 1 working day. Range in exposed 0.12–1.32 mg/m ³ ; referent not reported; although no measurements in referent, high concentration in exposed allows assumption of an adequate exposure contrast for comparison of exposed and referent.	Spirometry on Monday after 1 d unexposed and again at end of shift on 2 nd day. Half of referent tested before, and half tested after shift	Referents were “nonexposed” employees at same factory. All male, exposed slightly younger, 50% smokers; referent: 33% smokers. Analyses stratified by smoking status. Sampled for dust and solvents: Authors considered all exposures to be very low and not confounders	Mean values and difference from reference values by exposure group, and by smoking status among exposed. Change over 2 d by smoking status. Mean comparisons within exposure groups, Student’s t-test Cross-shift change: No comparison group	N = 38 exposed; N = 18 referent	Pre-shift <i>Medium</i> (↓) Potential for healthy survivor bias with attenuation in measure of association. Small sample size in referent. Cross-shift <i>Low</i> No comparison group
Gamble et al. (1976)	Of 68 workers exposed to hexamethylene-tetramine-resorcinol resin, 52	Area samples	Spirometry protocol described	Referent matched by age, race, sex, shift, and job; Exposure to multiple chemicals	Exposure group defined by use of hexamethylene-tetramine-	N = 19 exposed; N = 19 referent	<i>Not Informative</i> No quantitative analyses specifically for formaldehyde

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	(77%) completed questionnaire and lung function testing				resorcinol resin, not formaldehyde		
Herbert et al. (1994)	Participation 98% in exposed, 82% in referent. Excluded accidental hydrogen sulfide exposure (n = 14).	TWA continuous sample in breathing zone; 5 sites, 2 d. Range in exposed 0.09–0.33 mg/m ³ ; referent not reported; sampled for dust. Although no measurements in referent, formaldehyde exposure not expected for oil/gas field workers; adequate exposure contrast likely for comparison of exposed and referent.	Spirometric measures; best of 5 maneuvers, Snowbird criteria (Ferris, 1978); at start of work shift and after 6 hrs Cross-shift change not evaluated in referent	Preshift comparisons adjusted for age, height, and smoking; not dust levels, which authors considered to be low. Potential for exposure to other irritants among referent group (oil/gas field workers)	Exposed compared to referent using ANCOVA adjusting for age, height, and cigarette pack-years. Presented absolute values and <i>p</i> -values from ANCOVA. Unconditional logistic regression of FEV ₁ /FVC <75% controlling for age and cigarette pack-years. Presented odds ratios, 95% CI by smoking category. Cross-shift change: Presented absolute values of preshift and postshift with <i>t</i> -statistics and <i>p</i> -values; no comparison group	N = 99 exposed; N = 165 referent	Pre-shift <i>Medium</i> Potential for healthy survivor bias with attenuation in measure of association. Possible irritant exposure in referent; co-exposure to dust. Cross-shift <i>Low</i> No comparison group
Holmström and Wilhelmsson (1988)	70 (87% male) in formaldehyde products group (mean age 36.9 years), 100 furniture workers exposed for	Area samples in one group, 1979–1984, personal samples (1–2 hrs) in 1985 in all groups.	Spirometric measures (FVC, FEV ₁ /FVC) percent of expected normal based on age, sex,	Values compared to expected normal based on age, sex, smoking, height, and weight; respirable	Presented observed and expected values by exposure group, SD not reported. Statistical	N = 70 Group 1; N = 100 Group 2; N = 36 referent	<i>Medium</i> (↓) Potential for healthy survivor bias with attenuation in measure of association. Comparison groups selected from

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	formaldehyde and wood dust (93% male, mean age 40.5 years), 36 persons (56% male, mean age 39.9 years) government clerks. 100% participation among those recruited Potential selection for healthy workers	Estimated mean formaldehyde and dust exposure of every participant for each year of employment, dose-years. Range in Group #1 0.05–0.5 mg/m ³ , Group #2 0.2–0.3 mg/m ³ ; referent mean 0.09 mg/m ³ ; adequate exposure contrast likely for comparison of exposed and referent.	smoking, height, and weight.	particulates measured but not adjusted for in analysis. Comparison groups: Formaldehyde only, formaldehyde and wood dust, referent group. Referent group was composed of administrative workers who may not be comparable to exposed. Comparable smoking status between groups (data NR)	comparisons of observed and expected within exposure group (paired <i>t</i> -test); analyzed correlation with duration of exposure and cumulative dose but did not provide quantitative results		different source populations
Holness and Nethercott (1989)	Participants recruited from list of funeral homes, 86.6% participation; 79.8% of embalmers were active embalmers (healthy workers); community referent less similar?	2 area samples (impingers), during embalming, 30 to 180 min. Range in exposed 0.10–1.0 mg/m ³ , referent mean 0.025 mg/m ³ ; adequate exposure contrast likely for comparison of exposed and referent.	Lung function as percent predicted; measured at initial assessment and before and after embalming procedure among exposed and before, and after a 2–3 hr period in referents.	Analyses adjusted for age, height, and pack-years smoked Differences in source populations for exposed and referent groups lead to uncertainty in comparability, with no consideration of potential confounding.	Mean percent predicted (SD) presented by exposure group or by active or inactive embalmers, <i>p</i> -value from regression model adjusted for age, height, and pack-years smoked; percent change during embalming	N = 84 exposed; N = 38 referent	<i>Medium</i> Differences in source populations for exposed and referent groups lead to uncertainty in comparability, with no consideration of potential confounding.

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Horvath et al. (1988)	71% participation in exposed; 88% participation in referent. Age and sex distribution in participants similar to entire workforce in their respective companies. Evaluated and ruled out survivor bias using reasons for leaving employment among 54 former employees; evaluated characteristics of 30/45 nonparticipants who were younger and higher % male, with similar % smokers and mobile home residency.	8-hr TWA using personal and area sampling on day of exam. Range in exposed 0.32 to 4.48 mg/m ³ ; referent 0.037–0.15 mg/m ³ ; adequate exposure contrast likely for comparison of exposed and referent.	Spirometric measures (ATS methods); % predicted	Adjusted for age, sex, height, and smoking in analyses; particulates measured but not adjusted for in analysis. Smoking prevalence 53% in both groups; mean total particulates somewhat higher in referent. Other co-exposures not detected or a fraction of PEL (respirable particulates, phenol, CO, sodium hydroxide, NO ₂ and acrolein).	Variables evaluated as percent of predicted normal; mean % predicted (SD) compared between exposure groups, <i>t</i> -test; multiple regression on log concentration adjusted for age, sex, height, and smoking; for cross-shift change, paired <i>t</i> -test (before and after) of percent predicted values	N = 109 exposed; N = 254 referent	Pre-shift <i>High</i> Cross-shift <i>High</i>
Imbus and Tochilin (1988)	76% and 84.5% of employees tested at each plant	Area samples of formaldehyde and wood dust on same day as pulmonary testing. Sampling protocol (# samples and sampling period) not described. Range in exposed	Spirometry protocol described (ATS); cross-shift change	Within person change; values presented as percent predicted; descriptive data on study group were not given. No unexposed referent group.	Provided data, no statistical analyses presented	Plant A N = 94; Plant B N = 82	<i>Not Informative</i> Reporting deficiencies

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		<0.012–0.074 mg/m ³					
Khamgaonkar and Fulare (1991)	Lab workers in college anatomy and histopathology departments; selected every 2nd person from occupational list.	Multiple 30min area samples in the breathing zone in exposed (N = 43) and unexposed (N = 18) areas. Range in exposed 0.044–2.79 mg/m ³ ; referent mean 0.125 mg/m ³ , range ND–0.64 mg/m ³ ; adequate exposure contrast likely for comparison of exposed and referent.	Spirometry protocol not described; measured on Monday. Selected every second person on list from each exposure group.	Comparison group matched by age and sex (N = 74). Comparable for mean height and weight; smoking prevalence: 54% exposed, 59% referent. Other exposures in lab	Mean absolute values (SD not reported) compared between exposed and referent; <i>p</i> -values reported	N = 37 exposed; N = 37 matched referent	<i>Medium</i> (↓) Possible exposures in referent that affect pulmonary function; exposure to formaldehyde in referent labs
Kilburn et al. (1985b)	Concern for selection bias toward overestimating association. 41% participation, volunteers, nonrandom selection of participants in exposed.	No formaldehyde concentration measurements.	Spirometry protocol described; testing before and after work shift	Potential noncomparability of batt makers and administrative employees, calculated % predicted using reference population. Possible exposure to other contaminants among batt makers	Preshift absolute values and percent predicted, and postshift absolute values by smoking status (SD not reported) among batt makers and referent group	N = 44 exposed; N = 26 referent	<i>Not Informative</i> Low participation and nonrandom selection of exposed; no formaldehyde measurements and possible co-exposures
Kilburn et al. (1989a)	Attendees at 4 national	Formaldehyde sampling in 10	Spirometry protocol described (ATS);	Questionable comparability to	Exposure group defined by	N = 280	<i>Not Informative</i>

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	conventions in 4 different cities between 1982 and 1986, compared to lung function in a Michigan population. Participation <40%; not clearly presented	labs in Los Angeles (not representative of entire sample); very wide range of concentration	percent of "referent" value	Michigan referent population; exposure both to formaldehyde and solvents; probable confounding by local air pollution in Anaheim, CA	histology technician; not specific to formaldehyde		No quantitative analyses specifically for formaldehyde
Levine et al. (1984c)	94% participation among morticians attending a required postgraduate course	No sampling measurements; Rank order using reported # embalmings. Comparison to funeral home records for 5 persons indicated # embalmings was over-reported.	Spirometric measures (ATS methods), % predicted	% predicted based on age and height; all males and Caucasian	Regression model of lung function in relation to exposure rank, adjusted for age, height, pack-years. Table 6 in the paper: mean % predicted (SD) comparing low and high rank category by smoking status, low and high rank matched by age, Student's t-test	N = 90	<i>Medium</i> Uncertainty regarding assignment to exposure rank
Löfstedt et al. (2009)	86% participation in exposed and 69% participation in referent. Healthy survivor effect	Personal samples on all exposed participants over a single 8-hr shift on same day as lung function testing. Range in exposed 0.014–1.6 mg/m ³ ; referent not	Spirometry protocol described (ATS methods), cross-shift change, percent predicted using Swedish reference; testing on day after 2 unexposed days	Referent from the same industry; older age and smoking prevalence higher in exposed. Important confounders addressed in analysis.	Regression models of association of change over shift with log formaldehyde level among exposed, adjusted for smoking on test day and co-exposure to ICA or	N = 64 exposed; N = 134 referent	Cross-shift <i>Medium</i> (↓) Potential for healthy survivor bias with attenuation in measure of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		reported; major exposure was to isocyanates, low correlation with formaldehyde concentrations			MIC (in 2 models); compared mean change in % predicted across shift between exposed and referent		
Löfstedt et al. (2011a) (follow-up of Lofstedt (2009)	90% participation in exposed and referent. Evidence of survivor bias: prevalence of childhood allergy lower among exposed in 2005 (4% versus 31%). Higher prevalence of nasal symptoms among referents in 2005.	Personal samples on all exposed participants over a single 8-hr shift on same day as lung function testing. Range in exposed in 2001: 0.014–0.44 mg/m ³ , range in exposed in 2005: 0.01–0.19 mg/m ³ ; referent not reported	Spirometry protocol described (ATS methods), cross-shift change, percent predicted using Swedish reference; testing on day after 2 unexposed d	Referent from the same industry; comparable for age; smoking prevalence and work duration higher in referent. Exposure to formaldehyde, methyl isocyanate (MIC) and isocyanic acid (ICA) among exposed; correlation between formaldehyde and isocyanates low. Analysis within each exposure group	Compared preshift percent predicted values (SD) from 2001 and 2005 and change between the years (SD) within exposed and referent (Student's t-test). Multiple regression of changes in percent predicted across shift adjusted for MIC, formaldehyde, smoking (pack-years), and childhood allergy; authors stated no significant association but quantitative results were not reported.	N = 25 exposed; N = 55 referent	Pre-shift 2001 to 2005 <i>Low</i> Limited sample size to detect small changes between 2001 and 2005; concern for survivor bias; Co-exposure to MIC & ICA in exposed—unable to differentiate for comparisons of change from 2001 to 2005. Cross-shift <i>Medium</i> (↓)
Main and Hogan (1983)	All administrative personnel (exposed) and all workers on payroll (police personnel) who did	Three 1-hour area samples (impingers), 4 occasions (August,	Spirometric measures (ATS methods); Percent predicted	Percent predicted, stratified by smoking status; potential dissimilarity	Percent predicted by exposure group and smoking status; t statistic	N = 14 exposed; N = 17 referent	Pre-shift <i>Low</i> Comparison groups selected from different sources (possible

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	not work in trailers (referent) who were still employed at end of 34-mo period.	September, December, April) always on a Monday. Range in exposed 0.15–1.97 mg/m ³ ; limited sampling period in closed structure with no point formaldehyde emissions; sampling and analytic protocols referenced; referent not reported		between administrative employees and police officers; ETS more common among referent	and <i>p</i> -value presented		unmeasured confounding), ETS in referent; small sample size (low sensitivity)
Malaka and Kodama (1990)	Male employees at plywood company. Exposed workers randomly selected with stratification by smoking status and work duration (<5 and ≥5 years; 93% participation), mean age 26.6 years; Unexposed group matched for age, ethnicity, and smoking status (53%), worked in areas where formaldehyde was not used, and had	Personal and area sampling, duration not reported; JEM (cumulative measure); range in exposed 0.27–4.28 mg/m ³ , referent 0.004–0.09 mg/m ³ ; sampled for dust; adequate exposure contrast likely for comparison of exposed and referent.	Spirometric measures (ATS methods); % predicted and absolute values tested on Monday and cross-shift	Referent from same company; matched on age, ethnicity and smoking; analyses adjusted for age, height, weight, cigarettes per day, and dust.	Percent predicted by category of cumulative exposure (none, low, high) using ANCOVA; Linear regression of absolute value on cumulative exposure adjusted for age, height, weight, cigarettes/day, and dust. Cross-shift change: means of absolute values compared before and after shift in exposed and	N = 93 exposed; N = 93 referent	Pre-shift <i>Medium</i> (↓) Potential for healthy survivor bias with attenuation in measure of association. Cross-shift <i>Medium</i> (↓)

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	no previous or current exposure to formaldehyde based on occupational histories; 93% participation rate. Current workers. Healthy survivor effect				referent, paired <i>t</i> -test		
Milton et al. (1996)	Evidence of selection of healthy workers (some refusals to avoid working in basement area); direction toward underestimation of effect	Personal sampling on each participant during 5–6 d of PEF measurement, 4 hrs on 2 d, same day as lung function testing; calculated 8-hr TWA. Range in exposed 0.0012–0.265 mg/m ³	Spirometry protocol described (ATS criteria); tested before and after work after 2 d off work and 2 other work d. PEF using mini-Wright peak flow meter, measurements 5 per d during and off work, 6 d at work and 4 d off. Self-reported PEF correlated with spirometric PEF (88 person-days before (<i>r</i> = 0.91) and after (<i>r</i> = 0.93) shift	Within person change, cross-over design, also adjusted for night shift and PEF at home, multiple exposures including to endotoxin, phenol resin, and formaldehyde. Concentrations were correlated—difficult to differentiate individual risk	PEF variability (high minus low for the day as percent of mean over all days). Linear regression of FEV ₁ and FVC and home amplitude percent mean PEF adjusted for smoking, pack-years of cigarettes, and years since start of exposure. Cross-shift PEF and overnight PEF, logistic regression of ≥5% decline in PEF or linear regression of change in PEF on natural log of formaldehyde; models were GEE to account for repeated measures	N = 37	<i>Not Informative</i> Potential for confounding prevents differentiation of effect of formaldehyde from effect of other exposure(s). Potential for healthy survivor bias with attenuation in measure of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Neghab et al. (2011)	Participation 100%. Healthy survivor effect	Area samples (40 min, $N = 7$) in 7 workshops and 1 area sample in office area. Range not reported, mean (SD) 0.96 (0.49); Sampling results for referent not reported; adequate exposure contrast likely for comparison of exposed and referent.	Spirometric measures (ATS methods); testing before and at end of shift on first working day of the week; percent predicted	Referent from the same industry and comparable socioeconomic and demographic status; % predicted based on age and height; all male	Pre-shift values (percent predicted) (SD) compared between exposed and referent (Student's t-test) Regression models of lung function and association with duration of exposure adjusted for age, height, weight, and smoking. Cross-shift: Pre-and postshift percent predicted compared (paired t-test), Not compared to referent.	$N = 70$ exposed; $N = 24$ referent	Pre-shift <i>Medium</i> (↓) Potential for healthy survivor bias with attenuation in measure of association. Cross-shift <i>Low</i> No comparison group
Nunn et al. (1990)	Follow-up complete (1980–1985) for 76% of exposed and 74% of referent. Attempted to include former employees; evidence of survivor bias	Area samples (1–6 hrs) 1979–1985, personal samples for representative set of exposed workers, 1985–1987, estimated prior to 1979. Range in exposed 0.1–2.46 mg/m^3 and above.	FEV_1 values ($\text{FEV}_1/\text{height}^3$), adjusted for height	Referent group from same factory but exposed to other potential irritants (phenolic and epoxy resins, carbon fibers) and phenol- and urea-formaldehyde. Stratified results by smoking	Regression of $\text{FEV}_1/\text{height}^3$ on time of screening visit for each worker, adjusting for age in 1980, smoking status in 1980 and 1985, maximum and mean exposure rank, and total duration of exposure. Presented mean	$N = 125$ exposed; $N = 95$ referent	<i>Medium</i> (↓) Concern for selection bias: loss to follow-up higher among exposed with low lung function compared to referent; referent exposed to other potential irritants.

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		Uncertainty regarding formaldehyde levels in referent not reported			slope (95% CI) by exposure (exposed and referent), and smoking status.		
Ostojić et al. (2006)	16 physicians and lab technicians exposed daily in pathology/ anatomy lab (employed >4 years), source of referent not described (all male, matched for age and height)	Assessment of formaldehyde exposure was not described. No concentration data reported; exposed defined by work in pathology/ anatomy lab	Spirometry protocol described; morning measurements; percent expected	Referent matched by age and stature, all nonsmokers	Compared percent predicted (mean, SD) in exposed and referent using Student's t-test	N = 16 exposed; N = 16 referent	<i>Not Informative</i> Formaldehyde measurement protocols and data not reported. Selection and description of referent group not described. Small sample size.
Pourmahabadi an et al. (2006)	Selection and participation of study groups not described.	Area samples, 8 hr average, not measured in referent	Spirometry protocol not described	Differences by group for age, length of service, height, sex, education, and smoking; no adjustment for age, height, sex, weight, or smoking	Absolute values preshift and postshift (mean, SD), and mean difference across shift (SD) compared between exposed and referent using t-test. No adjustment for age, height, sex, weight, or smoking	N = 124 exposed; N = 56 referent	<i>Not Informative</i> Selection and participation of study groups not described. Reporting deficiencies in descriptions of data collection; concern for confounding.
Schoenberg and Mitchell (1975)	Participation 94%; current workers. Healthy survival effect	Formaldehyde measurements taken by insurance company during same month; 0.5–1 mg/m ³ ; 3 breathing zone	Spirometric measures; measured before and after shift on Monday and Friday.	% predicted based on age, height, and gender; standardized for 15 pack-years cigarette smoking; multiple exposures (phenol)	Compared % predicted (adjusted for cigarette smoking) across categories of duration	N = 48 exposed; N = 15 referent	<i>Medium</i> (↓) Potential for healthy survivor bias with attenuation in measure of association. Multiple exposures: formaldehyde, phenol. Phenol is an irritant but may not be associated

Supplemental Information—Formaldehyde (Inhalation)

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		samples, 10.6–16.3 mg/m ³ ; exposed categorized by duration; additional exposure to phenol (5–10 mg/m ³ ; OSHA PEL 19 mg/m ³). Concentrations for “never on line” not reported; adequate exposure contrast likely for comparison of exposed and referent.					with pulmonary function at these levels. Small sample size in comparison groups.
Sripaiboonkij et al. (2009)	100% and 71% participation in exposed and referent	Area samples; #, dates and protocol not described	Spirometry protocol described	Models adjusted for age, sex, education, smoking, and ETS. Co-exposures to other irritants (glass microfibers) and sensitizers (phenol resin, mineral oils)	Exposure group defined by glass microfibers or sensitizing agents; not specific to formaldehyde	N = 19 exposed; N = 159 referent	<i>Not Informative</i> Exposure group defined by glass microfibers or sensitizing agents; not specific to formaldehyde; small sample size in exposed.
Tanveer et al. (1995)	49 male workers exposed to formaldehyde resins (mean duration 15.6 year) and 29 male referents (security and administrative	8-hr TWA 0.03 mg/m ³ ; exposure protocols and measurements not described. (<i>concerned that TWA value may</i>	Respiratory questionnaire, standardized MRC, and spirometry (ATS protocol); baseline in morning and at end of workshift	Exposed and referent comparable for age, height, smoking, and alcohol; co-exposures not discussed	Compared preshift % predicted, exposed and referent, means, by smoking status and duration of exposure, Student’s	N = 49 exposed; N = 29 referent	<i>Not Informative</i> Unable to assess exposure assessment or recruitment and selection protocol.

Reference	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	staff). Recruitment and participation not described. Healthy survivor effect possible	<i>be a typo because of comment in discussion stated that findings by Dally et al. at 0.33–1.7 ppm supported by this study at 0.03 mg/m³</i>	(cross-shift measured in 31 exposed and 22 referent)		t-test; compared cross-shift change		

Controlled Exposure Studies in Humans

In addition to including shading (as described above for epidemiology studies), the controlled exposure studies in humans include italicized aspects of the study design or analysis highlight less significant limitations that had a minor impact on confidence classifications.

Table B-34. Evaluation of formaldehyde - pulmonary function controlled human exposure studies

Reference and Confidence	Exposure assessment (details on exposure quality evaluation in Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size
Andersen (1979); Andersen and Molhave (1983) Confidence: <i>Low</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0.24, 0.4, 0.81, 1.61 mg/m ³ <i>Adequate</i>	Spirometric measures, methods not described	Random assignment to order of exposure, <i>blinding not described</i> . 31.2% smokers.	Within person comparison	Difference between baseline and exposure periods; ANOVA	N = 16
Day et al. (1984) Confidence: <i>Low</i>	UFFI off gas products, dynamic chamber, analytical concentrations reported <i>Good</i>	Spirometric measures; method described	Nonrandom exposure assignment, blinding not described.	Within person comparison; between group difference	Change between before and after exposure, paired t-test. Difference between groups, Wilcoxon rank sums	I: N = 9 II: N = 9

Supplemental Information—Formaldehyde (Inhalation)

Reference and Confidence	Exposure assessment (details on exposure quality evaluation in Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size
Green et al. (1987) Confidence: <i>Medium</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0, 3.69 mg/m ³ <i>Good</i>	Spirometric measures; methods and ATS criteria cited	Random assignment to order of exposure, <i>single blinded</i> .	Within person comparison	Ratio of exposed value at time(n) to time(0) to ratio of clean air value at time(n) to time(0); t-test	N = 22
Green et al. (1989) Confidence: <i>Medium</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0, 3.69 mg/m ³ <i>Good</i>	Spirometric measures; methods and ATS criteria cited	Random assignment to order of exposure, double blinded.	Within person comparison	Ratio of exposed value at time(n) to time(0) to ratio of clean air value at time(n) to time(0); two-way ANOVA for repeated measures	N = 24
Kulle et al. (1987) Confidence: <i>Low</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported; I: 0, 0.62, 1.23, 2.46, II: 0, 1.23, 3.69 mg/m ³ <i>Good</i>	Spirometric measures; methods and ATS criteria cited	Random assignment to order of exposure, <i>blinding not described</i> .	Within person comparison	Ratio of exposed value at time(n) to time(0) to ratio of clean air value at time(n) to time(0); ANOVA	I: N=10; II: N=9
Lang et al. (2008) Confidence: <i>Low</i>	Paraformaldehyde, “quasi-static” chamber conditions, analytical concentrations reported; 0, 0.19, 0.37, 0.62, peaks to 1.23 mg/m ³ <i>Adequate</i>	Spirometric measures; method described	Random assignment to order of exposure, double blinded.	Within person comparison	Difference before and after exposure on day 10, Wilcoxon ranked sum test; Data and results of analysis were not reported.	N = 21
Sauder et al. (1986) Confidence: <i>Low</i>	Paraformaldehyde, dynamic chamber, analytical concentrations reported; 0, 3.69 mg/m ³ <i>Good</i>	Spirometric measures; methods and ATS criteria cited	Nonrandom exposure assignment, blinding not described.	Within person comparison	Ratio of exposed value at time(n) to time (0) to ratio of clean air value at time(n) to time(0); ANOVA	N = 9
Witek et al. (1986) ; Schachter et al. (1986a) Confidence: <i>Medium</i>	Paraformaldehyde over boiling 2-propanol, dynamic chamber, analytical concentrations reported	Expressed as % of predicted (mean, SD); method citation provided.	Random assignment to order of exposure, double blinded.	Within person comparison	Percent change from baseline; t-test	N = 15

Supplemental Information—Formaldehyde (Inhalation)

Reference and Confidence	Exposure assessment (details on exposure quality evaluation in Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size
	<i>Adequate</i>					
Schachter et al. (1987) Confidence: <i>Medium</i>	Paraformaldehyde over boiling 2-propanol, dynamic chamber, analytical concentrations reported.; 0, 2.46 mg/m ³ <i>Adequate</i>	Expressed as % of predicted (mean, SD); method citation provided.	Random assignment to order of exposure, double blinded. Participants had routine occupational formaldehyde exposure, <i>N</i> = 2 smokers.	Within person comparison	Percent change from baseline; t-test	<i>N</i> = 15

B.3.4. Immune-Mediated Conditions Including Allergies and Asthma

Details of the study evaluation approach methods are provided in Section 2.3.4 of the Toxicological Review. As discussed in that section, these reviews focused on human epidemiology and controlled human exposure studies, with animal hypersensitivity studies informing MOA (see Appendix B.3.6). Within the separate tables documenting the results of the study evaluations for observational epidemiology studies of allergies and asthma (see Table B-35), epidemiology studies of lower respiratory symptoms in infants and young children (see Table B-36), and controlled exposure studies in persons with asthma (see Table B-37), studies are arranged alphabetically. Gray shading is indicative of aspects of the study design or analysis that decreased EPA's confidence rating. EPA consulted with two groups of experts regarding issues pertaining to ascertainment of allergy sensitization and allergies in epidemiology studies and of asthma (see Section 2.3.4 of the Toxicological review).

Epidemiology Studies of Allergies and Asthma

Table B-35. Evaluation of allergy and asthma studies

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Annesi-Maesano et al. (2012) (France) Schools: children (prevalence survey)	Schools randomly selected from defined geographic area, ages 9–10 years. Participation rate 81% in initial survey, 69% with full protocol.	5-d samples in classrooms; sampling from 108 schools; all classes of specified grade level per school. Median (75th percentile) 0.027 (0.034) Maximum 0.055 mg/m ³ (estimated from figure). Tertiles (mg/m ³) Low ≤ 0.019 Medium > 9.1 - ≤ 28.4 High > 28.4 Protocol discussed.	ISAAC questionnaire Allergy: “sneezing and runny nose accompanied by itchy eyes out of cold in the past year” Asthma: asthma in past year (wheezing or whistling in the chest or wheezing or whistling in the chest at night-time or taken asthma treatment in the past year) Exercise induced asthma based on response to pulmonary function testing after exercise protocol. Exposure measurement blinded to outcome classification	Adjusted for age, gender, passive smoking, and paternal or maternal history of asthma and allergic diseases. Also examined dampness, gas appliances, ethnicity, socioeconomic status, and season. Other measures included: NO _x , PM _{2.5} , acetaldehyde, acrolein. No other pollutants associated with rhinoconjunctivitis. PM _{2.5} and acrolein were associated with asthma.	Generalized estimating equation modeling, accounting for nonindependence of observations within-area (schools) environment, including climate. OR (95% CI) (CI estimated from figure). Models took into account within city correlations among participants. Additional stratification of asthma analysis by atopy status. Sensitivity analysis: exercise induced asthma limited to measures in same week (n = 4,643)	N = 6,683	Allergy (rhinoconjunctivitis) and Asthma <i>High</i>
Billionnet et al. (2011) (France) Residences: adults	Nationally representative sample of residences (Indoor Air Quality Observatory study);	1-wk sample in bedroom; Median, 75th percentile (minimum, maximum)	ISAAC questionnaire: Rhinitis based on self-report of, in the past 12 mos, sneezing, running or blocked nose without	Covariates chosen if associated with asthma or rhinitis and affecting one or more effect estimates for	Generalized estimating equation modeling, accounting for nonindependence of within-area	N = 1,012 38.3% rhinitis 8.6% asthma	Allergy (rhinitis) and asthma <i>Medium</i> Low participation rate but potential for differential participation (by

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(prevalence study) October 2003–December 2005	13.6% participation rate (567 of 4,165 households). Low participation rate; no analysis of non-participants. Age range 15–89; median 44 years.	0.0194, 0.028 (0.013, 0.0863) mg/m ³ . None < LOD. Protocol discussed.	cold or respiratory infection. ECRHS: Asthma based on one of following criteria: (i) having an asthma attack in the last 12 mos; (ii) having been woken by an attack of shortness of breath in the last 12 mos; and (iii) currently using asthma medicine. Exposure measurement blinded to outcome classification	volatile organic compound exposure measures by 20% or more. Acrolein was not associated with asthma (OR 0.83). Adjusted for age, gender, smoking, education, relative humidity, time of survey, pets, mold, outdoor pollution sources within 500 meters. Did not specifically address correlation between formaldehyde and other exposures (other than noting that these were not among the higher correlations seen).	(dwellings) observations. OR (95% CI) (estimated from figure). Additional models took into account within dwelling correlations among participants. Compared nonparticipants (pollutant measures but no health questionnaire) and participants. Sensitivity analysis excluding relatives.		formaldehyde exposure and disease status) uncertain.
Branco et al. (2020a) (Portugul) School: children (prevalence study) 2013–2016	A total of 1,530 preschoolers (n = 648 3–5 years) and primary school children (n=882 6–10 years) were randomly recruited from urban and rural nursery (n =	Daily exposure based on time-averaged air concentration and reported time in specific school locations. Continuous monitoring in	The ISAAC questionnaire was completed by parents or guardians, which were validated by physicians. Spirometry measurements were taken in participants identified as asthmatic from the questionnaire	Potential confounders selected based on previous experience and included site (urban, rural), study phase, sex, age group, BMI	Multivariate logistic regression for each individual pollutant as continuous variable (per IQR) or dichotomized using median, or regulatory cutoffs. Models also for all	N = 1,530	Asthma diagnosis Dichotomized analysis: <i>Medium</i> Low participation rate, but potential for differential participation (by formaldehyde exposure and disease status) uncertain.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	17) and primary schools (n = 8) participating in the INAIRCHILD project. There were 2 phases in 2013/2014 and 2015/2016. Children <3 years were excluded. Participants represented 39% of the original sample. No comparisons of participants and nonparticipants. 42% were aged 3–5 years but no information on the proportion that was 3–4 years. with less specific asthma diagnosis. PFT was only conducted in the 49% who reported wheezing or asthma diagnosis. Missing PFT data for 269 of 763 selected (35%).	each room (24 hr to 9 d) (Branco et al., 2019). Time-activity obtained from parents' 24-hr daily diary, class timetables and teachers. Inhaled daily dose estimated using time-averaged exposure, inhalation rate for each activity and body weight. Mean HCHO concentration (SD) 35.3 (43.1) $\mu\text{g}/\text{m}^3$; 25 th percentile, Median, 75 th percentile: 0.0017, 0.0225, 0.0646 mg/m^3 Table in article also stated that these values were the median (IQR), but clarified with the author that the values	responses or reporting ever having one or more asthmatic symptoms (wheezing, dyspnea, or nocturnal cough with no upper respiratory infection) (of 763, missing or failed in 269). Spirometry before and after bronchodilator using ERS/ATS and Global Initiative for Asthma guidelines conducted by pediatric doctors with pulmonary specialization. Methods and QA described. Asthma diagnosed based on symptoms (≥ 1) and PFT results using GINA guidelines. Skin prick tests conducted on children with PFT results using several aeroallergens (n = 341, missing or failed for 153). Outcomes: reported active wheezing in last 12 mos (relevant to pre-schoolers) or reported asthma (does child have or ever had asthma?), followed by asthma diagnosis based on study physicians, FEV1/FVC	and parental history of asthma. Also controlled for surrogates of home indoor exposure including mother's education, living with smoker. Other covariates for contact with farm animals during 1 st year of life, pets at home in previous year &/or 1 st year of	pollutants simultaneously.		Continuous variable analysis: <i>Low</i> For continuous variable analysis: Uncertainty regarding interpretation of analysis as a continuous variable because of bimodal distribution.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		are mean and SD; author also provided data for 25 th and 75 th percentiles (email correspondence to Dr. Glinda Cooper) (Branco et al., 2020b)	<0.90, reduced FEV1 (<80% predicted), asthma diagnosed in 5.5%, asthma with or without aeroallergen sensitization, and no asthma.				
Choi et al. (2009) (Korea) Residences: children (and adults?) (case-control study) March–June 2006	Conducted in university outpatient clinic; recruitment procedure for cases or controls not described. Mean age cases 15.4 years (SD = 3.4; controls 16.2 years (SD = 4.1)	Household sample in living room at location away from sources of VOCs (sampling period not reported, but closed windows, no smoking or use of potential sources, and use of duplicates). Geometric mean 0.043 mg/m ³ , 75 th percentile 0.115 mg/m ³ in controls % above LOD: 92.3 controls 94.4 atopic dermatitis cases 100 asthma cases	Atopic dermatitis and allergic asthma: based on medical history, skin prick test and IgE (criteria not provided)	No information on socioeconomic status; higher percentage of cases lived near roads or in industrial area (21%, 34%, 44% of controls, dermatitis, and asthma cases, respectively). Housing age <3 years old in 29%, 40%, and 58% in controls, dermatitis, and asthma cases, respectively; and 50%, 56%, and 72% of controls, dermatitis cases and asthma cases lived in apartments.	Nonparametric (Mann-Whitney) comparison of formaldehyde by group; geometric mean, 25 th , and 75 th percentiles reported.	N = 50 atopic dermatitis cases, 36 asthma cases, 28 controls	Allergy (atopic dermatitis) and asthma <i>Low</i> Selection and recruitment process not reported; sampling period not reported and specific criteria for case definition not reported; potential confounders (age and type of housing and location differed between cases and controls, as measure of socioeconomic status) not addressed. Limited analysis.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Dannemiller et al. (2013) (United States) Residences: children (asthma control) July 2008–February 2010	Low-income homes in Boston, recruited from past allergy cohorts, asthma clinics, newspaper ads, and referrals from other participants. (Boston Allergen Sampling Study). 79% (37 out of 47) participated in this analysis. Mean age 10.5 years. Boston Allergen Sampling Study. Related reference on sampling: Sandel et al. (2014)	30-minute pumped air sample in kitchen. Median 0.044 mg/m ³ ; 31% >0.060 mg/m ³ ; maximum = 0.162 mg/m ³ . None < LOD. Protocol discussed; analysis of sources of exposure	Asthma control (5 questions) [based on validated questionnaire]; symptoms and inhaler use in past 4 wks	Examined season, temperature, and relative humidity as well as other potential confounders (<i>email from Karen Dannemiller to Glinda Cooper, May 6, 2015</i>)	Log ₁₀ -transformed formaldehyde; <i>t</i> -tests.	N = 37 asthma cases (out of 47 children in study, 79%)	Asthma control <i>Medium</i> Recruitment was not from a well-defined population. Limited exposure measurement period (but quality control details provided and none were < LOD).
Fransman et al. (2003) (New Zealand) Wood workers (prevalence study)	Plywood mill workers, participation rate 66%. Internal comparison by exposure level. Mean duration 4.7 years in mill, 2.7 years in current job. Workers' knowledge of formaldehyde exposure not discussed.	Personal samples (n = 22, 15-min samples); above 0.100 (geometric mean 0.260 mg/m ³). Limit of detection 0.030 mg/m ³ .	Allergy symptoms: self-report of sensitivity to house dust, food, animals or grasses/plants.	Allergy and asthma analysis: Adjusted for age, gender, ethnicity, and smoking for comparisons between high and low exposure within workplace. Weaker association seen with terpenes. Inhalable dust, abietic acid, and endotoxin also measured but not	Logistic regression, OR (95% CI)	N = 112	Allergy (allergy symptoms) <i>Low</i> Uncertain impact of outcome classification (inclusion of food allergies) and uncertainty regarding details of analysis; see asthma discussion for other limitations Asthma <i>Medium (↓)</i> Selection out of the exposed work force of "affecteds" possible in this type of prevalence study. "Low"

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
				clear if these were considered in the analysis of the allergy symptoms data			exposure group exposed to levels of formaldehyde up to 0.080 mg/m ³ . Either limitation could result in reduced (attenuated) effect estimate.
			Asthma: Current asthma medication use; past 12 mos, asthma attack or being woken by shortness of breath	Weaker association seen with terpenes. Inhalable dust, abietic acid, and endotoxin also measured but not clear if these were considered in the analysis of the allergy symptoms data			
Garrett et al. (1999a, 1999b) (Australia) Residences: children (prevalence study)	Combined analysis of cases and controls from a case-control study of asthma in 2 rural towns. Recruitment through schools and medical centers; additional advertisement for nonasthmatic children. 30 of the 95 controls were from same households as cases; the 65 other controls were from	4-day household samples (4 seasons), multiple locations; up to 0.139 mg/m ³ . Protocol discussed. Separate paper about exposure measures. 74% of children had lived in same house for at least 5 years.	Allergy and asthma: Exposure measurement blinded to outcome classification. Allergy: 12 allergen skin prick test (cat, dog, grass mix #7, Bermuda grass, house dust, 2 dust mite, 5 fungi). Asthma: Parent report of doctor-diagnosed asthma. Mean score 4.6 in asthma	Adjusted for parental asthma history, sex; other factors examined but not needed in final model (passive smoke, pets, indoor NO ₂ , fungal spores, house dust mite allergens)	Prevalence (n, %) by exposure group; logistic regression, OR (95% CI); figure showing wheal size and number of positive responses by exposure group. Evaluated relation between formaldehyde and NO _x , house dust, fungal spores, housing age. For asthma, incomplete reporting of results (adjusted results)	N = 145 in allergy analysis; 53 cases, and 95 controls in asthma case-control analysis	Allergy (skin prick tests) <i>Medium</i> (↓) Uncertainty about effect of recruitment process and ability to fully address household correlation of cases and controls; could result in attenuated effect estimate. Asthma <i>Low</i> Uncertainty about effect of recruitment process and ability to fully address household correlation of cases and controls; could result in attenuated effect

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	37 households. Ages 7–14 years.		cases, 0.7 in controls on respiratory symptom questionnaire completed at last home visit (symptom frequency, 4 categories, over past year of: cough, cough in the morning, shortness of breath, waking due to shortness of breath, wheeze/ whistling, asthma attacks, chest tightness, and chest tightness in the morning).		reported as “not statistically significant”)		estimate. Incomplete reporting of results
Herbert et al. (1994) (Canada) Wood workers (prevalence study) Related reference: Herbert et al. (1995)	Oriented strand board manufacturing, mean duration 5.1 years. Referent group = oil field workers, not exposed to gas or vapors, mean duration 10.0 years. Participation rate 98% in workers, 82% in comparison group. 99 exposed, 165 referents. Because both groups are “exposed” workers, healthy worker effect unlikely. Some uncertainty	Area samples. 21 hrs continuous sampling on 2 separate d); range 0.090 to 0.330 mg/m ³	Allergy and asthma: Exposure measurement blinded to outcome classification Allergy: 6 allergen skin prick test (wheat, rye, Alternaria, cat, house dust, birch). Asthma: International Union Against Tuberculosis and Lung Disease (1986) questionnaire, described and validated in (Ravault and Kauffmann, 2001): (asthma; lower respiratory tract symptoms (list includes woken by shortness of	Possible respiratory irritants in comparison group (oil sands workers); Adjusted for age and smoking; dust measured and reported as low, not included in analysis	Logistic regression, OR (95% CI); prevalence of “outcome” (positive responders) not reported	N = 99 exposed; 165 referents	Allergy (skin prick tests) <i>Low</i> Selection out of the exposed work force of “affecteds” possible in this type of prevalence study. Uncertainty about exposures in referent group. Uncertainty about time window of exposure measurement with respect to skin prick test results in adults. Asthma <i>Medium (↓)</i> Selection out of the exposed work force of “affecteds” possible in this type of prevalence study, and Uncertainty about exposures in referent group. Either

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	about effect of exposures in the referent group		breath; attacks of wheeze, wheeze with chest tightness.) [increased prevalence of lower respiratory tract symptoms associated with lower FEV ₁ or FEV ₁ /FVC in these workers]. Time frame of asthma definition interpreted to be relevant				limitation could result in reduced (attenuated) effect estimate.
Holness and Nethercott (1989) (Canada) Funeral home workers (prevalence study)	Participants recruited from list of funeral homes, 86.6% participation; 79.8% of embalmers were active embalmers (healthy workers); community referent (service organization and students)—potential differences (weight, smoking)	2 area samples (impingers), during embalming, 30 to 180 min. Range in exposed 0.10–1.0 mg/m ³ , referent mean 0.025 mg/m ³ ; adequate exposure contrast likely for comparison of exposed and referent.	American Thoracic Society (Ferris, 1978) questionnaire: wheeze (no details of questions)	Univariate analysis; did not consider other variables and referent may not be comparable to exposed with respect to possible confounders	Frequency by group and <i>p</i> -value from a logistic regression	N = 84 exposed; N = 38 referents	Asthma <i>Low</i> Uncertainty regarding asthma definition. Differences in source populations for exposed and referent groups lead to uncertainty in comparability. No consideration of potential confounding.
Hsu et al. (2012) (Taiwan) Residences: children (case-control)	Initially recr through randomly selected kindergartens and day care centers; Mean age 7.0 years. 73% of successfully	2-hr household sample (probably bedroom); Median 0.076 mg/m ³ ; 75 th	Initial screening through parent report of history of 2 or more diseases (asthma, allergic rhinitis) or symptoms (wheezing, coughing at night, eczema, sneezing, runny	None addressed in analysis. Similar season distribution in cases and controls	Mann-Whitney U test for case-control differences in exposure distribution. Median, 25 th and 75 th percentiles	N = 48 allergic rhinitis, 36 eczema, 9 asthma cases, and 42 controls	Allergy (rhinitis, eczema) and asthma <i>Low</i> Low and differential (at various steps) participation rate. Limited exposure

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
August 2008–September 2009	contacted agreed to send questionnaires to families and 68% of the questionnaires were completed. Selected for follow-up if had not moved or renovated house since birth. Of the 980 potential cases and 802 potential controls selected, 267 (27%) and 89 (11%) participated in clinical exam; 59 cases and 42 controls (22% and 47% of cases and controls, respectively, completing exam) also completed home exposure measures.	percentile 0.25 mg/m ³ ; Limited sampling period with no information on protocol.	or stuffy nose) during last 12 mos; confirmation of asthma, rhinitis, and eczema by clinical examination. Controls answered “no” to all of the disease and symptom questions. Exposure measurement blinded to outcome classification		given for cases and controls. <i>P</i> -values reported if <0.10. No additional modeling of the formaldehyde data undertaken		sampling period and no information on protocol. In addition, small sample size (<i>n</i> = 9) for asthma.
Huang et al. (2017) (Shanghai, China) Residences: children (case-control) March 2013–December 2014	Participants in a previous cross-sectional study (2011–2012) selected from 88 kindergartens located in 6 Shanghai districts (note: references for cross-sectional study stated 72	Continuous formaldehyde sampling in child’s bedroom, 24 hrs, in breathing zone (detection range: 0.012–0.08 mg/m ³). Mean (SD) concentration	ISAAC questionnaire, translated (selected questions) Current rhinitis: In the past 12 mos, has your child had a problem with sneezing, or a runny, or a blocked nose when he/she did not have a cold or the flu?	Covariates considered in models based on literature and previous analyses, included age, sex, family history of atopy, family annual income level, household ETS, household	Differences between cases and controls compared using Kolmogorov-Smirnov test. Multiple logistic regression models per IQR increment or quartile of formaldehyde concentration.	N = 409	Current rhinitis <i>Medium</i> Low participation rate, but potential for differential participation (by formaldehyde exposure and disease status) uncertain; uncertainty regarding validation of truncated version of ISAAC

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	kindergartens selected in 5 districts, N = 14,884). Included if homes were not renovated in the previous 2 years and agreed to an on-site home inspection, N = 454 residences, 4.5% of cross-sectional survey for 10,182 participants with contact information (409 of 454 residences assessed), 5–10 years old.	(mg/m ³), 24-hr 0.0215 ± 0.0130; 75th percentile 0.0275 mg/m ³ Range 0.006 – 0.06 mg/m ³ , with 3 bedrooms higher	Current eczema: In the past 12 mos, has your child had an itchy rash (eczema), which was coming and going for the last 6 mos? Current wheeze: Has your child had wheezing or whistling in the chest in the last 12 mos?	dampness-related exposures, antibiotics exposure during 1 st year of life, home decoration around time of birth, season of sampling. Higher proportion of homes with mechanical ventilation among current rhinitis cases compared to controls (77.5% versus 65%)			questionnaire in this population Eczema and Asthma <i>Low</i> Low participation rate, but potential for differential participation (by formaldehyde exposure and disease status) uncertain; larger uncertainty regarding truncated questions.
Hulin et al. (2010) (France) Residences: children (case-control)	2 samples: urban area, French 6 Cities Study (ISAAC). Random selection of 18 schools; nested case-control study of asthma Rural area; nested case-control study of asthma (FERMA) (rural sampling for regular contact with farm animals) Examined nonparticipants	7-d sample in living room. Protocol discussed. Median 0.019 mg/m ³ , maximum 0.075 mg/m ³	Ever asthma and current asthma (parent report of use of asthma medications or wheezing in past 12 mos). Exposure measurement blinded to outcome classification	Adjusted for age, sex, family history of allergy, passive smoke exposure during childhood, allergic rhinitis, and season. Considered nonindependence of participants in similar neighborhood. Assessed collinearity with other measures (NO _x , PM _{2.5})	OR (95% CI) by above and below median. Also included analysis stratified by location (urban, rural)	Urban: (N = 32 cases, 31 controls). Rural: (N = 24 cases, 27 controls). Combined: N = 56 cases, 58 controls (but 9 rural and 7 urban excluded, unspecified	Asthma <i>Low</i> Small sample size and uncertain interpretation of the stratified analyses (and unspecified n in analysis of current asthma).

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
						number excluded from analysis limited to current asthma	
Hwang et al. (2011) (Korea) Residences: children (case-control) May 2008	Case-control study, drawn from 1,005 elementary students (one school, all grades) (84% participation rate). 33 cases (out of 129?) and 40 controls (out of unspecified number) agreed to participate in environmental measurement study. Controls selected from respondents with no asthma symptoms or diagnosis, age and sex matched to cases.	3-d household sample (2 rooms) and personal sample. Geometric mean, controls: 0.036 mg/m ³ (no information on upper distribution reported).	Self-report asthma symptoms or physician-diagnosed asthma based on ISAAC questionnaire (no mention of modification or validation for this population)	Adjusted for age, gender, income, parents' education, passive smoking	Log-transformed; logistic regression, OR (95% CI)	N = 33 cases, 40 controls	Asthma <i>Low</i> Asthma definition does not distinguish between current asthma and ever asthma. Uncertainty regarding selection processes [high prevalence of family history of asthma in cases (86%) and controls (96%)]; uncertainty about analysis and distribution of formaldehyde levels.
Isa et al. (2020a) (Malaysia) Schools: children	8 randomly selected schools in Hulu Langat, Selangor, Malaysia, randomly selected students from 4	Formaldehyde concentrations measured during class time using PPM Formalde-meter	Asthma & allergy information and symptoms within defined period using ECRHS and ISAAC questionnaires.	Regression models controlled for atopy, sex, doctor's diagnosed asthma, parental asthma/ allergic	2-level hierarchic multiple logistic regression (school and student), OR (95% CI), adjusting for personal factors	N = 470	Allergy (rhinitis, dermal, skin prick tests) <i>Low</i> Uncertainty in exposure concentrations and distribution given short

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(prevalence study) August–November 2018 & February 2019	classes (Form 2, aged 14 years). Data collected Nov 2018–February 2019. Excluded students reporting smoking in last 12 months or treated with antibiotics in last 4 wks. Participation rate not reported but was 96% in similar study in other parts of Malaysia using similar design [Morback 2017 ref]	(accuracy of 10% at 2 ppm). Monitors 1 meter from ground in center, four 1-hr periods. Concentration (reported as mg/m ³ , but appears to have been µg/m ³) median (IQR) Urban 13.2 (9.3); Suburban 3.1 (5.2) Uncertainty in concentrations given short sampling duration	Responses were blind to environmental data. Allergy skin prick test for mites, fungi and cat allergens after 15 min measuring wheal diameter (atopy defined as ≥ 3 mm). Respiratory symptoms in last 12 mos: wheezing, daytime breathlessness, nocturnal attacks of breathlessness. Allergic symptoms in last 12 mos: rhinitis, skin allergy.	and location of schools.	and environmental pollutants associated with each outcome.		sampling duration; very low concentrations in half (suburban) schools.
Kim et al. (2011) (Korea) Schools: children (prevalence study) November–December 2004	12 schools, 2–3 randomly selected classrooms per school Participation rate 96%; 450 excluded based on missing data)	7-d samples in classrooms. Mean (±SD), (minimum, maximum) 0.028 (±0.0083) 0.016, 0.047 mg/m ³ . Protocol discussed, closed windows.	Current medication use or had asthma attack in past 12 mos. Exposure measurement blinded to outcome classification	Adjusted for age, sex, self-reported pet or pollen allergy, environmental tobacco smoke at home, other home environment (indoor dampness, remodeling, changing floor, age of home). All samples within same season.	Logistic regression, OR (95% CI) per 10 µg/m ³ increase; additional modeling to account for within school and within city correlations.	N = 1,028	Asthma <i>High</i>

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Krzyzanowski et al. (1990) (United States, Arizona) Residences: adults, children (prevalence study) Supplemental references: Quackenboss et al. (1989a) ; Quackenboss et al. (1989b)	Selected from 202 households (stratified sample from municipal employees). 2,322 completed baseline survey; subgroups selected based on housing characteristics (type, age, remodeling). Clusters within similar outdoor PM and pollen levels. Participation rate not reported but sampled nonresponders: higher proportion of current smokers among refusals (35% versus 27%)	Two 1-wk household samples (different seasons), multiple locations; Mean 0.032 mg/m ³ ; maximum 0.172 mg/m ³ (most <0.074, only a few above 0.110 mg/m ³) Protocol discussed (separate paper).	Asthma: American Thoracic Society (Ferris, 1978) questionnaire; doctor-diagnosed asthma (ever and current) and symptom questions: wheezing apart from colds, 2 or more attacks of shortness of breath with wheezing in last year. Exposure measurement blinded to outcome classification	Environmental tobacco smoke. Also examined NO ₂	Contingency tables, stratified by age group and for children, by environmental tobacco smoke exposure. For adults, incomplete reporting of results.	Adults: N = 613 Children: N = 298	Asthma, children and adults <i>Medium</i> For children, relatively small # (n = 21) in higher exposure categories. For adults, incomplete reporting of results.
Lajoie et al. (2014) (Quebec, Canada) Intervention study October 2008 – June 2011	Asthmatic children with exacerbation requiring medical care in the past year referred by physicians at tertiary care center, 3–12 years old, mean age 6 years (n = 83, 71.5% of those meeting inclusion criteria) in homes with low	Pre- and post-intervention. Passive air sampling for formaldehyde in bedroom, 6–8 d, during winter and summer seasons. Other measurements for NO ₂ , VOCs, dust, house dust mites, cat and	Variable number with complete data for each outcome. Participants were not blinded, although technicians were. Formaldehyde-specific Intervention/Control Proportion with ≥ 1 episode of wheezing over last 12 mos, ISAAC questionnaire	Potential confounders for asthma outcomes were age, gender, parents' level of education, and eczema. Comparing baseline concentrations formaldehyde, NO ₂ , and dust mites were	Power calculation reported. Multivariate linear models Formaldehyde analyses used results in intervention group only. Change from year 1 to year 2 in prevalence of asthma symptoms and medical care in	For ISAAC questionnaire, intervention N = 43, control = 39	Current asthma symptoms <i>Medium</i> Other coexposures that have been associated with asthma symptoms also declined in intervention group (toluene, ethylbenzene, styrene, limonene, alpha-pinene, airborne mold spores, although formaldehyde reduction was greatest

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	ventilation rates (<0.30 ACH). Randomly assigned to intervention to increase ventilation rates by 0.15 ACH (n = 43) and control (n = 40).	dog allergens, airborne mold spores. In controls, mean 0.037 mg/m ³ 25 th percentile 0.027 mg/m ³ 75 th percentile 0.051 mg/m ³	administered to parents: 43/39; Mean number of days with asthma symptoms per 14 d period (≥ 1 coughing, wheezing, chest tightness, disturbed sleep or trouble breathing Symptoms diary: 37/32; administered to parents 2 wks per month from November–March in 2010 and 2011; Asthma control over one month, Asthma quiz: 31/25;	comparable, Toluene and mold spores were higher in intervention group. Comparing year 1 to 2, reductions in formaldehyde, toluene, styrene, limonene, and alpha-pinene, airborne mold spore concentrations were significantly different for intervention group compared to control. NO ₂ concentrations increased. Allergens in mattress and rugs in bedroom did not change.	the past year associated with a 50% reduction in formaldehyde concentration analyzed using mixed linear models with repeated measures		
Liu et al. (2018a) (China) Hospital based case-control: children September 2016 to March 2017	Recruited 180 children with an asthma diagnosis from hospital and 180 healthy controls in same city (Changchun) during September 2016 to March 2017; mean age 10	Indoor area samplers placed 1–1.5 meters above ground, doors and windows closed 12 hrs prior. HCHO sampled in living room and bedroom	Current asthma symptoms (2 or more of cough, wheezing, and dyspnea for three or more consecutive days) based on ISAAC questionnaire; responses (2 or more incidents of cough, wheezing, and dyspnea for 3 or more	History of allergy, breast feeding, ETS and indoor plants were associated with asthma status. Included in model with PM _{2.5} and HCHO. Sex, mean age, mean BMI and race were	Associations with pollutant concentration (quartiles, entered as a single variable) analyzed with multivariate regression. Coexposures to PM and NO ₂ were	N = 180 cases; 180 controls	Current asthma symptoms <i>Medium</i> Uncertainty regarding interpretation of formaldehyde as a single variable representing 4 quartiles.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	years. Information on participation rate not provided but the “pass rate” of the questionnaire was 100% and equal number of cases and controls. Children excluded if medical treatment with vitamins or antibiotics within 3 mo, severe organ failure (heart, renal and other serious disorders).	with QC-2B sampler, Beijing Municipal Institute of Labor Protection method. Citation for method provided. Sampling period was 2 mos. Median (range) $\mu\text{g}/\text{m}^3$ HCHO Asthma 38.35 (12.04 – 142.12) Control 25.11 (12.26–94.34) NO_2 and PM also measured.	consecutive d). In addition, FEV_1 increased by >15% after β -agonist inhalation and persistent asthma was stable for 3 or more mos prior to study. Serum miR-155 also used as measure of asthma.	comparable between cases and controls.	simultaneously controlled.		
Madureira et al. (2016) (Porto, Portugal) Children, case-control, October 2012–April 2013	Random recruitment of 38 residences among asthmatic children and 30 residences among nonasthmatic children previously identified in a cross-sectional study (Madureira et al., 2015). Mean age 8.5 years. Excluded respondents with a recent renovation or who had moved	Continuous passive sampling for formaldehyde and other VOCs and aldehydes in bedroom over 7 d; PM _{2.5} , PM ₁₀ , bacteria, fungi, carbon dioxide (CO_2), temperature and relative humidity levels also measured both indoors	For asthma cases, parents responded yes to both of 2 questions in ISAAC questionnaire: (1) Has your child ever had asthma diagnosed by a doctor? and (2) In the past 12 mos, has your child had wheezing or whistling in the chest? Parents of controls responded no to both questions.	Higher proportion of cases were boys. Comparable for age, BMI and parental education level, family history of allergic disorders and number of siblings was slightly higher in cases. No other chemical or biological risk factors differed between groups	Concentrations (7-d means) compared between groups (unclear what test was used, other than Shapiro-Wilk test for normality before comparison. Differences in temperature and relative humidity not addressed in analysis.	Cases N = 38 Controls N = 30	Current Asthma <i>Low</i> Potential for selection bias, with greater environmental controls among asthmatic families. Differences in temperature and relative humidity not addressed in analysis.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	since responding. No information comparing participants to nonparticipants. Potential exists for selection bias with greater environmental controls among asthmatic families. Although extent of bias impact unknown, TVOCs, acetaldehyde and ventilation rates higher in control homes, but not PM or bacteria and fungi counts.	and outdoors. Sampling and analysis methods described. Formaldehyde concentrations all above the detection limit. Controls mean (sd) 0.016 (0.009) mg/m ³ 25th percentile 0.009 mg/m ³ 75th percentile 0.022 mg/m ³ Median 0.015 mg/m ³		(except limonene was higher in control).			
Malaka and Kodama (1990) (Indonesia) Wood workers (prevalence study)	Plywood mill workers, random sample of exposed workers (based on measurements), stratified by smoking, work duration (<, ≥ 5 years), (random sampling process not specified). Random sample of nonexposed (defined based on area measures and job history),	Personal and area samples (duration not reported); above 200 (mean 910, up to 3,480 µg/m ³). Nonexposed areas based on measurements (e.g., warehouse, saw mill)	American Thoracic Society (Ferris, 1978) questionnaire. Asthma defined as “Ever had attack of wheezing that made you feel short of breath?” or ever had asthma and if so, do you currently have asthma? Also included “occupational asthma” (not defined). Since purpose of study was the impact of occupational exposure, asthma definition is interpreted	Adjusted for age, smoking, dust	Percent by exposure status, OR, <i>p</i> -value 95% CI not reported (but could be calculated for crude OR estimate)	N = 93 exposed; 93 referents	Asthma <i>Medium</i> Selection out of the exposed work force of “affected” possible in this type of prevalence study. “Unexposed” exposure group exposed to levels of formaldehyde up to 0.086mg/m ³ . Either limitation could result in reduced (attenuated) effect estimate. Unclear definition of asthma used in the analysis: Occupational asthma” not defined, and

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	matched to exposed by age, duration, and smoking. 93% participation rate and mean duration about 6 years in both groups.		to be relevant to current status. [Increased prevalence of asthma associated with lower FEV ₁ or FEV ₁ /FVC in these workers].				lack of clarity in asthma definition pertaining to current prevalence.
Matsunaga et al. (2008) (Japan). Residences: adults (Prevalence study)	Pregnancy cohort, enrolled 2 nd trimester. Recruited through pregnancy clinics and obstetrics departments. 17% of pregnant women in the city participated; recruitment extended to other areas. Low participation rate. Internal comparison group.	24-hr personal sample; 60 th , 90 th percentile and maximum: 0.033, 0.058, and 0.161 mg/m ³	Exposure measurement blinded to outcome classification. Allergy: Self-report of medical treatment (medication use) for atopic eczema or allergic rhinitis in past 12 mos. Asthma: Self-report of medical treatment (medication use) for asthma in past 12 mos.	Adjusted for age, gestation, parity, family history (of asthma, atopic eczema, allergic rhinitis), smoking status, current passive smoking at home and work, mold in kitchen, indoor domestic pets, dust mite antigen level, family income, education, and season of data collection. Also examined NO ₂	Logistic regression, OR (95% CI) by 4 exposure categories (30 th , 60 th and 90 th percentiles); also presented dichotomized at 90 th percentile. Results also stratified by family history of allergies.	N = 998; 21 asthma cases, 57 eczema, 140 rhinitis cases	Allergy (atopic eczema, rhinitis) and asthma <i>Medium</i> Low participation rate but potential for differential participation (by formaldehyde exposure and disease status) uncertain. Some uncertainty pertaining to sensitivity and specificity of outcome assessments.
Mi et al. (2006) (China) Schools: children (prevalence study)	10 schools, 3 classrooms (7 th grade) per school. Participation rate 99%	4-hr (school day) air samples from 30 classrooms; protocol discussed. Minimum = 0.003 mg/m ³ ; (unclear if this is	ECRHS questionnaire, Medication use or asthma attack in past 12 mos; additional questions on lower respiratory tract symptoms (in past 12 mos, wheeze or whistling in the chest,	Adjusted for age, gender, smoking, observed water leakage and indoor moulds. Also examined temperature, relative humidity, indoor CO ₂ , indoor	Logistic regression, OR (95% CI) per 0.010 mg/m ³ increase. Uncertainty about exposure distribution and analysis (e.g., percent <LOD and	N = 1,414	Asthma <i>Low</i> Short exposure measurement period and uncertainty about exposure distribution and analysis (e.g., percent <LOD and treatment in analysis as continuous variable)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
November–December 2011		½ of LOD?; 1 SD above mean = 18 µg/m³; maximum = 20 µg/m³.	daytime breathlessness attack at rest or after exercise, nighttime breathlessness attack). Exposure measurement blinded to outcome classification	O ₃ , and examined collinearity of exposures.	treatment in analysis as continuous variable)		
Neamtiu et al. (2019) (Romania) Children: schools	Schools Indoor Pollution and Health: Observatory Network in Europe (SINPHONIE) project, 2010 to 2012. The authors analyzed the data for Romania, which included 5 primary schools in one county (2 rural, 3 urban), and 3 classrooms per school were selected. Questionnaire responses for October to December 2011 for 139 male and 141 female students; 89.7% response rate for children	Formaldehyde measured in each classroom, 5 d sampling period. Passive samplers, Radiello cartridges, impregnated with 2,4-dinitrophenylhydrazine using ISO 16000-2 protocol. Analysis within 48 hrs using a validated method from European Commission. Detection limit was 0.1 µg/m³; median = 0.035 mg/m³; maximum = 0.066 mg/m³; 75 th percentile = 0.045 mg/m³	ISAAC questionnaire. Allergy-like symptoms in past week: defined as skin conditions (e.g., rash, itch, eczema), eye disorders (e.g., red, dry, swollen, itching, or burning eyes, or sensation of “sand in the eyes,” and rhinitis symptoms (e.g., itching nose, sneezes, and/or stuffy or blocked Nose). Asthma-like symptoms: defined as difficult breathing, dry cough and wheezing in the past week (any symptom.	Analyses controlled for age, sex, ETS in the past week, microclimate parameters (NO ₂ , CO, CO ₂ , temperature, relative humidity, ventilation rate.	Multivariate analysis of formaldehyde categorized as high (> 35 µg/m³) and low (≤ 35 µg/m³) based on the median.	N = 280	Asthma-like symptoms, Allergy-like symptoms <i>Medium</i> Outcome definition for allergy-like symptoms using ISAAC questionnaire included combined symptoms of rhinitis (nose), eye and skin conditions, which could mask an effect in one of these categories; skin condition question not specific for eczema. Asthma-like symptoms limited to the past week.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Neghab et al. (2011) (Iran) Workers: melamine-formaldehyde resin plant (prevalence study)	Exposed: melamine-formaldehyde resin plant workers. Referent group: office workers from same plant, no present or past exposure to formaldehyde or other respiratory irritant chemicals. Participation rate 100%. Duration ≥2 years	Area samples (40 min) in 7 workshops and 1 area sample in office area. Exposed (mean ± SD) 0.96 (±0.49) mg/m ³ ; unexposed = nondetectable.	American Thoracic Society (Ferris, 1978) questionnaire (modified): wheezing symptoms (no details of questions)	No covariates considered in the symptom analysis. Similar in demographics and current smoking (but smoking frequency higher among exposed)	Fisher's exact test, OR (p-value)	N = 70 exposed, 24 unexposed	Asthma <i>Low</i> Selection out of the exposed work force of "affecteds" possible in this type of prevalence study; could result in reduced (attenuated) effect estimate. Potential low specificity and low sensitivity of outcome measure; no covariates considered in analysis.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Norback et al. (1995) (Sweden) Residences: adults (nested case-control)	Recruited from 154 randomly selected members of general population; 64% participation rate for cases, 57% for controls	2-hr household sample (bedroom). Limited sampling period in closed residence with no point formaldehyde emissions; sampling and analytic protocols referenced Andersson et al. (1981) . Mean (Min, Max) 0.029 (<0.005, 0.110) mg/m ³ ; LOQ = 0.1 mg/m ³)	Positive response to: asthma attack in past 12 mos, nocturnal breathlessness in past 12 mos, or current use of asthma medication. Controls answered no to all questions	Adjusted for age, sex, current smoking, wall-to-wall carpets, and house dust mites. Formaldehyde measure reported to be strongly correlated with total volatile organic compounds.	Log-transformed, logistic regression, OR (95% CI) per 0.001 mg/m ³ increase. Mean subtracted from each observation to reduce collinearity with VOCs	N = 47 cases, 41 controls	Asthma <i>Low</i> (↑) Uncertainty about exposure (most values <LOQ). Similar results for volatile organic compounds, and not possible to distinguish effects of formaldehyde and these other compounds; could result in inflated effect estimate.
Norbäck et al. (2017) (Malaysia) Schools: children 2007	8 randomly selected schools in Johor Bahru, Malaysia, randomly selected 15 students each from 4 randomly selected classes (Form 2, aged 14 years). Data	Sampling and analytical methods were described. Formaldehyde sampled continuously over 7 d in each classroom using diffusion samplers.	Standardized questionnaire completed by students with parents blinded to environmental measurements. Rhinitis defined by 2 questions combined regarding nasal catarrh or nasal congestion. Ocular symptoms defined on	There were no significant correlations between CO ₂ , NO ₂ or formaldehyde and any of the measured VOC. Models adjusted for other indoor chemical	Stepwise multiple logistic regression for symptoms including indoor exposures (CO ₂ , NO ₂ , formaldehyde and VOC personal factors (sex, race, current smoking, atopy, parental	N = 462	Allergy <i>Medium</i> Results for rhinitis were reported as “not statistically significant” without providing quantitative effect estimates. Very low indoor formaldehyde concentrations

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	collection in 2007. Participation 96%	Samplers placed 2 meters above floor. Mean concentrations formaldehyde indoor 4.2 µg/m ³ , max 18.0 µg/m ³ , 100%>DL Outside 5.5 µg/m ³ , max 6.0 µg/m ³ , 100%>DL. Combined indoor and outdoor measures used in the analysis.	one question on ocular symptom (dry eyes or itchiness in the eyes). Cases defined by reporting symptoms weekly over a 3-mo period.	exposures, personal factors and home environment factors.	asthma/allergy) and home environment factors (ETS, dampness/ mold, recent indoor painting). 3-level logistic regression models (child, school, classroom) including significant exposure variables from first model, all personal factors and all environment factors. Quantitative results not reported for formaldehyde if not statistically significant.		
Palczynski et al. (1999) (Poland) Residences: adults, children (prevalence study)	Random sample of 120 households with children ages 5–15 years, built 10 years before study. Participation rate not reported (i.e., were more than 120 households originally recruited?)	24-hr household sample, area not specified; Mean (±SD) (minimum, maximum): 0.026 (±0.011) (0.002, 0.067) mg/m ³ ; 2% > 0.050 mg/m ³ Calibration: 0.005 to 0.100 mg/m ³	Allergy: 5 allergen skin prick test (dust, dust mites, feathers, grasses); serum IgE positive if ≥ 0.35 kU/l RAST (uncertainty regarding time window of exposure for this outcome in adults) Asthma: Bronchial asthma diagnosis based on American Thoracic Society criteria (Ferris, 1978) (additional details)	Environmental tobacco smoke	Contingency table analysis, prevalence (n, %) by age (adult; children) exposure group, and environmental tobacco smoke exposure. Highest exposure group very sparse.	N = 278 adults, 186 children	All outcomes <i>Not informative</i> above 0.050 mg/m ³ because of sample size (≤5). Allergy (skin prick tests), children <i>Medium</i> Allergy (skin prick tests) in adults <i>Low</i> Uncertainty about time window of exposure measurement with respect

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
			not reported). Diagnosis interpreted to be for current status. Exposure measurement blinded to outcome classification				to skin prick test results in adults. (greater uncertainty in adults than in children); Asthma, children and adults <i>Medium</i> Uncertainty regarding asthma definition
Smedje and Norback (2001) (Sweden) Schools: children (nested case-control design) 1993–1997 Related reference: Smedje et al. (1997) ; however, this baseline study of prevalence of current asthma used measures taken in 1993, which ranged from <0.005 to 0.010 mg/m ³ , with >50% less than LOD.	Nested case-control in school-based cohort study, 1 st , 4 th , and 7 th grades at baseline (1993); follow-up in 1997. Excluded if history of allergy at baseline. 78% participation in follow-up. Schools randomly selected in Uppsala, Sweden; 2–5 classrooms selected from schools for exposure measures. Participants compared to nonparticipants on baseline characteristics.	4-hr (school day) samples, 2–5 rooms per school (chose frequently used rooms), 1993 and 1995; <0.005 to 0.042 mg/m ³ . Mean 0.008, geometric mean 0.004 mg/m ³	Allergy: Parent report of incident allergy to hay fever/pollen or pet dander. Asthma: Parent-report of incident physician diagnosis (validation study: specificity >99%, sensitivity 73% compared with physician's assessment). Exposure measurement blinded to outcome classification	Adjusted for age, sex, history of atopy (eczema) at baseline, changes in smoking habits. Collinearity among measures (including VOC, mold) assessed; did not attempt adjustment for multiple exposures but pattern of results differed among the exposures examined.	Logistic regression, OR (95% CI) per 0.010 mg/m ³ increase [high proportion below detection limit of 0.005 mg/m ³ , 54% of 1993 samples and 24% of 1997]. Results were reported to be similar when students who were no longer in the school were excluded (about 2/3 left the school at mean of 1.5 years before follow-up)	N = 88 incident pollen allergy; 50 incident pet allergy cases; 56 incident asthma cases out of 1,258 at baseline.	Allergy (incidence of allergies) and asthma (incidence) <i>Low</i> Exposure measures in only 2 of the 4 years and 2/3 of students left the school more than a year before follow-up; uncertainty about distribution; relatively high percentage <LOD. Confounding by other exposures not fully addressed but pattern of results differed among the exposures examined. (Information on percent below detection limit and individual student exposures provided in email from Dr. Greta Smedje, March 22, 2012)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Thus, this analysis did not meet EPA's inclusion criteria.							
Tavernier et al. (2006) (United Kingdom) Residences: children (case-control) Related reference: Gee et al. (2005)	Cases from 2 primary care practices, age- and sex-matched controls from same practices. Ages 4–16 years. Participation rate 50%. 95 additional cases excluded because no matching control identified. [Note: Gee et al. (2005) described the age range as 4–16 years]	7-d sample in living room and bedroom. Did not report any information on exposure distribution. [Note: Gee et al. (2005) described this as a 5 d sample; median values 0.037 and 0.049 mg/m³ in living room and bedroom, respectively]	Positive responses to three questions on screening questionnaire: (1) wheezed in the last 12 mos; (2) woken at night by cough in the absence of a cold or respiratory infection in the last 12 mos; (3) received more than 3 courses of antibiotics for respiratory symptoms (both upper and lower respiratory tract) in the last 12 mos; (4) history of hay fever or eczema; (5) family history of asthma in first degree relatives. In validation study, positive predictive value 84% for meriting trial for asthma medication. Exposure measurement blinded to outcome classification. [Note: Gee et al. (2005) described the positive predictive value from the validation study as 79%]	Adjusted for measured exposures (e.g., endotoxin, Der p 1, particulate matter, NO₂, and other risk factors.	Logistic regression, OR (95% CI) by tertile (but exposure levels by tertile not reported)	N = 105 cases, 95 controls	Asthma <i>Low</i> Uncertainty regarding selection process and loss of almost half of the cases. Outcome classification includes questions that are not specific to asthma. Uncertainty as to exposure range, particularly upper tertile (no response from email to corresponding author).

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Venn et al. (2003) (United Kingdom) Residences: children (nested case-control and symptom control among cases) October–May 1998	Participants in air pollution study 1993–1995, 85% response rate; 835 potential cases (positive wheeze question) and 860 potential controls recontacted in 1998; 54% responded. ages 9–11 years. From this, 243 eligible cases and 383 eligible controls identified. Participation rate 79% cases, 59% controls. Supplemental reference: Venn et al. (2000)	3-d sample in bedroom in 1998 concurrent with data collection on outcomes; median 22 $\mu\text{g}/\text{m}^3$; 75th percentile 32 $\mu\text{g}/\text{m}^3$	Asthma: Parent report of persistent wheeze (1995–1996 and 1998); validation by medical record review of prescription for asthma medication. Symptom frequency: 1 mo daily diaries recording symptoms, including daytime and nighttime wheezing, chest tightness, breathlessness, and cough, each measured on 0 to 5 scale. Exposure measurement blinded to outcome classification	Adjusted for age, sex, Carstairs deprivation index (based on postal code). Also examined and addressed other variables, including NO_2 , moisture, mold, season. No evidence of an adverse effect of NO_2 or VOCs other than formaldehyde on children's respiratory health.	Logistic regression, OR (95% CI) by quartile. Examined effect modification of symptom frequency by atopy	N = 190 cases, 214 controls	Asthma <i>Medium</i> Uncertainty regarding impact of loss of subjects between 1995 and 1998. Asthma control <i>High</i>
Yeatts et al. (2012) (United Arab Emirates) Residences (survey) October 2009 to May 2010	Nationally representative sample of households, stratified by geographic area and population density. 628 households, household participation rate 75%. Age-stratified sample selected from households.	7-d sample in living room. 71% <LOQ (0.0074 mg/m^3); 95 th percentile 0.059 mg/m^3 ; 99 th percentile 0.114 mg/m^3 (converted from ppm)	Symptom questionnaire (last 4 wks), drawn from standard questionnaires. Mothers responded for children. Exposure measurement blinded to outcome classification	Moderate correlation between formaldehyde and sulfur dioxide ($r = 0.63$); formaldehyde strongly associated with frequency of incense use. Adjusted for sex, urban/rural area, age group,	Logistic regression, above versus below detection limit, OR (95% CI). Does not separate analysis of children and adults; only 29% above LOD—analyzed as above versus below LOD	N = 1007 adults, 330 ages 11–18 years, 253 ages 6–10 years	Asthma -children and adults (combined) <i>Low</i> (↑) Difficult to disentangle possible effects of sulfur dioxide from those of formaldehyde (similar effect sizes; moderate-strong correlation; could result in inflated effect estimate. Does not report separate analysis of children and adults; only 29% above

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
				household tobacco smoke exposure.			LOD—analyzed as above versus below LOD
Yon et al. (2019) (Seongnam City, Korea) Prevalence study	5 th and 6 th grade students were recruited from 22 randomly selected classrooms at 11 elementary schools (n = 620), aged 10–12 year. 68.9% participation rate.	Formaldehyde sampling in each classroom using monitors with pumps during the 1 st and 2 nd half of the school year. Mean 0.028 ± 0.0077 mg/m ³ ; as high as 0.060 mg/m ³ in some classrooms. Duration and sampling methods were not described.	Current asthma or rhinitis definition: presence of characteristic symptoms and /or signs during the previous 12 mos using ISAAC questionnaire (some uncertainty regarding validation in this population) Rhinitis severity categorized using Allergic Rhinitis and Its Impact on Asthma guidelines.	Models for asthma or rhinitis adjusted for age and sex a priori. Also adjusted for variables based on statistical significance in model (<i>p</i> < 0.10). Covariates were BMI z-score, height, prematurity or low birth weight, home renovation, environmental tobacco smoke, keeping a pet at home, and physician-diagnosed atopic dermatitis, allergic rhinitis, and parental asthma	Analysis used generalized linear mixed models with robust variance estimates and post hoc Bonferroni correction. Accounted for classroom (random effect)	N = 427 (n = 246 with rhinitis; n = 10 with asthma)	Rhinitis in last 12 months and rhinitis severity <i>Medium</i> Uncertainty regarding validation of ISAAC in this population; uncertainty regarding exposure measurement period and other protocol details. Current asthma <i>Low</i> Uncertainty regarding validation of ISAAC in this population; uncertainty regarding exposure measurement period and other protocol details; few (n = 10) children with asthma contributed to analyses.
Zhai et al. (2013) (China) Residences (survey) January 2008 to December 2009	Provided criteria for selection of homes in defined area; evaluated 186 homes in Shenyang, China; homes were decorated in last 4 years and occupied	Cited Code for indoor environmental pollution control of civil building engineering (GB50325-2001); samples	Asthma: based on American Thoracic Society (Ferris, 1978) questionnaire	Univariate analysis; confounding unlikely explanation of the results in children	Univariate results for asthma outcome [multivariate modeling of “respiratory symptoms”; not clear what is included in this category)	N = 186 homes 186 adults, 82 children; only n = 22 adults with asthma	Asthma, Children <i>Medium</i> Uncertainty regarding exposure measurement period. Although potential confounders were not considered in asthma only analysis, the magnitude of

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	within the last 3 years. Participation rate of households not reported (i.e., were more than 186 households originally recruited?) Participants within houses were randomly selected	in 3 rooms per house (bedroom, living room, kitchen); sampling time not specified (no response from email to corresponding author); N = 558 samples in 186 homes. Exposure groups “polluted” homes: >0.08 mg/m³, mean 0.09–0.13 mg/m³ in three rooms; “nonpolluted” ≤0.08 mg/m³, mean 0.04–0.047 mg/m³. 64% of the 186 houses, and 24% of the 82 houses with children were >0.08 mg/m³ (“polluted”)					the results is unlikely to be explained by confounders. Asthma, Adults <i>Low</i> See notes above, for children. In addition, for adults, small number (n = 2) of positive responses.

Epidemiology Studies of Lower Respiratory Symptoms in Infants and Young Children

Table B-36. Evaluation of lower respiratory symptoms in infants and young children (up to age 3 years)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Li et al. (2019) (Hong Kong) Prospective study of birth cohort September 2013 to April 2014 and Yu et al. (2017) (Hong Kong) Prospective study of birth cohort November 2009 to April 2011	Infants aged < 4 mos attending maternal and child health clinics history of asthma, family history of allergy and no family history. Excluded if congenital disease, cared for at child-care center > 20 hrs/wk, moving after recruitment. 14 clinics used in 2013–2014 cohort; 29 clinics used in 2009–2011 cohort. Participation rate 67% in 2013–2014 cohort and 76% in 2009–2011 cohort. ONo comparisons of participants with nonparticipants.	Air sampling (NO₂, formaldehyde) using standardized diffusion samplers at 6 mos of age. NO₂ 10–14 d sampling period. From Li, 2019: Formaldehyde 72 hr sampling period using ISO 16000-4 method. Concentrations not reported. From Yu, 2017 REF: Mean (SD) concentrations Formaldehyde 0.051 (0.075) mg/m³	Baseline information obtained using validated ISAAC questionnaire completed by parents prior to age 4 mos. Weekly respiratory health diary and monthly health telephone survey blinded to exposure status until 18 mos of age. New onset wheeze (time to event) measured from 6 to 18 mos of age. 120 (12.5%) infants had new onset wheeze at an average of 13.2 mos.	Potential confounders selected from baseline characteristics associated with formaldehyde concentrations using log-rank test, $p < 0.25$. Stepwise adjustment, final models adjusted for NO₂, sex, neonatal respiratory illness, having a sibling, family history allergy or asthma, pets, or cooking fuel.	Cox regression in entire sample; formaldehyde modeling as continuous variable	2013–2014 cohort: N = 963 2009–2011 cohort: N = 535	Time to onset of wheeze event <i>Medium</i> Low participation rate but potential for differential participation (by formaldehyde exposure and disease status) uncertain. Authors did not respond to queries; EPA assumed exposure measurement details and exposure levels were similar in these 2 studies.
Raaschou-Nielsen et al. (2010) (Denmark) Infants (birth cohort) 1998–2003	Copenhagen Prospective Study on Asthma in Childhood. 378 out of 411 (92%) participants at 18m o followup; 343 with formaldehyde data.	Three 10-wk bedroom sampling periods from birth to 18 mos (aimed for 6, 12, and 18 mos). Median 0.018 mg/m³, 95th	Daily diary kept by parents on respiratory symptoms. Training and definitions provided. Wheezing = any symptom severely affecting	Adjusted for sex, area of residence, education of mother, baseline lung function	Logistic regression of “ever had at least one symptom day” (88% = yes) and linear regression of number of symptom days	N = 343	Lower respiratory tract conditions in infants and toddlers <i>Medium</i> Analysis does not take into account important features of the data (e.g., temporal

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		percentile 0.037 mg/m ³ . Within individual variance 69% of total variance	the child's breathing, such as noisy breathing (wheeze or whistling sounds), breathlessness, shortness of breath, or persistent, troublesome cough). Reviewed by study personnel every 6 th month and after a 3-d period of respiratory symptoms. Outcome defined as "ever had at least one symptom day"; sensitivity analysis defined outcome as three or more consecutive days with wheezing symptoms.		(excluded 78 with 0 d). Analyzed by quintile of exposure (reference = <0.012 mg/m ³)		variations in symptoms and large within individual variability formaldehyde)
Roda et al. (2011) (France) Residences: infants (birth cohort) 2003–2006	Infants (singletons, >2,500 g) from 5 maternity hospitals in Paris. N = 3,840 out of 4,177 (92%) initially enrolled completed 1 or more questionnaires; 2,940 had baseline and 12 mo questionnaire (70% of initial enrollees; 76% of	Questionnaire on home characteristics at baseline and updated at 3, 6, 9, and 12 months. N = 196 randomly selected for predictive modeling analysis. Based on 4 1-wk measures at 1, 3, 6, and 9 months.	Parent questionnaire at 1, 3, 6, 9, and 12 mos: Upper respiratory infections Lower respiratory infections Eczema wheezing episodes (frequency) At 12 mos, also includes shortness	Examined sex, older sibling, parental asthma, history, socioeconomic status (4 levels, based on parents' occupation), prenatal and postnatal tobacco smoke exposure, dampness, breast	Exposure prediction model for high versus low (based on median): sensitivity 72.4% specificity 73.6%. Exposure prediction model by tertile: sensitivity 57.4% specificity 82.1%.	N = 2,940	Lower respiratory tract conditions in infants and toddlers <i>Medium</i> Did not test predictive model on separate sample (may overestimate sensitivity and specificity)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	those with 1 or more questionnaire)	LOD 0.008 mg/m ³ . Median 0.020 mg/m ³ ; IQR 0.014, 0.027 mg/m ³ . Predictors included measures of continuous formaldehyde exposure, intermittent exposure, home characteristics, and air flow	of breath, dyspnea, dry cough at night without cold Used to define lower respiratory infections with and without wheeze	feeding <3 mos, day care, pets in home	Outcome examined as LRI versus no LRI, and as 3-level variable in multinomial logistic regression (LRI-with wheeze; LRI-no wheeze, no LRI)		
Rumchev et al. (2002) (Australia) Residences: children (case-control) Related reference: Rumchev et al. (2004)	Limited to ages 6–36 mos; recruitment process not described for cases or controls; cases from emergency room and controls (age matched) from area health department, representing the catchment area of the hospital	8-hr samples, bedroom and living room, two seasons. Mean 0.030 and 0.28 and maximum 0.224 and 0.190 mg/m ³ , respectively, in bedroom and living room.	Emergency room discharge diagnosis of asthma, ages 6–36 mos.	Adjusted or considered age, allergies, family history of asthma, dust mites, relative humidity, temperature, atopy, environmental tobacco smoke, pets, air conditioning, use of gas appliances	Generalized estimating equation modeling for repeated measures	N = 88 cases, 104 controls	Lower respiratory tract conditionsn infants and toddlers <i>Medium</i> Recruitment process not described; uncertainty as to what is included within this case definition and length of time between emergency room visit and subsequent exposure measure.

Controlled Exposure Studies in People with Asthma

Table B-37. Evaluation of controlled acute exposure studies among people with asthma

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure assessment (details on exposure quality evaluation in Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size	Confidence
Casset et al. (2006)	Formalin, 30 min, 0.032 (background) and 0.092 mg/m ³ , achieved concentrations analyzed. Includes allergy challenge. Nose clipped during exposure (mouth breathing)	Spirometry; FEV ₁ , FEF ₂₅₋₇₅ , PEF (protocol not mentioned) and bronchial challenge-airway reactivity test (PD ₂₀ FEV ₁ Der p1) (standard protocol) Testing pre- and every hour up to 6 hrs postexposure.	Mild asthma, ages 19–35 years, no respiratory infections for 2 wks; not in relevant allergy season or living with a pet if allergic. Random assignment to order of exposure (3 wks between experiments); double blinded	Within-person	Individual data values and <i>t</i> -tests	N = 19	<i>High</i> Randomized, double blinded, detailed data presentation; applies to mouth breathing
Ezratty et al. (2007)	Formalin, 60 min, 0 and 0.500 mg/m ³ , achieved concentrations analyzed. Includes allergy challenge	Spirometry; FVC, FEV ₁ (ECRHS protocol), and bronchial challenge-airway reactivity test (PD ₁₅ FEV ₁ grass) (standard protocol) Testing pre- and every hour up to 6 hrs postexposure.	Intermittent asthma (dyspnea < twice per week and night symptoms < twice per month with PEF > 80%), ages 18–44 years; not in allergy season. Random assignment to order of exposure (2 wks between experiments); double blinded.	Within-person	Individual data values and Wilcoxon sign rank test	N = 12	<i>High</i> Randomized, double blinded, detailed data presentation
Green et al. (1987)	Paraformaldehyde, 60 min, clean air and 3 ppm, achieved concentrations analyzed.	Spirometry; FVC, FEV ₁ , SGaw (ATS protocol), testing pre- and during exposure period, ≈15 min intervals.	Asthma (clinical history), no respiratory infection for 2 wks, age 19–35 years. Random assignment to order of exposure; two 15-min exercise segments in 60-min exposure period; single blinded.	Within person	Group means and SE	N = 16	<i>Medium</i> Randomized, single blinded

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure assessment (details on exposure quality evaluation in Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size	Confidence
Harving et al. (1990) Related Reference: Harving et al. (1986)	Formalin, 90 min, filtered air (8), 0.120 and 0.850 mg/m ³ , achieved concentrations analyzed.	Spirometry; FEV ₁ , R _{aw} , SGaw (protocol not mentioned), testing pre- and near end of exposure period. Bronchial challenge-airway reactivity test, immediately after exposure PEF by home peak flowmeter every 2 hrs after exposure and next morning	Asthma (substantial bronchial hyperreactivity to histamine), age 15–36 years. Random assignment to exposure order (one per week); double blinded	Within-person	Group means and SD	N = 15	<i>High</i> Randomized, double blinded, detailed analysis
Krakowiak et al. (1998)	Formalin, 2 hrs, 0.5 mg/m ³ , achieved concentrations analyzed.	Spirometry FEV ₁ (testing 2 hrs pre- and immediately after, 5 hr, and 24 hr) PEF (testing at beginning of exposure, every hour for 12 hrs, 24 hrs after)	Formaldehyde-exposed workers with asthma, age 23–53 years. Order not randomized (1 wk between experiments); single blinded	Within person	Group means (bar graph)	N = 10	<i>Low</i> Not randomized, single blinding, SE or SD not reported
Sauder et al. (1987)	Paraformaldehyde, 3 hrs, clean air and 3 ppm (0 and 3.69 mg/m ³), achieved concentrations analyzed.	Spirometry; FVC, FEV ₁ , SGaw (ATS protocol), testing at 0, 15, 30, 60, 120, 180 min during exposure.	Asthma (clinical history), no respiratory infection for 6 wks, age 26–40 years. Order not randomized; clean air followed by formaldehyde (one week apart); blinding not specified	Within person	Grouped means and paired <i>t</i> -tests for most measures, individual FEV ₁ data	N = 9	<i>Low</i> Not randomized, blinding not specified
Sheppard et al. (1984)	Paraformaldehyde, 10 min, 0, 1, and 3 ppm (0, 1.23, and 3.69 mg/m ³), achieved concentrations analyzed.	Spirometry; SGaw, testing before and 2 min after exposure.	Asthma (clinical history), age 18–37 years. Randomization of order not reported; two protocols (at rest and during exercise) ≥1 d apart; blinding not specified	Within person	Grouped means and SD and paired <i>t</i> -tests	N = 7	<i>Low</i> Randomization and blinding not specified

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure assessment (details on exposure quality evaluation in Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure)	Consideration of likely confounding	Results presentation	Size	Confidence
Witek et al. (1986) ; Witek et al. (1987)	Paraformaldehyde (with 2-propanol?), 40 min, 0 and 2 ppm (0 and 2.46 mg/m ³)	Spirometry; FVC, FEV ₁ , R _{aw} , testing during and at 10 and 30 min postexposure; PEF assessed from 1 to 24 hrs post exposure.	Mild asthma (ATS definition), age 18–35 years. Random assignment to order of exposure; two protocols (at rest and during exercise); double blinded	Within person	Individual data values and paired <i>t</i> -test	N = 15	<i>High</i> Randomized, double blinded; nonparametric analysis could be preferred but individual data provided

B.3.5. Respiratory Pathology

The details of the study evaluation approach methods are provided in Section 2.3.5 of the Toxicological Review. Within Tables B-38 (epidemiology studies in school and occupational settings), B-39 (controlled exposure studies in humans), and B-40 (animal studies) below documenting the results of the study evaluations for respiratory tract pathology, studies are arranged alphabetically. Gray shading is indicative of aspects of the study design or analysis that decreased EPA's confidence rating.

Studies in Humans

Epidemiology Studies

Table B-38. Respiratory pathology study evaluations: epidemiology studies

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/estimated power	Confidence
School Settings							
Norback et al. (2000) (cross-sectional study)	Exposure measurements in 2 randomly selected classrooms at each school on 2 occasions; Measurements of respirable dust, CO ₂ , temperature, humidity, formaldehyde (4-hr sample), airborne microorganisms, viable molds, and bacteria, NO ₂ (only in 1993); all staff assigned school mean concentration. Formaldehyde	Interview for symptoms, nasal lavage and acoustic rhinometry; use of both subjective and objective measures enabled evaluation of information bias	Primary school personnel at 12 of 18 randomly selected schools (out of 62) and with restriction to schools with classes 1–6 and no changes in ventilation or redecoration during study period (March 1993–March 1995). 234 current employees (84%) working 20 hr/wk or more.	Multiple linear regression models adjusted for age, sex, smoking, atopy, and mean classroom temperature; Co-exposure: Nasal patency measures were inversely associated with dust, NO ₂ , and Aspergillus. Elevations in nasal lavage biomarkers associated with	Multiple linear regression models; reported regression coefficients and whether statistically significant ($p < 0.05$); uncertainties in analysis: use of school-based mean concentration as unit of analysis	N = 234 individuals, but unit of analysis was school means, N = 12	<i>Low</i> Unknown correlation between co-exposures (dust, NO ₂ , and Aspergillus) which also were inversely associated with nasal patency and biomarkers, potential confounding; some schools with mean < LOD; less robust analytic approach given unit of analysis

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Confidence
	concentration: mean 0.0095 mg/m ³ ; min-max of means, 0.003–0.016 mg/m ³ ; provided citation for analysis; LOD 0.005 mg/m ³ (Smedje et al., 1997)		Excluded those on sick leave or otherwise off duty. High participation reduced likelihood of selection bias.	NO ₂ , Aspergillus, and yeast; correlation between indoor levels of pollutants or microbials not reported; correlated with ventilation? No indoor sources of combustion—NO ₂ levels higher in schools near traffic			
Occupational Settings							
Ballarin et al. (1992) Prevalence study	Personal sampling; 8-hr TWA (NIOSH, 1977) Warehouse (N = 3), 0.39 ± 0.20 mg/m ³ , range 0.21–0.6 mg/m ³ Shearing-press (N = 8), 0.1 ± 0.02 mg/m ³ , range 0.08–0.14 mg/m ³ Sawmill (N = 1), 0.09 mg/m ³ Inspirable wood dust: 0.11–0.69 mg/m ³ , 0.73 in sawmill	Cytopathology analysis of nasal respiratory mucosa cells by 2 trained readers blinded to exposure status; scoring and classification analogous to Torjussen et al. (1979) and Edling et al., 1988 ; most severe score present assigned.	Details of participant recruitment not described. Nonsmokers in plywood factory (N = 15) compared to nonsmoking university or hospital clerks (N = 15) matched by age and sex. Excluded heavy drinkers. Potential confounding by wood dust	Addressed potential confounding by age and sex through matching and smoking and heavy alcohol use by exclusion.	Mean histological scores in exposed and referent compared using Mann-Whitney U test and frequency by classification using chi-square test	N = 15 exposed/unexposed pairs	<i>Medium</i> Uncertainty regarding exposure levels during previous years. Small sample size increases potential for unreliable results. Inclusion only of current workers raises possibility of healthy worker survival effect due to irritation effects.

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Confidence
	Uncertainty regarding concentrations during previous years.						
Berke (1987) Cross-sectional study	Exposure measurements since the mid 1970s using personal monitoring (monitoring protocol not described). Group 1 ranging from 0.02–1.3 ppm. Group 2 plant 0.05–2.0 ppm	Clinical exam and nasal cytology by pathologist blind to exposure or clinical status. System for classifying atypical and typical metaplasia not defined.	52 volunteers from 3 paper plants (currently employed, participation 95% of available exposed) Group 1&2: 42 exposed, Group 3: 10 referent workers. Group 3: 28 additional referent white-collar employees (36% atypical squamous metaplasia in this group)—not clearly representative	Mean age in exposed higher than employee referent group, comparable to additional white-collar referent group. Smoking prevalence 60% in Groups 1, 2, and 3; 20% in white-collar referent. Statistical analysis excluding smokers	Exposed (Groups 1 and 2) compared to referent (Groups 3 and 4); chi-square test with adjustment for age and smoking; analysis of combined groups not appropriate (exposures different and very different demographic characteristics)	N = 42 exposed, 10 employee referents, 28 white-collar referents	<i>Not Informative</i> Methods were not well described. Comparisons of dissimilar groups. Nonstandard outcome definition and analyses that cannot be interpreted. Inclusion of only current workers and long duration of employment (mean >15 years) raises possibility of healthy worker survival effect
Boysen et al. (1990) Cross-sectional, study	Formaldehyde monitoring conducted after 1980. Before 1980, exposure assigned by plant health officer with knowledge of the production process, recent measurements, and worker sensations.	Slides evaluated by two authors blinded to clinical or occupational status. Histology: Scoring and classification of histologic samples per variation of Torjussen et al. (1979) protocol.	37/74 volunteers from a chemical company producing formaldehyde (50% of exposed workforce). Referents: 37 age matched subjects without overt nasal disease (office staff,	Exposed and referent comparable for age, smoking, or previous nasal disease.	Comparison of histological results between exposed and referent groups using Wilcoxon rank sum test, evaluated associations with age, smoking, intensity and	N = 37 exposed, 37 referents	<i>Medium</i> Inclusion only of current workers and long duration of employment raises possibility of healthy worker survival effect due to irritation effects.

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Confidence
	Range of formaldehyde 0.5 ppm to >2 ppm (0.62–2.5 mg/m ³); no measurements in referent; however, exposure contrast likely adequate.	Rhinocopy: Scoring according to Boysen and Solberg (1982)	hospital laboratory personnel, and EN&T outpatients). Use of referent group with different occupations results in less similar comparison groups.		duration of exposure; comparison of rhinoscopic results using chi-square test		
Edling et al. (1987a, 1988) Prevalence Study Related studies: Odkvist et al. (1985)	Past TWA formaldehyde measurements by plant industrial hygienists sporadically between 1975 and 1983. Levels of formaldehyde in air ranged from 0.1–1.1 mg/m ³ , with peaks up to 5 mg/m ³ . No measurements available before 1975, but estimated levels higher during the 1960s and early 1970s. No measurements in referent; however, exposure contrast likely adequate.	Rhinocopy: Nasal mucosa histological grading by pathologist blinded to exposure using Torjussen et al. (1979) grading system	75 of 104 exposed male factory workers from 3 plants (72% of eligible). Referents: 25 men with similar age and no known industrial exposures to formaldehyde; source of referent group not described. Evaluated characteristics of nonparticipants at 1 plant, age and exposure time similar, % with symptoms higher in nonparticipants, % smokers lower.	Exposed mean age: 38 years; 35% smokers. Referent mean age: 35 years, 48% smokers. Histological score was higher among exposed smokers compared to ex-smokers and nonsmokers; possible confounder	Exposed groups compared to referent group using Wilcoxon rank sum test, no adjustment for age or smoking	N = 75 exposed, 25 referents	<i>Medium</i> Inclusion of only current workers and long duration of employment (mean 10.5 years) and high prevalence of symptoms raises possibility of healthy worker survival effect due to irritation effects. Potential for confounding by smoking.

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/ estimated power	Confidence
Holmström and Wilhelmsson (1988) ; Holmstrom et al. (1989c) Cross-sectional study	Personal sampling in breathing zone for 1–2 hrs in 1985. Chemical Plant: 0.05–0.5 mg/m ³ , mean 0.26 [SD 0.17 mg/m ³]. Furniture Factory: 0.2–0.3 mg/m ³ , mean 0.25 [SD 0.05 mg/m ³]. Referent 0.09 mg/m ³ formaldehyde. Total dust and respirable dust also measured. Uncertainty regarding measured exposure levels and levels in etiologically relevant period.	Nasal symptoms questionnaire, nasal volume flow rate using rhinomanometry; mucociliary clearance using green dye to measure time for spot to reach rhinopharynx. Histological changes in nasal mucosa graded by a pathologist blind to exposure according to Torjussen et al. (1979)	Nasal specimens in 62 of 70 formaldehyde exposed, 89 of 100 formaldehyde/ wood dust exposed, and 32 of 36 referents. Excluded subjects with upper airway infections; Apparent high participation and outcome assessment blinded to exposure status reduced likelihood of selection bias. Use of referent group with different occupations results in less similar comparison groups.	Formaldehyde exposed were slightly younger than formaldehyde-dust exposed or referent; smoking status higher in exposed; higher % male in exposed groups. Duration of exposure and smoking status were not correlated with histology score, therefore confounding not a concern	Compared exposure groups using 2-tailed t-test for symptoms, nasal flow rate, and histology, and chi-square test for mucociliary clearance	N = 62 of 70 Group 1, N = 89 of 100 Group 2, N = 32 of 36 Referent	<i>Medium</i> Inclusion of only current workers and long duration of employment raises possibility of healthy worker survival effect due to irritation effects
Löfstedt et al. (2011b) Cross-sectional Study Related study: Westberg et al. (2005) (exposure methods)	Personal sampling over a single 8-hr shift. Formaldehyde concentration, mean (SD), range: 0.051 (0.049) mg/m ³ , 0.013–0.190 mg/m ³ ; 71.4% of exposed	Nasal symptoms and signs; questionnaire; examination by rhinologist blind to exposure status	43 exposed employees at 3 brass foundries producing cores using Hot Box method (90%) Referent: 82 assembly workers	Evaluated impacts of confounding by exclusion of smokers, females, or asthmatic and allergic subjects	Logistic regression, single-pollutant analyses, OR (95% CI); cut-point for categories of formaldehyde	N = 69 unexposed, 30 low and 12 high exposure	<i>Low</i> Formaldehyde levels among exposed were low (30 of 43 exposed at <LOD). Possible confounding of formaldehyde associations by ICA or MCA, but correlation for pollutant pairs was not reported.

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/estimated power	Confidence
	<LOD for formaldehyde (<0.02 mg/m ³).		and storage workers with no chemical exposure (98%); high participation reduced likelihood of selection bias. Use of referent workers from same companies increased similarities between groups. Possible healthy worker survival selection because of inclusion only of current workers and irritant exposures, but authors said there was no evidence.	from analysis. Other exposures also associated with nasal signs: isocyanic acid (ICA) and methyl isocyanate (MIC) and dust; correlations between co-exposures ranged between -0.08 and 0.65 (except ICA and MIC, $r = 0.92$); analyses using metric for combined exposure to multiple irritants.	exposed was LOD.		

Controlled Exposure Studies in Humans

Table B-39. Respiratory pathology study evaluations: controlled exposure studies in humans

Reference	Exposure measures and range (exposure quality evaluations in B.3.1)	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Size/estimated power	Confidence
Andersen and Lundqvist from Andersen and Molhave (1983) ^a	Paraformaldehyde. Dynamic chamber; analytic concentrations; clean air followed by 0.3, 0.5, 1.0, and 2.0 mg/m ³ formaldehyde. <i>Adequate</i>	Nasal airflow resistance and nasal mucociliary flow	Subjects assigned to four groups, each group with four different exposures over four consecutive days, order decided by Latin square design. <i>Clean air exposure order not randomized. Blinding not described</i>	Within-person comparison	Results presented with statistical analyses	N = 16	<i>Medium</i> Clean air exposure order not randomized. Blinding not described
Falk et al. (1994)	Formalin exposure; analytic concentrations, mean: Group 1: 0.021, 0.028, 0.073, 0.174; Group 2: 0.023, 0.029, 0.067, 0.127 <i>Deficient</i>	Nasal mucosa swelling measured using rhinostereometry (summary of changes for both turbinates)	Double blind exposures, exposure-order stochastically distributed and separated by 2 d.	Within-person comparison	Results presented in graphs	N = 6–7 per group	<i>Medium</i> Formalin source; small group size
Pazdrak et al. (1993)	Test article characterization and exposure generation method not described; clean air followed by 0.5 mg/m ³ formaldehyde <i>Deficient</i>	Stage 1 evaluation of symptoms, morphological changes, and biochemical changes in nasal washings. Stage 2 clinical comparison of nasal mucosa by group.	Two-stage, single-blind examination with nonrandom order of exposure assignment.	Within-person comparison	Results presented with statistical analyses	N = 8–11 per group	<i>Low</i> Formalin source of exposure; nonrandom order of exposure assignment; investigators not blinded. Small group size.

^aAt the request of EPA's Office of Chemical Safety and Pollution Prevention, EPA's Human Studies Review Board review of four intentional exposure studies for potential use in assessing acute formaldehyde inhalation determined the ethical conduct of this study to be adequate.

Studies in Animals

Animal studies in Table B-40 are grouped by exposure duration, and then organized alphabetically by first author. Specific study details (or lack thereof) that highlight a limitation or uncertainty in answering each of the domain criteria are noted in the table cells. For those identified as having a substantial limitation likely to influence the study results (major limitations), the relevant study details are

bolded. Italics are used to highlight other limitations not viewed as substantial in the context of respiratory tract pathology (minor limitations). A ‘Good’ domain rating is denoted by ‘++’; an ‘Adequate’ rating by ‘+’; and a ‘Deficient’ rating by gray shading. See Appendix B.3.6 for documentation of evaluations on studies of cellular proliferation or mucociliary function in the respiratory tract.

Table B-40. Evaluation of controlled inhalation exposure studies examining respiratory pathology in animals

Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations and statistics	Confidence classification
Respiratory Pathology—Chronic						
(Appelman et al., 1988) Rat	++	+ <i>Small N (N=10)</i>	++	+ <i>Lesion severity provided for 13-wk but not 52-wk sacrifice</i>	++	<i>Medium</i> [small N; limited reporting of lesion severity]
(Dalbey, 1982) Hamster	++	++	++ Note: single concentration study	+ <i>Lesion severities NR</i>	++	<i>Medium</i> [failure to report lesion severities]
(Holmstrom et al., 1989b) Rat	++ Note: high concentration exposure (15.3 mg/m ³ -d)	+ <i>Small N (N=16/group)</i>	++ Note: single concentration study	<i>Lesion severities NR; nonstandardized histological characterization makes interpretation of effect difficult</i>	Incidence of metaplasia and dysplasia reported together	<i>Not Informative</i> [small N; failure to report lesion severities; incidence of metaplasia and dysplasia reported together]
(Kamata et al., 1997) Rat	+ <i>Formalin</i> ; methanol concentration was reported and a methanol control was used.	+ <i>Small number of animals for interim sacrifices (N = 5)</i>	++	+ <i>Lesion severities NR; prevalence of neoplastic lesions complicates assessment of nonneoplastic lesions</i>	++	<i>Medium</i> [formalin; small N for interim sacrifices; failure to report lesion severities]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations and statistics	Confidence classification
(Kerns et al., 1983) Mouse See also (Swenberg et al., 1980a; Swenberg et al., 1980b; Battelle, 1982)	++	+ <i>Survival to 18 mos was <33% in all groups (N>25)</i>	++ Note: data from this study based on a GLP study (Battelle, 1982)	<i>Lesion severities NR; incidence NR; only three nasal sections (II, III, and V) evaluated</i>	++	<i>Medium</i> [limited sampling, high mortality, and failure to report lesion incidence and severities]
(Kerns et al., 1983) Rat See also (Swenberg et al., 1980a; Swenberg et al., 1980b; Battelle, 1982)	++	+ <i>Transient viral infection at wks 52–53 was considered unlikely to influence study outcome because of its short course</i>	++ Note: data from this study based on a GLP study (Battelle, 1982)	++ Note: incidence and severity data by nasal section extracted from the full GLP report	++	<i>High</i> [Note: transient viral infection]
(Monticello et al., 1996) Rat	++	++	++	<i>Lesion severities NR; lesion incidence NR</i>	+ <i>Insufficient data to verify magnitude of concentration-response</i>	<i>Low</i> [Failure to report lesion incidence and severities; insufficient data to verify magnitude of concentration-response]
(Sellakumar et al., 1985) Rat see also (Albert et al., 1982)	+ <i>Formaldehyde was generated by heating a slurry of paraformaldehyde in paraffin oil (kerosene), which could cause co-exposure to paraffin oil. [Note: high concentration exposure (18.2 mg/m³-d)]</i>	++	++ Note: single concentration study	+ <i>Lesion severities NR</i>	++	<i>Medium</i> [Likely co-exposure to paraffin oil (kerosene); testing at a single high concentration; failure to report lesion severities]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations and statistics	Confidence classification
(Woutersen et al., 1989) Rat	++	++	++	+ <i>Lesion severities NR</i> ; significant incidence of lesions in controls	++ Statistical analyses of lesions NR	<i>High</i> [Failure to report lesion severities]
Respiratory Pathology—Subchronic						
(Andersen et al., 2010) Rat	++	+ <i>small N (N = 8)</i>	++	++	+ <i>Data for levels III-V NR</i> ; <i>statistical analyses of lesions NR</i>	<i>Medium</i> [Small N; data for levels III-V NR]
(Arican et al., 2009) Rat	<i>Analytical method and concentrations NR</i>	++	++ Note: single concentration study	<i>Lesion severity NR</i> ; lesion incidence NR	+ <i>Qualitative descriptions only</i>	<i>Not Informative</i> [Failure to report analytical method and analytical concentrations; failure to report lesion incidence and severities]
(Bansal et al., 2011) Rabbit	<i>Formalin; Analytical method and concentrations NR</i>	Small N (N = 2 controls and N = 6 exposed)	Continuous exposure (24 hrs/d)	<i>Lesion severity NR</i> ; lesion incidence NR	+ <i>Qualitative descriptions only</i>	<i>Not Informative</i> [Deficient exposure quality; Small N; failure to report lesion incidence and severities]
(Casanova et al., 1994) Rat	++	Small N (N = 3)	++	<i>Lesion severity NR</i> ; lesion incidence NR	+ <i>Qualitative descriptions only</i>	<i>Not Informative</i> [Small N; failure to report lesion incidence and severities]
(Coon et al., 1970) Dog	++	Small N (N = 2)	Continuous exposure (22 hrs/d) Note: single concentration study	<i>Lesion severity NR</i> ; lesion incidence NR	+ <i>Qualitative descriptions only</i>	<i>Not Informative</i> [Small N; single concentration tested; failure to report lesion incidence and severities]
(Coon et al., 1970) Guinea pig	++	++	Continuous exposure (22 hrs/d) Note: single concentration study	<i>Lesion severity NR</i> ; lesion incidence NR	+ <i>Qualitative descriptions only</i>	<i>Not Informative</i> [Continuous (high) exposure; failure to report lesion incidence and severities]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations and statistics	Confidence classification
(Coon et al., 1970) Monkey	++	Small N (N = 3)	Continuous exposure (22 hrs/d) Note: single concentration study	<i>Lesion severity NR;</i> <i>lesion incidence NR</i>	+ <i>Qualitative descriptions only</i>	<i>Not Informative</i> [Small N; continuous (high) exposure; failure to report lesion incidence and severities]
(Coon et al., 1970) Rabbit	++	Small N (N = 3)	Continuous exposure (22 hrs/d) Note: single concentration study	<i>Lesion severity NR;</i> <i>lesion incidence NR</i>	+ <i>Qualitative descriptions only</i>	<i>Not Informative</i> [Small N; continuous (high) exposure; failure to report lesion incidence and severities]
(Coon et al., 1970) Rat	++	++	Continuous exposure (22 hrs/d) Note: single concentration study	<i>Lesion severity NR;</i> <i>lesion incidence NR</i>	+ <i>Qualitative descriptions only</i>	<i>Not informative</i> [Continuous (high) exposure; failure to report lesion incidence and severities]
(Feron et al., 1988) Rat	++ Note: exposure in the high concentration group was excessive (24.4 mg/m ³ -d)	++	++	+ <i>No quantitative interim sacrifice data</i> to inform lesions immediately after exposure	++ Note: recovery period data informs persistence of lesions	<i>High</i> [Note: only tested high formaldehyde levels]
(Horton et al., 1963) Mouse	+ <i>Analytical concentrations NR</i> Note: extremely high concentration exposure (200 mg/m ³ -d)	++	+ Early mortality in high exposure group by 11 th d of exposure	Nose was not examined; <i>lesion severity NR</i> Note: lesions are of questionable adversity	++	<i>Low</i> [Analytical concentrations NR; early mortality in the high concentration group, which had an extremely high concentration; nose was not examined; failure to report lesion severity]
(Maronpot et al., 1986) Mouse	+ Formalin; methanol concentration was not reported and a methanol control was not used. [Note: high concentration exposure (49.2 mg/m ³)]	+ <i>Small N (N=10)</i>	++	++	++	<i>Medium</i> [Formalin; small N]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations and statistics	Confidence classification
(Rusch et al., 1983) Rat	++ Note: concentrations tested were very low (0.23–3.6 mg/m ³ -d), and unlikely to elicit a response	++	++	+ <i>Lesion severity NR</i>	incidence of squamous metaplasia and hyperplasia reported together; data reported for only one nasal section	<i>Medium</i> [Failure to report lesion severity; incidence of squamous metaplasia and hyperplasia reported together; data reported for only one nasal section]
(Rusch et al., 1983) Monkey	++ Note: concentrations tested were very low (0.23–3.6 mg/m ³ -d), and unlikely to elicit a response	++	++	+ <i>Lesion severity NR</i>	Incidence of squamous metaplasia and hyperplasia reported together; data reported for only one nasal section	<i>Medium</i> [Failure to report lesion severities; incidence of squamous metaplasia and hyperplasia reported together; data reported for only one nasal section]
(Rusch et al., 1983) Hamster	++ Note: concentrations tested were very low (0.23–3.6 mg/m ³ -d), and unlikely to elicit a response	++	+ <i>Limited study design: only endpoint evaluated was squamous metaplasia</i>	++	Specific incidence data NR, so lack of effect could not be verified	<i>Medium</i> [Specific incidence data NR; only squamous metaplasia was evaluated]
(Wilmer et al., 1989) Rat	+ <i>Analytical concentrations NR</i>	++	++	+ <i>Lesion severity NR</i>	++	<i>Medium</i> [Analytical concentrations NR; failure to report lesion severities]
(Woutersen et al., 1987) Rat	++ Note: high concentration exposure (24.4 mg/m ³ -d)	++	++	++	++	<i>High</i> [Note: the high concentration level was excessive]
(Zwart et al., 1988) Rat	++	++	++	+ <i>Lesion severity NR</i>	+ <i>Lesion incidence incompletely reported</i>	<i>Medium</i> [Failure to completely report lesion incidence; severity NR]
Respiratory Pathology—Short-term						

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations and statistics	Confidence classification
(Andersen et al., 2008) Rat	+ ≈30% variations in chamber concentrations	+ Small N (N = 8)	++	++	+ Statistical analyses of lesions NR	Medium [Small N; variation in chamber concentrations]
(Bhalla et al., 1991) Rat	Analytical method and concentrations NR	+ Small N (N = 6)	++ Note: single concentration study	Lesion severity NR; lesion incidence NR	++	Not Informative [Failure to report analytical method and FA concentrations; small N, failure to report lesion incidence and severities]
(Buckley et al., 1984) Mouse	+ Formalin; methanol concentration was not reported and a methanol control was not used	++	++ Note: single concentration study	Lesion incidence NR	+ Statistical analyses of lesions NR	Low [Formalin; failure to report lesion incidence]
(Cassee and Feron, 1994) Rat	++	++	++ Note: single concentration study	++	Statistical analyses of lesions NR Incidence and severity of hyperplasia and metaplasia reported together	Medium [Incidence and severities of hyperplasia and metaplasia were reported together]
(Cassee et al., 1996b) Rat	++	+ Small N (N=6)	++	++	Statistical analyses of lesions NR; Data NR for 7.9 mg/m ³ group	Medium [Small N, failure to report data for the 7.0 mg/m ³ group]
(Chang et al., 1983) Rat	++	Sample size N unclear	Note: single concentration study; this study measured reflex bradypnea	Lesion severity NR; lesion incidence NR	+ Statistical analyses of lesions NR	Low [Sample size unclear, failure to report lesion incidence and severity]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations and statistics	Confidence classification
(Chang et al., 1983) Mouse	++	Sample size N unclear	Note: single concentration study; this study measured reflex bradypnea	<i>Lesion severity NR</i> ; lesion incidence NR	+ <i>Statistical analyses of lesions NR</i>	<i>Low</i> [Sample size unclear, failure to report lesion incidence and severity]
(Ionescu et al., 1978) Rabbit	Test article characterization NR; <i>analytical concentrations NR</i> ; formaldehyde generation method NR	Test subject strain and number NR	++ Note: single concentration study	<i>Lesion severity NR</i> ; lesion incidence NR	++	<i>Not Informative</i> [Analytical concentrations NR; test article characterization NR; FA generation method NR; test subject strain and number NR; failure to report lesion incidence and severity]
(Kamata et al., 1996b) Rat	+ <i>Formalin</i> ; no methanol control or concentration was reported. [Note: high concentration exposure (179.1 mg/m ³)]	+ <i>Small N (N = 5) for histo-pathology</i>	++	<i>Lesion severity NR</i> ; lesion incidence NR	+ <i>Statistical analyses of lesions NR</i>	<i>Low</i> [Formalin; small N for histopathology; failure to report lesion incidence and severities]
(Kuper et al., 2011) Rat	+ Appears to be freshly made formalin; although <i>formaldehyde generation method NR</i>	+ <i>Small N (N = 8)</i>	++ Note: GLP-compliant study	++	++	<i>Medium</i> [Small N; unclear test article]
(Kuper et al., 2011) Mouse	+ Appears to be freshly made formalin; although <i>formaldehyde generation method NR</i>	+ <i>Small N (N = 6)</i>	++ Note: GLP-compliant study	++	++	<i>Medium</i> [Small N; unclear test article]
(Lima et al., 2015) Rat	Test article characterization NR; concentrations NR- [Note: likely high levels]	+ <i>Small N (N = 7)</i> ; males only	Short (20 min × 3) daily exposures; controls did not appear to be chamber exposed. Note: 5 d exposure	<i>Lesion severity NR</i> ; lesion incidence (nonmorphometric analyses) NR Note: randomized, but blinding NR	+ <i>Statistical analyses of lesions NR</i>	<i>Not Informative</i> [Failure to characterize the test article and report levels; short periodicity; lesion data NR]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations and statistics	Confidence classification
(Ohtsuka et al., 1997) Rat	Test article characterization NR; concentrations NR- [Note: likely high levels]	<i>Small N (N=5 total, but only N = 2 nasal and N = 3 other levels examined); males only</i>	+ <i>Short (3 hr) daily exposure for 2 wk (5 d/wk) with no long-term follow up</i>	<i>Lesion severity NR; lesion incidence NR</i>	+ <i>Qualitative descriptions only</i>	<i>Not Informative</i> [Failure to characterize the test article and report levels; small N; lesion data NR]
(Monteiro-Riviere and Popp, 1986) Rat	++	+ <i>Small N (N = 5; note: only 3/ treated group examined in "detail")</i>	++	<i>Lesion severity NR; lesion incidence NR</i>	+ <i>Statistical analyses of lesions NR</i>	<i>Medium</i> [Small N; lesion incidence and severity NR]
(Monticello et al., 1989) Monkey	+ <i>Analytical concentrations NR</i>	++	++ Note: single concentration study	<i>Lesion severity NR; lesion incidence NR</i>	++	<i>Medium</i> [Analytical concentrations NR; lesion incidence and severity NR]
(Murta et al., 2016) Rat	Test article characterization NR; concentrations NR- [Note: likely high levels]	+ <i>Small N (N = 7); males only</i>	Short (20 min × 3) daily exposures note: 5 d exposure	<i>Lesion severity NR; lesion incidence (nonmorphometric analyses) NR</i> Note: randomized, but blinding NR	+ <i>Statistical analyses of lesions NR</i>	<i>Not Informative</i> [Failure to characterize the test article and report levels; short periodicity; lesion data NR]
(Morgan et al., 2017) Mouse	+ <i>Analytic concentrations NR</i>	++ Note: "randomly assigned"; Males only; ~25 mice/ group; genetically modified (Trp53+/-)	++ Note: 8 wk exposure duration with 32 wk follow up was not a notable issue for these outcomes as numerous lesions found	<i>Blinding NR; only 3 nasal sections evaluated (and 1 larynx)</i>	+ <i>Statistical analyses of lesions NR</i>	<i>Medium</i> [limited sampling and minor reporting limitations]
(Reuzel et al., 1990) Rat	++	++	++	++	+ <i>Statistical analyses of lesions NR</i>	<i>High</i>

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations and statistics	Confidence classification
(Schreiber et al., 1979) Hamster	Test article characterization NR; <i>analytical concentrations NR</i> ; formaldehyde generation method NR Note: high concentration exposure (307.5 mg/m ³)	+ <i>Small N (N = 3 to 5)</i>	++ Note: single concentration study	<i>Lesion severity NR</i> ; lesion incidence NR	+ <i>Statistical analyses of lesions NR</i>	<i>Not Informative</i> [Failure to characterize the test article, describe the generation method, and report analytical concentrations; failure to report lesion incidence and severities]
(Speit et al., 2011b) Rat	+ Formalin; methanol concentration was not reported and a methanol control was not used	+ <i>Small N (N = 6)</i>	++	++	++	<i>Medium</i> [Small N; formalin]
(Wilmer et al., 1987) Rat	+ <i>Analytical concentrations NR</i>	++	++	<i>Lesion severity NR</i> ; lesion incidence NR	++ Note: intermittent versus continuous exposures compared	<i>Medium</i> [Analytical concentrations NR; failure to report lesion incidence and severities]
(Yorgancilar et al., 2012) Rat	Test article characterization NR; <i>analytical concentrations NR</i> ; formaldehyde generation method NR	+ <i>Small N (N = 8)</i>	++ Note: single concentration study	<i>Lesion severity NR</i> ; lesion incidence NR	+ <i>Statistical analyses of lesions NR</i>	<i>Not Informative</i> [Failure to characterize test article; failure to report analytical concentrations and generation method; small N; failure to report lesion incidence and severities]

NR = not reported; N/A = not applicable.

^a Although some notes on exposure levels are included, considerations and interpretations related to the tested exposure levels are discussed as part of the evidence synthesis in the Toxicological Review.

B.3.6. Mechanistic Information Related to Inflammation and Immune Effects

As described in Section 2.3.6 of the Toxicological Review, this broad mechanistic evaluation encompassed studies relating to multiple noncancer health outcomes as well as cancer. It includes the evaluation of studies identified through the searches documented in Appendix B.2.6 as well as animal studies of hypersensitivity responses, and studies of cell proliferation and mucociliary function, some of which were identified through other health effect-specific searches. Details of the study evaluation approach methods are provided in

Section 2.3.6 of the Toxicological Review. The approach to documentation in Table B-41 to Table B-48 highlights important study details regarding the design of each experiment and summarizes the results. The utility of each set of study findings to evidence synthesis and integration judgments is summarized, with supporting documentation highlighting the study details leading to each judgment. The documentation of the review of human studies with both mechanistic and apical health effect evidence that were evaluated in other sections is based on those more detailed health effect evaluations. Studies on the implications of altered endogenous formaldehyde levels are not extracted into the tables below, although there may be some contextual discussion (e.g., to inform potential susceptibility) in the Toxicological Review. The study evaluation tables are first organized according to mechanistic effect type, and then within each effect type into observational and controlled exposure studies organized alphabetically. Specific study details (or lack thereof) that highlight a limitation or uncertainty in answering each of the domain criteria are noted in the table cells. For those identified as having a substantial limitation likely to influence the study results (major limitations), the relevant study details are bolded. Italics are used to highlight other limitations not viewed as substantial in the context of respiratory tract pathology endpoints (minor limitations).

Evaluation of Mechanistic Evidence Related to Changes in the Upper Respiratory Tract (URT)

Cell Proliferation and Mucociliary Function Studies in Animals

Cell proliferation and mucociliary function animal studies in Table B-41 were evaluated using the criteria applied to respiratory tract pathology studies (see Appendix B.3.5).

Table B-41. Evaluation of controlled inhalation exposure studies examining cell proliferation and mucociliary function in animals

Experimental feature categories						
Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
Cell Proliferation						
(Andersen et al., 2008) Rat	+ ≈30% variations in atmospheres	++	++	++	++	High

Supplemental Information—Formaldehyde (Inhalation)

Experimental feature categories						
Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
(Andersen et al., 2010) Rat	++	+ Variable sample size (N = 1 to 8)	++	++	++	High
(Casanova et al., 1994) Rat	++	++	Relevance of exposure scenario unclear (Note: nasal regions selected for analysis may not be relevant to humans)	++	++	Medium
(Cassee and Feron, 1994) Rat	++	+ Number of cells analyzed NR	++ Note: single concentration study	++	++ Qualitative data only	Medium
(Cassee et al., 1996b) Rat	++	+ Small N (N = 3 to 5)	++	+ Data for 7.9 mg/m ³ NR	++	High
(Chang et al., 1983) Rat	++	+ Variable sample size (N = 4 to 9)	Unclear description of study design Note: single concentration study	++	++	Medium
(Chang et al., 1983) Mouse	++	+ Variable sample size (N = 4 to 10)	Unclear description of study design Note: single concentration study	++	++	Medium
(Kuper et al., 2011) Rat	++ Formaldehyde generation method NR	++	++ Note: GLP-compliant study	++	++	High
(Kuper et al., 2011) Mouse	++ Formaldehyde generation method NR	++	++ Note: GLP-compliant study	++	++	High
(Meng et al., 2010) Rat	+ Analytical concentrations NR	++	++	++	++	High

Supplemental Information—Formaldehyde (Inhalation)

Experimental feature categories						
Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
(Monticello et al., 1991) Rat	++	+ Variable sample size (N = 4 to 6)	++	++	++	High
(Monticello et al., 1989) Monkey	+ Analytical concentrations NR	++	+ Note: single concentration study	+ Qualitative data only for nasal region	++	Medium
(Monticello et al., 1996) Rat	++	+ Variable sample size (N = 3 to 8)	+ Nonstandard selection of nasal regions; Note: regions may not be relevant to humans	++	+ Statistical analyses of cell proliferation NR	Medium
(Reuzel et al., 1990) Rat	++	++	++	++	++	High
(Roemer et al., 1993) Rat	++	++	++	++	++	High
(Speit et al., 2011b) Rat	+ Formalin exposure; no methanol controls and concentration NR	++	++	++	++	Medium
(Wilmer et al., 1987) Rat	+ Analytical concentrations NR	Small and variable sample size (N = 1 to 3)	++	++	++	Medium
(Wilmer et al., 1989) Rat	+ Analytical concentrations NR	++	++	++	++	High
(Feron et al., 1987) Rat	++ Note: high concentration exposure (24.4 mg/m ³ -d)	Small N (N=2)	++	++	+ Statistical analyses of cell proliferation NR	Medium

Supplemental Information—Formaldehyde (Inhalation)

Experimental feature categories						
Reference and species	Exposure quality ^a (details in Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
(Zwart et al., 1988) Rat	++	++	++	++	+ Cell proliferation data not readily accessible from graphic form	High
Mucociliary Function						
(Fló-Neyret et al., 2001) Frog	Not an inhalation study. Exposure based on immersion into formaldehyde solution (i.e., formalin)	+ frogs	Ex vivo amphibian study; experiments carried out three days after sacrifice; mucus removed from palate during preparation and returned to palate for testing	++	++	Not Informative
(Morgan et al., 1984) Frog	+ Analytical concentrations within 20% of nominal	+ frogs	Ex vivo amphibian study; method of sacrifice (anesthesia) and palate harvest NR	+ Inter-animal variation observed at several concentrations	++	Low
(Morgan et al., 1986a) Rat	++	++	++ Note: mucociliary function assessed using dissected nasal cavities	++	+ Statistical analyses of mucociliary function data NR	High
(Morgan et al., 1986c) Rat	++	++	++ Note: mucociliary function assessed using dissected nasal cavities	++	+ Statistical analyses of mucociliary function data NR	High

NR = not reported; N/A = not applicable.

^a Although some notes on exposure levels are included, considerations and interpretations related to the tested exposure levels are discussed as part of the evidence synthesis in the Toxicological Review.

Other Changes in the Upper Respiratory Tract

Table B-42. URT-specific structural modification, sensory nerve-related changes, or immune and inflammation-related changes

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
<i>Observational Epidemiology Studies</i>					
(Lyapina et al., 2004)	Symptomatic and nonsymptomatic human workers with carbamide-FA glue (n = 29)	Exposed workers: 0.87 ± 0.39 mg/m ³ (n = 21 nonexposed); duration mean: 12.7 ± 9.6 years	Assessment of chronic URT inflammation	Statistically significant increase in subjective symptoms and objective clinical findings of chronic, URT inflammation (e.g., hypertrophy/atrophy of mucus membranes; rhinitis) and decreased neutrophil function (but N/C in leukocyte cell counts) in workers; symptomatic workers exhibited decreased resistance to infections (increased frequency, duration)	High or Medium Confidence [mixture exposure]
(Bono et al., 2016)	Human plastic laminate workers (n = 50) and office personnel controls (n = 45); males only	Controls (mean $\pm$ SE and range): 0.035 ± 0.0034 (0.016–0.11) mg/m ³ ; Workers: 0.211 ± 0.015 (0.049–0.444); duration unclear	Nasal epithelial ROS (M ₁ dG adducts; a marker of oxidative stress and lipid peroxidation)	Increased adducts with increasing formaldehyde exposure (<i>p</i> trend = 0.002), with statistically significant increases at > 0.066 mg/m ³ (i.e., <0.025 mg/m ³ = 47.6; 0.025–0.066 mg/m ³ = 59.2; and >0.066 mg/m ³ = 105.5 adducts)	High or Medium Confidence [unknown duration]
(Holmström and Wilhelmsson, 1988) (note: mucociliary function data below)	Two exposed groups (n = 170 total; $\approx$ 90% male); 70 formaldehyde production workers; 100 workers exposed to wood dust and	Exposed workers: chemical plant: 0.05–0.5 mg/m ³ , mean 0.26 [SD 0.17 mg/m ³]. Furniture factory: 0.2–0.3 mg/m ³ , mean 0.25 [SD 0.05 mg/m ³]. Referent mean 0.09 mg/m ³ (based on 4 measurements in 4	Symptoms of URT inflammation Histopathology scores	Symptoms of nasal obstruction and nasal watery discharge more frequent in exposed (<i>p</i> < 0.05). When divided into subgroups based on exposure time, there were no signs of increasing nasal restrictivity after employment >5 years. Formaldehyde-only nasal specimens mean histological score: 2.16 (range	Low Confidence [Inclusion of only current workers and long duration of employment raises possibility of healthy worker survival effect due to irritation effects; referent group not well matched (different type of work activity; undersampled males); crude measures of effect]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
	formaldehyde at five furniture factories; Referent: (n=36; ~55% male) from government, with no history of formaldehyde or wood dust exposure	seasons); duration of employment >10 years		0–4) ($p < 0.05$) compared to referent group 1.56 (range 0–4); while formaldehyde-dust group had mean score 2.07 (range 0–6) ($p > 0.05$). No correlation observed between smoking habits and biopsy score, nor was a correlation found between the duration of exposure and any histological changes.	
(Norback et al., 2000)	Primary school personnel in Sweden (n = 234)	0.003–0.016 (mean= 0.0095) mg/m ³ ; duration unclear (working at least 20 hr/wk; assumed length mos or more)	Assessment of acoustic rhinometry and factors in nasal lavage	Formaldehyde was significantly associated with multiple measures of nasal obstruction. Formaldehyde was positively associated with biomarkers for eosinophils (eosinophil cationic protein; lysozyme); N/C in a neutrophil marker (myeloperoxidase) or albumin	Low Confidence [mixture exposure (formaldehyde was independently associated with these changes, but so were NO₂ and Aspergillus)-did not evaluate confounding; some school measures below the limit of detection]
(Priha et al., 2004)	Human MDF board workers (n = 22) versus wood dust (n = 23) and nonexposed (n = 15)	0.19± 0.11 mg/m ³ (MDF board) versus 0.11 ± 0.08 mg/m ³ (note: VOCs 3-fold higher in MDF than wood); pre- and post-8-hr workshift	Nasal lavage cell and cytokine counts	N/C in cell counts Increased postshift total protein vs. unexposed controls Increased post- vs. preshift NO (nitrite) in wood and MDF workers Decreased post- vs. preshift TNFα in wood workers	Low Confidence [short duration; minimal exposure differential; role of VOCs not accounted for] NOTE: ACUTE (8 hr; cross-shift)
<u>Controlled-Exposure Studies in Humans or Primary Human Cells</u>					
(Pazdrak et al., 1993)	Human occupationally exposed (n = 10 males and females) with positive reaction to FA: “allergic”;	Formalin (assumed: test article NR): 0.5 mg/m ³ for 2 hr with follow-up out to 16–18 hr	Nasal lavage cell and protein counts Note: changes were associated with scoring measures of nasal symptoms	Increased number of eosinophils, albumin, and total protein; N/C basophils Increased proportion of eosinophils and decreased proportion of epithelial cells; N/C in proportion of basophils, neutrophils, or	Low Confidence [formalin; short duration; somewhat small sample size; lack of investigator blinding (nonissue for automated albumin measures)]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
	11 “nonallergic” control males		(e.g., sneezing; edema)	mononuclear cells (i.e., lymphocytes and monocytes) Effects max 10 min after exposure and declining, but still significant, at 16–18 hr; effects observed regardless of “allergy”	NOTE: ACUTE; authors noted albumin changes may indicate increased mucosal permeability: albumin percentage, also called the “permeability index,” was elevated at 10 min postexposure only
(Krakowiak et al., 1998)	Human workers with bronchial asthma or healthy subjects (n = 10 each)	Formalin (assumed: test article NR): 0.5 mg/m ³ for 2 hr with follow-up out to 24 hr	Nasal lavage cell and protein counts Note: changes were associated with scoring measures of nasal symptoms (e.g., sneezing; edema)	Increased eosinophils, leukocytes, total cell counts, and permeability index at 30 min after exposure, but not at 4 hr or 24 hr after exposure; N/C in basophils (changes were observed regardless of asthmatic designation) N/C in mast cell tryptase or eosinophil cationic protein	Low Confidence [formalin; short duration; small sample size; lack of investigator blinding (nonissue for automated albumin measures)] NOTE: ACUTE; albumin percentage, aka “permeability index” was used to indicate mucosal permeability; no effect on FEV ₁ , etc.
(Falk et al., 1994)	Human symptomatic for nasal distress (n = 7) or controls (n = 6)	Formalin (assumed from description of test article) Symptomatic: 0.021, 0.028, 0.073, 0.174 mg/m ³ ; ≤2 hr Healthy: 0.023, 0.29, 0.067, 0.127 mg/m ³ ; ≤2 hr	Nasal mucosa swelling by rhinostereometry	FA increased mucosal swelling at ≥0.073 mg/m ³ in symptomatic persons, but swelling was unchanged in healthy controls	Low Confidence [formalin; short duration; small sample size] NOTE: ACUTE; assay is relevant to inflammation, but limited in scope and exposure contrast
(He et al., 2005)	Human student volunteers (n = 10)	Ocular exposure to wood-panel generated formaldehyde gas 0, 1, 2, or 3 mg/m ³ ; 5 min/d for 4 d	Nasal lavage substance P	Substance P was increased significantly at 3 mg/m ³	Low Confidence [exposure route- unknown relevance of ocular exposure route to inhaled exposure level, but considered to be reasonable due to similarities in access of

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
					gas to trigeminal nerve endings for this endpoint; short duration and periodicity; somewhat small sample size]
(Bardet et al., 2014)	In vitro (human primary nasal cells); n=5 experiments (cells: one donor)	Formalin gas: 0.2 mg/m ³ for 1 hr/d for 1, 2, or 3 d	Nasal cell cytokine secretion (at 72 hrs for all exposures)	Slight, statistically significant, decreased IL-8 with 3 exposures only; N/C in IL-6	Not Informative [in vitro; formalin; short duration; small sample size; comparable in vivo inhaled exposure level unknown]
<i>Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells</i>					
(Fujimaki et al., 2004b)	Female C3H mice (n = 5–6 per group)	PFA 0, 0.098, 0.49, or 2.46 mg/m ³ ; 12 wks Sensitization: i.p. 10ug OVA prior to FA exposure; aerosol OVA boost for 6 min on wks 3, 6, 9, and 11	Serum cytokines and neuropeptides (see explanation at right)	D/D increased Substance P without OVA (no change + OVA) at 2.46 mg/m ³ ; FA decreased OVA-induced NGF elevation at 0.098–0.49 mg/m ³ (N/C with FA alone) Body weight decreased at ≥0.49 mg/m ³	High or Medium Confidence [small sample size] Note: although serum measure, discussed in the context of changes in the URT, so included here
(Monticello et al., 1989)	Young adult male rhesus monkeys (n = 3/group)	PFA 0 or 7.38 mg/m ³ for 1 or 6 wk (6 hr/d, 5 d/wk)	Nasal histopathology	Goblet cell loss, hyperplasia, and neutrophil inflammatory response at 1 wk	High or Medium Confidence [high exposure level] Note: n = 3 monkeys/group considered a reasonable sample
(Andersen et al., 2010)	Male F344/CrlBR rats (n = 7–8)	PFA 0, 0.86, 2.46, 7.38, 12.3, or 18.5 mg/m ³ for 1, 4, or 13 wk (6 hr/d, 5 d/wk)	Nasal histology Nasal mRNA analyses (Note: modeling results not considered)	mRNA changes: altered cellular immune response at 1 wk at 12.3–18.5 mg/m ³ , with changes in DNA repair and cell cycle at ≥ 2.46 mg/m ³ ; by 4 wk, immune/injury response is lost; by 13 wk, pervasive changes noted	High or Medium Confidence Note: unclear, indirect interpretability of mRNA profiling
(Andersen et al., 2008)	Male F344 rats (n = 8 for	PFA 0, 0.86, 2.46, or 7.38 mg/m ³ for up to 3	Nasal histopathology, and	Inflammatory cell infiltration was observed at 7.38 mg/m ³ at ≥1-d	High or Medium Confidence NOTE: unclear, indirect

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
	histopath; n ≥5 for genomics)	wks (6 hr/d, 5 d/wk); also acute (18.5 mg/m ³) and instillation	microarray (high flux regions)	exposure; microarray changes at ≥2.46 mg/m ³ at 5 d, but only at 7.38 mg/m ³ at 15 d (1 gene at 2.46 mg/m ³ , 1 d); mostly stress-response related	interpretability of genomic endpoints; note: nasal instillation caused more robust changes
(Woutersen et al., 1989)	Male Wistar rats (n>20/ group)	PFA 0, 0.12, 1.23, or 12.3 mg/m ³ for 28 mos (6 hr/d, 5 d/wk)	Nasal pathology	No treatment-related changes at 0.12–1.23 mg/m ³ ; evidence of damage, inflammation, proliferation at 12.3 mg/m ³	High or Medium Confidence
(Rager et al., 2014)	Male Fischer rats (n = 3 biological replicates/group)	PFA 0 or 2.46 mg/m ³ for 7 d, 28 d or 28 d with 7 d recovery (6 hr/d)	miRNA microarray of nasal respiratory epithelium	Nasal miRNAs were changed after 7 d or 28 d (84 or 59 transcripts), not with recovery; associated with inflammation and immunity, or tumor suppression	High or Medium Confidence [very small sample size] NOTE: unclear, indirect interpretability of endpoints
(Tsubone and Kawata, 1991)	Male Wistar rats (n = 6/ group; each rat received 2–4 exposures of PFA or control air)	PFA 0.39–5.78 mg/m ³ through upper airway for 22 sec (under anesthesia)	Ethmoidal nerve activity (nasal trigeminal nerve branch)	Afferent nerve activity was increased by FA, with a 50% increase in activity at ≈2.2 mg/m ³ (although FA stimulated nerve activity at all levels- ≈20% at 0.62 mg/m ³)	High or Medium Confidence [short duration] NOTE: ACUTE: surgical procedures considered internally controlled (since rats served as own controls)
(Kulle and Cooper, 1975)	Male SD rats (n = 5)	PFA 0.62, 1.23, 1.85, or 2.46 mg/m ³ for 1 hr or 0.62–3.08 mg/m ³ for 25 sec (with anesthesia)	Nasopalantine nerve responses (similar to ethmoidal in preliminary tests)	Sensory threshold from 25 sec exposure: 0.31 mg/m ³ Trigeminal response to an odorant (amyl alcohol) is decreased at ≥0.62 mg/m ³ FA	High or Medium Confidence [slightly small sample size; short duration] NOTE: ACUTE; surgical procedures internally controlled
(Yonemitsu et al., 2013)	TRPA1 knockout (KO) or wild type (WT) mice (n = 3–5)	Formalin at up to 123 mg/m ³ (varied by experiment and chamber location, but all exposures considered “excessive”); ACUTE	Responses related to effects on the trigeminal nerve	Formalin vapor (3 min) activated secondary trigeminal system neurons (according to c-fos activity) in WT but not KO mice. Consistent with this, formalin vapor accelerated wakefulness and induced avoidance behaviors in WT but not KO mice; and labeling studies	High or Medium Confidence [small sample size; short duration; formalin; excessive levels; see below for explanation] NOTE: ACUTE; effects of related chemicals such as acrolein were similarly blocked in KO mice.

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
				confirmed TRPA1 expression on trigeminal afferents innervating the nasal mucosa	Given the difficult nature of studying this event, the consistency of effects across related chemicals, and the well-accepted role for TRPA1 in acrolein-induced sensory effects (based largely on Bautista et al., 2006), these results are judged to provide indirect evidence interpreted with high or medium confidence and not direct evidence interpreted with low confidence.
(Rager et al., 2013)	Male cynomolgus macques (n = 2–3/group)	PFA 0, 2.46, or 7.38 mg/m ³ for 2 d (6 hr/d)	Nasal miRNA screen and molecular target verification	3 and 13 miRNAs were dysregulated by exposure, including associations with decreased apoptosis signaling (at 2) and increased epithelial proliferation (at 6)	Low Confidence [short duration; n=2 primates: small sample size] NOTE: Unclear direct relevance of miRNA changes
(Clement et al., 1987)	Female Wistar Rats (n=10)	PFA 0 or 18.5 mg/m ³ for 12 wks (6 hr/d, 5 d/wk)	URT epithelial structure and junctional proteins by IHC and TEM	Basal lamina degeneration, and goblet cell hypertrophy of respiratory epithelium FA reduced levels of junctional proteins but did not cause destroy the junctional complex when assessed by TEM Note: body weight significantly decreased by FA (<5%)	Low Confidence [excessive exposure levels]
(Cassee et al., 1996b)	Male Wistar albino rats (≥3/group)	PFA 0, 1.23, 3.94, or 7.87 mg/m ³ for 1 or 3 d (6 hr/d)	Nasal histopathology and biochemistry	Evidence of damage and inflammation at 3 d, ≥3.94 mg/m ³ Increased GPx and NPSH (3 d, ≥3.94 mg/m ³ ; latter at 1 d, 7.87 mg/m ³ too), not GST, FDH, ADH, or GR in respiratory epithelium	Low Confidence [short duration; very small sample size] NOTE: ACUTE or 3 d; NPSH: nonprotein sulfhydryl groups

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Cassee and Feron, 1994)	Male Wistar rats (n = 20/ group; n = 6+/endpoint)	PFA 4.43 mg/m ³ for 3 d (intermittent) Note: weights decreased in all groups	Nasal enzyme activity Nasal GSH	Increased GPx N/C in ADH, GST, G6PDH, GR, or FDH N/C in cytosolic GSH (slightly increased) Note: rhinitis and necrosis also reported	Low Confidence [short duration and unclear periodicity; high exposure level]
(Abreu et al., 2016)	C57BL/6 mice (n = 12 M+F/ treatment group and n = 6 M+F/control)	Formalin (assumed) 0, 0.25, 1.2, and 3.7 mg/m ³ for 8 hr (aldehyde mixture data not included herein; authors noted some exposure cross-contamination)	Nasal epithelial histology (morphology only) (blinded measures 6–8 hr postexposure)	N/C in nasal epithelium, except small, but significant, decreases in cilia at 0.25 mg/m ³	Low Confidence [formalin; short duration and periodicity; some coexposure to acetaldehyde possible but unclear] Note: ACUTE
(Monteiro-Riviere and Popp, 1986)	Male F344 rats (n = 3 examined in detail)	PFA 0, 0.62, 2.46, 7.38, or 18.5 mg/m ³ for up to 4 d (6 hr/d); controls not air-exposed	URT respiratory epithelium ultra-structural pathology	Inflammation (neutrophil infiltration; goblet cell hypertrophy) at ≥7.38 mg/m ³ ; duration-dependency shown	Low Confidence [short duration; very small sample size; controls not air exposed] NOTE: no statistical comparisons of structural changes
(Mcnamara et al., 2007)	In vitro mouse and rat dorsal DRG neurons (n = 300+ neurons) or HEK293 cells (n ≥ 5); (note: relevance is as URT stimulus)	Formalin or methanol controls (levels irrelevant to inhalation exposure); ACUTE experiments	Activation and specific inhibition of “sensory nerve cell” activity	Formalin, but not methanol, specifically activated TRPA1 in vitro. This specific activation was confirmed using TRPA1 knockout DRG neurons as well as specific pharmacologic inhibitors. TRPA1 inhibition also reduced formalin-induced pain behaviors in vivo.	Low Confidence [in vitro; unknown exposure level relevance; short duration] Note: ACUTE; methanol controls; categorized as low confidence rather than excluding due to less concern for methanol effects on receptors in nasal mucosa
(Tani et al., 1986)	Male rabbits (strain unspecified) n= unclear	Formalin 12.3 mg/m ³ (acute) directly infused into either the URT (nasal) and/ or LRT (lung)	Pharmacologic intervention studies on respiratory and cardiac function (compared to	The effects of formaldehyde on respiration and heart rate were only observed with nasal exposure, not lung. Inhibition of afferent sensory	Low Confidence [formalin; short duration; unknown sample size] NOTE: ACUTE; categorized as low confidence rather than

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
			acrolein and ammonia)	nerve activity abrogated the formaldehyde effects.	excluding due to less concern for methanol effects on receptors in nasal mucosa
(Kunkler et al., 2011)	In vitro trigeminal root ganglia (rat) neurons (n = 9–15)	Formalin (levels irrelevant to inhalation exposure); ACUTE experiments	Agonist/antagonist studies of TRP channel-mediated CGRP release	Formaldehyde stimulated release of CGRP from adult trigeminal neurons (Note: inhibitor studies not tested on FA, but acrolein was through TRPA1)	Low Confidence [in vitro; formalin; short duration; high, unknown exposure level] NOTE: ACUTE; categorized as low confidence rather than excluding due to less concern for methanol effects on receptors in nasal mucosa
(Zhao et al., 2020)	Male Balb/c mice (n = 3, pooled into single sample for nose and lung samples); 2 experiments by different researchers	Formalin 0, 3 mg/m ³ for 2 wks (8 hr/d, 5 d/wk)	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies in nose, lung, spleen, and bone marrow	Nose (ex vivo) results: Decreased formation of BFU-E in both experiment I and II Decreased formation of CFU-GM in experiment I; N/C in experiment II Nose (in vitro treatment): 400 uM formaldehyde significantly decreased BFU-E not CFU-GM formation (both nonsignificantly decreased across doses)	Low Confidence [formalin; small sample size; in vitro (for cell treatments)]
(Hester et al., 2003)	Male F344 rats; n = 3–4	Formalin (assumed, based on description); nasal instillation (400 mM in 40 µL aliquot/nostril)	Respiratory epithelium gene expression	24 of 1,185 genes upregulated, and 22 downregulated	Not Informative [formalin; short duration; very small sample size; high, unknown exposure level; exposure route] NOTE: ACUTE
(Ohtsuka et al., 2003)	Male BN and F344 rats; n = 4/group	Formalin aerosol 1% for 3 hr/d for 5 d vs. water	Nasal mucosa cytokines and structure	Degeneration and neutrophil inflammation (F344> BN) Decreased IFN-γ and IL-2 in BN; N/C in F344; N/C in IL-4 or IL-5 in BN or F344	Not Informative [formalin; short periodicity; small sample size; high, unknown exposure levels]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Macpherson et al., 2007)	In vitro; n ≥ 7; transfected cells (HEK293T cells neuroendocrine; immortalized human kidney)	Formalin (levels irrelevant to inhalation exposure); ACUTE experiments	Activation and specific inhibition of “sensory nerve cell” activity	Formalin activated TRPA1. This selective activation was confirmed by inhibition of pain-related behaviors induced by formalin in vivo.	Not Informative [in vitro; formalin; short duration; high, unknown exposure level; limited reporting] NOTE: ACUTE

Evaluation of Mechanistic Evidence Related to Changes in Lower Respiratory Tract (LRT)

Markers of Structural Changes, Immune response, Inflammation or Oxidative Stress

Table B-43. LRT (e.g., lung, trachea, BAL) markers of structural modification, immune response, inflammation, or oxidative stress

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
Observational Epidemiology Studies					
(Franklin et al., 2000)	Human healthy children (n = 224; age ≈9.5 year);	FA levels in bedroom and living room were dichotomized into > or < 0.062 mg/m ³ ; duration unknown	exhaled nitric oxide (eNO); Note: technique used excludes NO originating from the upper airway	eNO (“reflects airway inflammation”) significantly increased in children of homes with higher FA levels, after correcting for multiple other variables	High or Medium Confidence [limited exposure contrast; accuracy of single measure questionable] Note: authors suggest species differences in inflammation locale
(Bentayeb et al., 2015)	Human elderly (>65 years) European nursing home individuals (n = 600 from 20 homes)	Indoor FA levels in main common room ranged from approximately 0.005–0.01 mg/m ³ (median ≈0.006) over 1 wk of sampling; duration unknown	eNO (marker of lower airway inflammation) eCO (marker of CO exhalation and smoking)	FA was not associated with eNO FA was associated with increased eCO Note: FA was associated with increased reported COPD and FVC, but not FEV1, asthma diagnosis or symptoms, or cough	High or Medium Confidence [limited exposure contrast; unclear whether adjusted for co-exposures] Note: PM co-exposure was not associated with eNO or eCO; NO ₂ was associated with decreased eNO
(Flamant-Hulin et al., 2010)	Human school children (34 asthmatics; 70 nonasthmatics);	[Low] yards: 0.0036 (0.0024–0.0044) mg/m ³ and rooms: 0.025 (0.013–0.036) mg/m ³	Fractional exhaled nitric oxide (FeNO)—“reliable, noninvasive marker of airway inflammation” [Note:	FeNO significantly increased in both nonasthmatics and asthmatics with high versus low FA exposure in classrooms, but not schoolyards; in nonasthmatics, a	High or Medium Confidence [accuracy of single measure questionable] Note: authors hypothesized that atopic status might modify airway

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
		[High] yards: 0.0058 (0.0049–0.0068) mg/m ³ and rooms: 0.044 (0.038–0.047) mg/m ³ ; unknown duration	“nasal contamination” was prevented]	stronger association was found for atopic versus nonatopic children	response to formaldehyde; called changes “bronchial inflammation”
(Roda et al., 2011)	French infants (n = 2,940 with assessment at birth and 12 mos)	Median 0.020 mg/m ³ ; IQR 0.014–0.027 mg/m ³ ; LOD 0.008 mg/m ³ .	LRT infections (with or without wheeze) Note: although URT infections were queried, these data were NR	Significantly increased LRT infection: 32% or 41% increase per 0.0124 mg/m ³ increase in formaldehyde (without and with wheeze, respectively)	High or Medium Confidence [specificity and sensitivity of predictive model not tested on a separate sample]
(Rumchev et al., 2002)	Australian children (ages 6–36 mos); 88 cases, 104 controls	Mean 0.030 and 0.028 and maximum 0.224 and 0.190 mg/m ³ , respectively, in bedroom and living room.	Lower respiratory tract infection involving wheezing (assuming misclassification of a many of the discharges as asthma rather than infection)	Increased emergency room visits for this case definition	Low Confidence [recruitment process not described; uncertainty as to how well this case definition describes LRT infection and the length of time between emergency room visit and subsequent exposure measure]
Controlled-Exposure Studies in Humans or Primary Human Cells					
(Casset et al., 2006)	Human (n = 19 with mild asthma and allergy to mite allergen)	Formalin 0.1 mg/m ³ for 30 min; placebo at ≈0.03 mg/m ³ double-blind randomized; restricted to mouth breathing only	Sputum (lower airway mucus) eosinophils and ECP	Authors note a trend, not statistically significant, towards increased eosinophil counts (≈38 ± 9% vs. 11 ± 3%, FA vs. air controls), and an increase in ECP (439 ± 171 vs. 156 ± 58 µg/l, FA vs. air controls)	Low Confidence [formalin; short duration; not clear that restriction to mouth breathing is realistic for typical inhalation] NOTE: ACUTE; within-subjects comparison between air and FA
(Ezratty et al., 2007)	Human (n = 12 intermittent asthmatics with allergy to pollen)	Formalin 0.5 mg/m ³ for 60 min; randomized allocation (no nonexposed controls)	Sputum (lower airway mucus) cell counts and released factors	N/C in sputum Total cell counts, WBC subtypes, or factors (e.g., ILs, MCP, TNF)	Low Confidence [formalin; short duration] NOTE: all exposed to both air and FA; internally controlled
Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells					
(Fujimaki et al., 2004b)	Female C3H mice (n = 5–6 per group)	PFA 0, 0.098, 0.49, 2.46 mg/m ³ ; 12 wks	BAL cell counts BAL cytokines and neuropeptides	No significant changes in cell counts with FA alone; macrophages and eosinophils increased at 2.46 mg/m ³ with OVA+FA; N/C in neutrophils or lymphocytes	High or Medium Confidence [small sample size for some groups/endpoints] Note: MIP-1α, eotaxin, MCP-1, BDNF, and Substance P levels insufficient for testing
		Sensitization: i.p. 10 µg OVA prior to FA exposure; aerosol OVA boost for 6 min on wks 3, 6, 9, and 11		No significant changes in cytokines with FA alone (NGF was D/D increased)	

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
				FA with OVA D/D decreased IL-1 β at 2.46 mg/m ³ and NGF at 0.098–0.49 mg/m ³ ; N/C in TNF- α , GM-CSF, or IL-6; MCP-1, MIP-1a, and eotaxin were not detectable Body weight decreased at ≥ 0.49 mg/m ³	
(Riedel et al., 1996)	Female Dunkin-Hartley guinea pigs (n = 3)	Formaldehyde (bottled pressurized gas) 0, 0.13, 0.31 mg/m ³ for 5 d (8 hr/d)	Airway histology and morphometry	With FA, lung bronchi had intramural edema (wall thickening) by morphometry; no evidence of cellular lower airway inflammation by histology	High or Medium Confidence [small sample size] Note: histology after FA with OVA not examined
		Sensitization: 0.5% inhaled OVA; OVA boost at 2 wk Challenge: 1% inhaled OVA 1 wk later			
(Ito et al., 1996)	Male Wistar rats (n = 7)	Formalin (with MeOH controls) 2.46, 6.15, 18.5, or 55.4 mg/m ³ for 10 min	Airway microvascular leakage (Evans blue) in trachea and main bronchi	D/D increased leakage at ≥ 6.15 mg/m ³ , which resolved in <20 min Leakage at 18.5 mg/m ³ was inhibited by NK1 receptor antagonism, but not by histamine H1 or bradykinin B2 R antagonists 55.4 mg/m ³ MeOH alone induced slight leakage in main bronchi, but not trachea)	High or Medium Confidence [short duration] Note: figure comparisons presented against room air, not MeOH, controls, but comparisons made to MeOH controls in text
(Jakab, 1992)	In vivo and Ex vivo Female Swiss mice (n = 5+ mice/determination)	PFA 0, 0.62, 1.23, 6.15, 12.3, or 18.5 mg/m ³ for 4–18 hr or 4 d (4 hr/d); $\pm$ carbon black	Pulmonary bactericidal activity to inhaled Staphylococcus And ex vivo alveolar macrophage function	Pulmonary antibacterial activity was reduced: at 1.23 mg/m ³ for 18 hr before and 4 hr postbacterial challenge (postexposure alone reduced at 18.5 mg/m ³) N/C in ex vivo alveolar macrophage Fc receptor-mediated phagocytosis of RBCs at 6.15 mg/m ³ for 4 d (FA + carbon black, but not FA alone, caused a robust decrease)	High or Medium Confidence [short duration]—in vivo pulmonary bactericidal activity Note: ACUTE Low Confidence [ex vivo; short duration]
(Swiecichowski et al., 1993)	Male Hartley guinea pigs (n = 5–12/group)	PFA at 4.18 mg/m ³ for 2 or 8 hrs (multiple experiments)	Airway Histology (trachea)	No change histological evidence of cell infiltration or epithelial damage up to 96 hr after exposure to 4.18 mg/m ³ for 8 hr	High or Medium Confidence at 1.23 mg/m³ and above [short duration] Low Confidence below 1.23 mg/m³ and ex vivo [ex vivo; sample size of 5 at 1 or more levels below 1 ppm] NOTE: ACUTE

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Ozen et al., 2003a)	Male albino Wistar rats (n = 6)	PFA at 6.15 and 12.3 mg/m ³ for 4 or 13 wks (8 hr/d)	Lung tissue homogenate measures of trace elements	Zn was dose-dependently decreased (≥6.15 mg/m ³ for both exposure durations) Fe was dose-dependently increased (≥6.15 mg/m ³ with 13 wk; significant only at 12.3 mg/m ³ after 4 wk); Cu was unchanged	High or Medium Confidence [high levels] NOTE: unclear relevance of endpoints; authors claim Fe change linked to oxidative stress and Zn change linked to decreased DNA synthesis, but no direct evidence
(Aydin et al., 2014)	Male SD rats (n=6/group)	Test article unclear, but appears to be formalin in this experiment at 0, 6.48 (low), 12.3 (moderate), or 18.7 mg/m ³ for 4 wk (8 hr/d, 5 d/wk)	Lung tissue total antioxidant and total oxidant levels (TAS and TOS; kit uses vitamin E and H ₂ O ₂ as reference, respectively) Lung tissue oxidative stress index (OSI: TOS/TAS) and apoptotic index Lung irisin (hormone may regulate obesity)	Increased TOS and OSI, and decreased TAS and irisin, at ≥ 12.3 mg/m ³ formaldehyde Increased lung apoptotic index at ≥6.48 mg/m ³ Note: Carnosine supplementation reduced changes.	Low Confidence [formalin; high levels]
(Luo et al., 2013)	In vitro and ex vivo (intact trachea) from SD rats (sex NR); n= as low as 4 (some inhibitor assays), as high as 28 (trachea)	Formalin (assumed, test article NR; levels irrelevant to inhalation exposure); ACUTE (bath application) experiments	Isc currents in trachea and epithelium from trachea with various inhibitors TRPV channel expression and labeling	Formaldehyde caused a dose-dependent, sustained increase in currents in isolated trachea and airway epithelia TRPV-1 channels were localized to intraepithelial nerve endings and inhibition of TRPV-1 or substance P activity (blocking NK-1R) inhibited current increases Cl ⁻ released in response to formaldehyde was blocked several Cl channel blockers and involved cAMP	Low Confidence [in vitro and ex vivo (intact trachea); formalin; unknown exposure level relevance] Note: ACUTE, some inhibition experiments had n = 4, but magnitude of inhibition was robust with small variability
(Lundberg and Saria, 1983)	Male SD rats (sample size NR)	Direct injection of formaldehyde (assumed to be formalin); 50 µL volume unknown comparison to inhalation exposure	Tracheal mucosal reactivity (Evans blue extravasation)	Formaldehyde injection caused extravasation which was reduced or abolished by capsaicin pretreatment	Low Confidence [formalin; inferred high levels; short duration; nonspecific reporting] NOTE: ACUTE
(Larsen et al., 2013)	Male BALB/cA mice (n = 10/group)	PFA 0.49, 2.21, or 4.9–7.0 (dry vs. humid air) mg/m ³ ; 60 min	BAL counts	FA did not affect BAL “degree of lung inflammation” (data not shown; unclear if this reflects comparisons of total cell	Low Confidence [short duration; for BAL endpoints: poor reporting; FA alone groups data NR; OVA

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
		Sensitization: pre-FA i.p. 1 µg OVA, with 0.1 µg OVA boosts i.p. on days 14 and 21 (note: FA on Day 31) Challenge: 0.2% OVA aerosol for 20 min on Days 29 and 30		counts or comparisons of individual cell types, as data were presented for OVA, i.e., neutrophils, lymphocytes, eosinophils, macrophages)	without FA and OVA with FA groups combined] NOTE: ACUTE
(Wu et al., 2013)	Male Balb/c mice (n = 8/group)	Formalin 0 or 3 mg/m ³ for 4 wk (6 hr/d, 5 d/wk) with or without OVA aerosol	BALF cell counts Lung tissue cytokines, neuropeptides, and histology/IHC	Total cells, eosinophils, and lymphocytes were increased in BALF by FA alone, and all of these cells (minus lymphocytes but plus neutrophils) were increased more robustly by FA+ OVA Histopathology: increased inflammation FA increased lung IL-4, IL-1β, substance P, and CGRP, but not IFNγ; more robustly by FA+OVA (peptide changes by IHC also) TRPA1 and TRPV1 antagonists reduced FA+OVA-induced eosinophil counts (anti-TRPA1 also decreased neutrophils), and lung factors (except IL-1)	Low Confidence [formalin; pharmacological interventions did not include effects of FA alone]
		Sensitization: s.c. 80 µg OVA on Days 10, 18, and 25 Challenge: 1% OVA aerosol 30 min/d on Days 29–35			
(Qiao et al., 2009)	Male Wistar rats (n = 8/group)	Formalin 0, 0.51 or 3.08 mg/m ³ for 3 wk (6 hr/d)	BALF cell counts Lung histology and cytokine levels	“slight but insignificant pulmonary abnormalities” with FA alone; OVA 3.18 mg/m ³ changed airway structure N/C in BAL total cells or eosinophils with 3.18 mg/m ³ , but ≥0.51 mg/m ³ dose-dependently increased both in presence of OVA; 3.18 mg/m ³ FA alone increased IFNγ and decreased IL-4; FA+OVA increased IL-4	Low Confidence [formalin]
		Sensitization: i.p. OVA on Days 10 and 18 Challenge: 1% OVA 30 min/d for 7 d			
(Liu et al., 2011)	Male Balb/c mice (n = 6/ group)	Formalin 0, 0.5, or 3 mg/m ³ for 21 d (6 hr/d)	BALF cell counts Pulmonary histology and cytokines	Cell infiltration and airway remodeling in 3 mg/m ³ FA + OVA Increased % Eosinophils at ≥ 0.5 mg/m ³ , which is amplified by OVA; N/C IFNγ Increased lung IL-4 and IL-6 at 3 mg/m ³ ; with OVA, this is observed at 0.5 mg/m ³	Low Confidence [formalin]
		Sensitization: i.v. 20 mg OVA on Day 10 and 1 Challenge: 1% OVA aerosol for 30 min/d for 7 d			
(Ye et al., 2013)	Male Balb/c mice (n ≥9/ group/ endpoint)	Formalin 0, 0.5, 1, or 3 mg/m ³ for 7 d (8 hr/d)	ROS (dichlorohydro-flourescein and MDA) and GSH in Lung	Dose-dependent decrease in GSH levels in lung at ≥0.5 mg/m ³ Dose-dependent increase in DCFH and MDA in lung at ≥1 mg/m ³ Co-administered GSH attenuated effects	Low Confidence [formalin]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Abreu et al., 2016)	C57BL/6 mice (n = 12 M+F/ treatment group and n = 6 M+F/ control)	Formalin (assumed) 0, 0.25, 1.2, and 3.7 mg/m ³ for 8 hr (aldehyde mixture data not included herein; authors noted some exposure cross-contamination)	Lung histology (cells and morphology) (blinded measures 6–8 hr postexposure) Lung cytokine, catalase, and SOD levels/ activity	FA increased distended alveoli at 3.7 mg/m ³ ; N/C in total mononuclear or polymorphonuclear cells N/C in IL-1, IL-6, TNF, CCL2, or MIP-2, or in antioxidants; increased keratinocyte chemoattractant at 0.25 mg/m ³ only Note: N/C in lung mechanics except increased airway inertance (might indicate an impedance of airflow) at 3.7 mg/m ³	Low Confidence [formalin; short duration and periodicity; some coexposure to acetaldehyde possible- unclear] Note: ACUTE
(Sandikci et al., 2007b)	SD rats (n = 6/ group) at GD1 [I], PND1 [II], PND28 [III] or adults [IV]	Formalin (assumed: test article NR): 0 or 7.38 mg/m ³ for 6 wks (8 hr/d, 7 d/wk)	BALT T lymphocyte CD4+, CD8+ counts (by IHC)	Increased BALT T lymphocytes (ANAE+ as marker); CD4+ T cell counts and size of BALT increased in Groups III and IV; CD8+ T cell counts increased in Group III Note: body weight was significantly decreased in Groups I and II	Low Confidence [formalin; high exposure levels] Note: limited assays
(Sandikci et al., 2007a)	Female SD rats at GD1 [i], PND1 [ii], PND 28 [iii], or PND90 [iv] (n = 6)	Formalin (assumed; test article NR) 0, 7.38 mg/m ³ for 6 wks (8 hr/d, 7 d/wk)	BALT T lymphocyte counts; BALT size Note: body weight decreased by FA in groups i and ii	CD4+ cell counts increased in groups iii and iv; CD8+ cell counts increased in group iii (group iv N/S increased) Increased size of BALT in adults (iii & iv)	Low Confidence [formalin; high exposure levels]
(Sandikci et al., 2009)	SD rats (n = 9/ group) at GD1 [I], PND28 [II] or adults [III]	Formalin (assumed: test article NR): 0 or 7.38 mg/m ³ for 6 wks (8 hr/d, 7 d/wk)	Apoptotic cell counts in lung and BALT	Apoptotic cells increased in lung and BALT in Groups II and III, but not Group I (authors speculated apoptotic BALT cells were lymphocytes)	Low Confidence [formalin; high exposure levels]
(Jung et al., 2007)	Female C57BL/6 mice (n = 10/ group)	Formalin (assumed; test article NR) 0, 6.15, 12.3 mg/m ³ for 2 wk (6 hr/d, 5 d/wk)	Lung oxidative stress (intracellular, by flow) BAL and lung homogenate counts, and histopath. Cytokine mRNA and protein	Oxidative stress (DCFH-DA) at ≥6.15 mg/m ³ Total BAL cells increased (2-fold) at 12.3 mg/m ³ ; Slight changes in B220+ B cells (↓) and CD3+ or CD4+ T cells (↑) were not interpreted as significant; CD8+ T cells were ↑, only slightly; N/C in neutrophils Large increase in eosinophil counts from BAL, and in flow counts and gene expression of lung tissue at 12.3 mg/m ³ , eosinophil infiltration, and epithelial damage, by histopath at ≥6.15 mg/m ³	Low Confidence [formalin; high exposure levels; statistical significance of flow data NR] Note: Th2 cytokines

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
				Increased IL-4, IL-5, and IL-1 β (not IL-13) in lung at 6.15 and 12.3 mg/m ³ body weights decreased $\approx$ 10%	
(Sul et al., 2007)	Male SD rats (n = 10/group)	Formalin (assumed; test article NR) 0, 6.15, 12.3 mg/m ³ for 2 wks	Lung tissue oxidative stress and mRNA array	Lipid peroxidation (MDA) and protein oxidation were increased at 12.3 mg/m ³ Changes in 21 genes, including D/D decrease in 3 immune-related genes: HSP70 _{1a} , complement 4 binding protein, and Fc receptor IgG low affinity III	Low Confidence [formalin; high levels] NOTE: utility of mRNA results by themselves unclear
(Lu et al., 2005)	Male Kun Ming mice (n = 5)	Formalin 0, 0.5, 1, or 3 mg/m ³ for 10 d (6 hr/d)	BALF IL-4 (undetected in serum)	D/D Increased IL-4 at $\geq$ 1 mg/m ³ FA Blocked by vanilloid (TRPV) receptor antagonist, CPZ	Low Confidence [formalin; small sample size]
(Ahn et al., 2010)	Male SD rats (n = 4/group)	Formalin (assumed; test article NR) 0, 2.46, or 24.6 mg/m ³ for 2 wk (6 hr/d)	BAL fluid proteomic analysis	6 proteins increased (3 inflammatory serpins, anti-inflammatory annexin, an erythrocyte protein associated with trauma or inflammation, and a metabolic enzyme); 5 proteins were decreased	Low Confidence [formalin] NOTE: unclear utility of measures
(Kimura et al., 2010)	Male Wistar (n = 5–6)	Formalin 1.23, 6.15, 18.5, or 55.4 mg/m ³ for up to 45 min	Airway microvascular leakage (lung- main bronchi and trachea) BALF counts of leukocytes Shed epithelial cells in BALF	D/D increase leakage by 15 min at $\geq$ 1.23 mg/m ³ ; not exacerbated with longer/repeated exposure Note: Leakage induced by substance P was not inhibited by pre-FA exposure, but preinhalation of the same mg/m ³ abolished FA-induced leakage and pre-FA inhibited capsaicin-induced leakage; however, 20 hr between exposures allows for recovery of tachykinins and leakage by FA exposure Inhibition of mast cell activation (H1 receptor antagonist), but not cyclooxygenase products (indomethacin), blocked FA leakage at 6.15 mg/m ³ ; increased shed epithelial cells 20 hr, but not immediately, after 6.15 mg/m ³ for 30 min Increased BALF neutrophils with preinhalation at 6.15 mg/m ³ , but N/C eosinophils or mononuclear cells	Low Confidence [formalin; small sample size; short duration] Note: Authors hypothesize preinhalation of FA depletes the amount of tachykinins available at the target site (but not desensitization of NK1 receptors), in part b/c capsaicin can no longer induce a response; also, because of recovery, up to 6.15 mg/m ³ does not cause irreversible damage to airway sensory nerves, but that prolonged exposure ($\geq$ 7 d) might exacerbate neurogenic airway inflammation

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Dallas et al., 1987)	Male SD rats (n = 2/ timepoint; unclear reporting)	PFA 0, 0.62, 3.69, or 18.5 mg/m ³ for 1 wk to 24 wk (6 hr/d, 5 d/wk)	Flow cytometry DNA/RNA analysis of alveolar cell proliferation/ health	Increased RNA index in alveolar cells at all FA levels at 1 wk; only at ≥ 3.69 mg/m ³ at 8 wk; N/C in DNA (e.g., % S phase) [Note: same alveolar samples had chromatid breaks at 18.5 mg/m ³]	Low Confidence [small sample size; unclear reporting] NOTE: unclear specificity/ utility of methods
(Kim et al., 2013a)	Female C57BL/6 mice (n = 5 “experiments”; number of mice/ group unclear)	Formalin (assumed; test article NR) 0, 6.15, or 12.3 mg/m ³ for 2–3 wk (6 hr/d, 5 d/wk)	Lung cell counts BAL cell counts Ex vivo cellular functional assays	N/C in lung tissue total cells, but number of NK1 cells markedly decreased (this recovered by 2 wks postexposure) at 12.3 mg/m ³ Lung NK1 cell mRNA and protein markers (IFN γ , perforin, and CD122) were D/D decreased at ≥ 6.15 mg/m ³ BAL total cells increased, but number of NK cells decreased at 12.3 mg/m ³ N/C in other lung or BAL lymphocyte populations (e.g., % CD4+ or CD8+ cells)	Low Confidence [formalin; high levels; small sample size] Not Informative: ex vivo experiments or in vitro FA treatment of NK precursors showing reduced differentiation to mature cells
(Sadakane et al., 2002)	Male ICR mice (n = 9 or 18)	Formalin 0.5% for 4 wk (15 min/wk) Sensitization: i.p. with 3 mg/mL Der f (house dust mite allergen) prior to FA Challenge: intratracheal 10 μ g Der f 3 hr after last exposure (note: measures 3 d later)	Lung IHC cell counts and cytokine analysis	N/C in lung eosinophil recruitment or goblet cell proliferation by FA alone, but Der f-induced eosinophil recruitment was exacerbated by FA Increased RANTES in lung by FA alone, and exacerbated increase to Der f-changes with FA for IL-5 and RANTES; N/C in lung IL-2 or IL-4	Low Confidence [formalin; unquantified high levels; short periodicity]
(Sandikci et al., 2007a)	Female SD rats at PND1, PND28, or PND90 (n = 3)	Formalin (assumed; test article NR) 0 or 7.38 mg/m ³ for 6 wk (8 hr/d, 7 d/wk)	Lung and BALT histology	N/C in exposed PND1 group Increased apoptotic cells in lungs and BALT of PND28 and PND90 groups Authors: apop. cells likely lymphocytes	Low Confidence [formalin; high level; small sample size]
(Matsuoka et al., 2010)	Male ICR mice (n ≥ 7)	Formalin at 0.12 mg/m ³ for up to 24 hr; also, a single experiment at 3.69 mg/m ³ for 24 hr	lung ROS (8OHdG) and NO metabolites (nitrates/ nitrites); at 3.69 mg/m ³ : LPS response	Decreased ROS lung; N/C in NOs or lung NOs after LPS injection	Low Confidence [formalin; short duration] NOTE: ACUTE
(Yan et al., 2005)	Male Kun Ming mice (n = 6)	Mixture (test article wood panels) 0, 0.5, 1, or 3 mg/m ³ for 72 hr (24 hr/d)	Lung NOS activity and NO measurement	Increased NOS activity at 3 mg/m ³ FA (p = 0.06 at 1 mg/m ³)	Low Confidence [wood panel exposure; lack of controls for co-exposure; short duration]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
				NO was detected more frequently in samples from 3 mg/m ³ FA group (50% vs. 17%)	NOTE: NO detection did not include statistical comparisons
(Dinsdale et al., 1993)	Male SD rats (n = 4, 6, or 10)	PFA or Formalin 12.3 mg/m ³ for 4 d (6 hr/d)	Lung enzymes (in BAL or tissue) Lung histology	Increased cytochrome P450 and decreased γ-glutamyl transpeptidase with PFA exposure (not with formalin) No abnormalities (i.e., signs of injury or repair) by histology	Low Confidence [small sample size; excessively high levels; short duration] NOTE: Endpoints not very informative for inflammation (injury response, possibly)
(Rager et al., 2011)	In vitro (human lung cancer cell line); n = 6 replicates	PFA 1.23 mg/m ³ for 4 hr or air controls	In vitro epithelial cell miRNA microarray and IL-8 secretion	Increased IL-8 release >16-fold with FA 89 miRNAs were downregulated by FA; the 4 most robust were associated with inflammatory response pathways	Low Confidence [in vitro; short duration; exposure level comparability to inhalation unclear]
(Zhao et al., 2020)	Male Balb/c mice (n = 3, pooled into single sample for nose and lung samples); 2 experiments by different researchers	Formalin 0, 3 mg/m ³ for 2 wks (8 hr/d, 5 d/wk)	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies in nose, lung, spleen, and bone marrow	Lung (ex vivo) results: Decreased formation of BFU-E in experiment II; N/C in experiment I Decreased formation of CFU-GM in experiment II; N/C in experiment I Lung (in vitro treatment): Up to 400 uM formaldehyde caused N/C in BFU-E not CFU-GM formation	Low Confidence [formalin; small sample size; in vitro (for cell treatments)]
(Maiellaro et al., 2014)	Pregnant Wistar rats (n = 5; note: individual pup data for n = 10 pups did not appear to account for litters)	Formalin 0.92 mg/m ³ from GD1-GD21: 1 hr/d, 5 d/wk	BAL cell counts and factors Lung factors	N/C in parental BAL total cells, monocytes, lymphocytes, or granulocytes N/C in parental lung IL-4, IL-6 or IL-10; Decreased birth weight in offspring 24 hr after OVA challenge, offspring have: decreased BAL total cells, mononuclear cells, neutrophils, and eosinophils; Increased BAL IL-10, but decreased IL-6 and TNFα (N/C in IL-4)	Not Informative [formalin, short periodicity; small sample size; offspring comparisons do not include FA alone; did not appear to account for litter effects]
		Sensitization: s.c. 10 µg OVA with sc boost after 7 d Challenge: 7 d later, 1% OVA aerosol 15 min/d, 3 d			
(Maiellaro et al., 2016)	Pregnant Wistar rats (n = 5 dams; note: individual pup data for n = 10 pups did not appear to account for litters)	Formalin 6.13 mg/m ³ from GD1-GD21: 1 hr/d, 5 d/wk	BAL cell counts and factors in pups on ≈PND45	Increased (amplified) total BAL leukocytes Increased (amplified) BAL mononuclear cells and neutrophils Increased (amplified) myeloperoxidase Decreased (slightly reduced) eosinophils and eosinophil peroxidase	Not Informative [formalin, short periodicity; small sample size; offspring comparisons do not include FA alone; did not appear to account for litter effects]
		Sensitization on PND 30: s.c. 10 µg OVA Challenge: 14d later, 1% OVA aerosol 15 min/d, 3 d			

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Silva Ibrahim et al., 2015)	Pregnant Wistar rats (n = 5 dams; 10 pups/group for experiments; note: individual pup data for n = 10 pups did not appear to account for litters)	Formalin 0.92 mg/m ³ from GDs 1–21: 1 hr/d, 5 d/wk	Cell number, cytokine and neutrophil marker (MPO) in BAL Function of BAL cells Lung gene and proteins	24 hr after LPS challenge, offspring exposed to formaldehyde have reduced immune responses to LPS (i.e., decreased BAL cells and granulocytes- N/C in lymphocytes or monocytes; decreased MPO and oxidative burst- N/C in phagocytosis; decreased IL-6 and increased IFN and IL-10; decreased TLR4 and NFkB)	Not Informative [formalin; short periodicity; offspring comparisons do not include FA without LPS; small sample size; did not appear to account for litter effects]
		Randomly assigned pups all received 5 mg/kg lipopolysacharride (LPS) injections at PND 30			
(Ibrahim et al., 2016)	Pregnant Wistar rats (n = 5 dams; 10 pups/ group for experiments; note: individual pup data for n = 10 pups did not appear to account for litters)	Formalin 0.92 mg/m ³ from GDs 1–21: 1 hr/d, 5 d/wk	Total BAL cell number and cytokine gene expression	Increased cell number by LPS was reduced in offspring exposed to formaldehyde Formaldehyde increased IFN expression, decreased IL-6, TLR4, and NF-kB expression, and caused N/C in IL-10, as compared to LPS	Not Informative [formalin; short periodicity; offspring comparisons do not include FA without LPS; small sample size; did not appear to account for litter effects] Note: effects rescued by vitamin C
		Randomly assigned pups all received 5 mg/kg lipopolysacharride (LPS) injections at PND 30			
(da Silva et al., 2015)	Male Wistar rats (n = 6/ group)	Formalin 1% for 3 d (90 min/d); rats exposed in static chambers 5 rats/ time	BAL cell counts Lung vascular permeability BAL and lung cytokines (all measures at 24 hr postexposure except permeability, which was immediate)	FA increased total BAL cells, activated mast cells, and neutrophils (latter based on myeloperoxidase activity) FA did not change trachea permeability (Evans blue), but did increase it in lung parenchyma and bronchii FA increased TNF, IL_6, and N/C IL-10 in BAL, and increased IL-10, but not IL-6 mRNA in lung tissue Note: while reduced effects were reported as reduced with laser therapy, laser therapy-only controls were not used	Not Informative [formalin; unquantified high levels; static exposure chamber and group exposure; short duration and periodicity]
(Murta et al., 2016)	Male Fischer rats (n = 7)	Formalin (assumed) 1%, 5%, or 10% for 5 d (3 × 20 min/d)	BAL cell counts Lung histopathology and chemokine levels	FA increased total leukocyte, macrophages at 10%, and lymphocytes at ≥5%; N/C in neutrophils or eosinophils; ≥5% caused lung parenchyma damage; ≥1% increased CCL5 and 10% CCL2 (N/C in CCL3)	Not Informative [formalin; unquantified high levels; static exposure chamber; short periodicity]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Kilburn and Mckenzie, 1978)	Male and female Syrian golden hamster (n = 6–14)	PFA “low”: 3.69 or 7.38 mg/m ³ or “high”: ≥246 mg/m ³ for 4 hr; alone, with carbon dust, or evaporated onto carbon	Lower airway PMN Leukocyte recruitment and cellular changes by histology	Although cytotoxic effects were observed at ≥3.69 mg/m ³ , FA alone did not induce PMN leukocyte recruitment; FA + carbon caused leukocyte recruitment 2 hr postexposure, which peaked at ≈20 hr and resolved by 1 wk; recruitment was similar at “low” and “high” levels	Not Informative [short duration, precision of exposure levels unclear; reporting difficult to follow, and data NR for all exposure levels indicated as tested; nonexposed controls did not appear to be included]
(Persoz et al., 2010)	In vitro (human immortalized lung cells); n = 4 experiments	Formalin gas: 0.050 mg/m ³ for 30 min, ± TNFα sensitization	Lung cell Cytokine secretion (at 24 hr post-FA)	N/C in IL-6, IL-8, or MCP-1 without TNF α sensitization Increased IL-8 only with sensitization Note: air exposure alone increased IL-8	Not Informative [formalin; in vitro; short duration; unknown exposure level relevance; small sample size; controls exhibited effects from air-only exposure]
(Persoz et al., 2011)	In vitro (human immortalized lung cells); n = 4 experiments	Formalin gas: 0.050 mg/m ³ for 30 min, with or without aspergillus spores (Asp)	Lung cell cytokine secretion (at 24 hr post-FA)	N/C in IL-8 or MCP-1 mRNA or protein	Not Informative [formalin; in vitro; short duration; unknown exposure level relevance; small sample size; controls exhibited effects from air-only exposure]
(Persoz et al., 2012)	In vitro (human immortalized lung cells); n ≥3 experiments	Formalin gas: 0.050 mg/m ³ for 30 min; treatment with sensitizers (i.e., TNFα or MCM)	Bronchial or alveolar cytokine secretion (at 24 hr post-FA)	IL-8 production in alveolar cells induced by TNFα or macrophage-conditioned media (MCM) increased by FA MCP-1 production in bronchial cells induced by sensitizers increased by FA N/C on IL-8 or MCP-1 otherwise Note: expression affected by air alone	Not Informative [formalin; in vitro; short duration; unknown exposure level relevance; small sample size; controls exhibited effects from air-only exposure]
(Kastner et al., 2013)	In vitro (human immortalized lung cells); n = 3 experiments	Formalin gas: 0.2 mg/m ³ for 30 min, 1 hr, or 2 hr/day once or for 4 d	Lung cell cytokine secretion and epithelial barrier function/ viability (at 24 hr post-FA)	N/C in IL-6 or IL-8 release, or TEER (measures disruption to epithelial cell monolayer) by FA alone Note: viability affected by air exposure	Not Informative [formalin; in vitro; short duration; unknown exposure level relevance; small sample size; controls exhibited effects from air-only exposure]
(Lino-Dos-Santos-Franco et al., 2013a)	Female Wistar rats (n = 5)	Formalin 1% or methanol vehicle for 3 d (90 min/ d), ± ovariectomy	BAL counts Ex vivo lung IL-10	1 d after challenge: FA/OVA versus OVA alone decreased total cell counts, including mononuclear cells, neutrophils, and eosinophils FA/OVA versus OVA alone: Robust IL-10 increase	Not Informative [formalin (MeOH controls); naïve not chamber exposed; unquantified high levels; FA alone untested; small sample size]
		Sensitization: After FA, s.c. 10 µg OVA, with s.c. boost 7 d later Challenge: After 7 d, 1% OVA aerosol for 15 min			

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Lino-Dos-Santos-Franco et al., 2010)	Male Wistar rats (n = 5–6)	Formalin 1% for 3 d (90 min/d)	Lung cellular oxidative burst (flow) and tissue oxidative stress-peroxynitrite (3-NT)	Increased cellular oxidative burst (DFFH, $\pm$ OVA) Increased lung nitration (peroxynitrite formation; without OVA)	Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity] Note: vitamin C, E blunted effects
		Sensitization: immediately post-FA, s.c. 10 μ g OVA; boost 1 wk later with s.c. 10 μ g OVA injection Challenge: 1 wk later with 1% aerosol OVA (15 min)			
(Macedo et al., 2016a)	Male Wistar rats (n = 6)	Formalin 1% for 3 d (90 min/d)	Lung (or lung cells) oxidative stress indicators: H ₂ O ₂ , nitrites, oxidative burst, enzyme activity and gene expression of redox-related proteins	Formaldehyde exposure increased H ₂ O ₂ and NO ₂ , but not DCFH-DA (oxidative burst), and exposure increased expression of cNOS and iNOS, SOD and catalase, but did not affect the activity of enzymes associated with detoxification processes (e.g., glutathione reductase)	Not Informative [formalin; unquantified high levels; short duration and periodicity] Note: Photobiomodulation (laser) therapy blunted effects
(Lima et al., 2015)	Male Fischer rats (n=7)	Formalin 1, 5, or 10% for 5 d (20 min $\times$ 3/d)	Trachea or diaphragm muscle (DM) oxidative stress indicators: carbonyl protein, lipid peroxidation, and catalase activity; and inflammatory cell influx	In Trachea: increased lipid peroxidation at 1 and 5, but not 10%; N/C in catalase or inflammatory cell influx; increased mucus deposits at 5%, and increased metaplasia and ulceration at 10% In DM: increased lipid peroxidation at 1 and 5, but not 10%; increased carbonyl protein and increased inflammatory cell influx at 10%; decreased catalase at $\geq$ 1%	Not Informative [formalin; unquantified high levels; short duration and periodicity; controls not chamber exposed]
(Lino dos Santos-Franco et al., 2009)	Male Wistar rats (n = 5)	Formalin 0, 1% for 3 d (90 min/d)	BAL nitrites	FA increased BAL nitrites, which was exacerbated with OVA sensitization	NotInformative [formalin; unquantified high levels; small sample size; short duration and periodicity]
		Sensitization: immediately post-FA, i.p. 10 μ g OVA; boost 1 wk later with s.c. injection Challenge: 1 wk later with aerosolized OVA			
(Lino-Dos-Santos-Franco et al., 2013b)	Male Wistar rats (n = 5–8)	Formalin 1% or naive for 3 d (90 min/ d), with or without subsequent OVA	Lung mRNA Ex vivo Lung factors	FA increased iNOS and COX-1, but not COX-2, expression in lung (OVA and FA seemed to attenuate induction by other) FA/OVA vs. OVA increased NO and LTB ₄ (both inhibited by inhibition of NOS or by inhibition of COX), but not TXB ₂ or PGE ₂ Note: suggests mast cell- and NO-mediated effects	NotInformative [formalin; unquantified high levels; small sample size; short duration and periodicity; comparisons reported did not include all relevant controls (e.g., FA alone; air alone)]
		Sensitization: after FA inhalation, s.c. 10ug OVA with same boost 7 d later Challenge: after 1 wk, 1% OVA aerosol for 15 min			

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Lino-Dos-Santos-Franco et al., 2011b)	Male Wistar rats (n = 5/group)	Formalin 1% for 3 d (90 min/d)	BAL cell counts Lung ROS Ex vivo lung cytokines in explants or cultured BAL cells	FA increased total BAL cells, mononuclear cells, and neutrophils FA decreased SOD, but not catalase, GPX, GR, or GST activity in lung tissue; mRNA expression for SOD, catalase, NOS, and COX was increased FA increased IL-1 β and IL-6 in explants; increased NO ₂ and H ₂ O ₂ in BAL cells	Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity; some ex vivo]
(Lino dos Santos-Franco et al., 2006)	Male Wistar (n = 5–6)	Formalin 1% or methanol vehicle for 4 d (30, 60, or 90 min/d)	BAL cell counts Lung IHC Ex vivo BAL nitrites	Increased BAL Total cells (90 min only), mononuclear cells (60 and 90 min), and neutrophils (30, 60, or 90 min) Increased ex vivo cultured BAL cell release of nitrites Lung IHC showed mast cell degranulation and neutrophil infiltration Note: number of cells recovered in BAL was significantly reduced by capsaicin (depletes neuropeptides from sensory nerve endings), but bronchial hyporesponsiveness not altered; conversely L-NAME (inhibits NO synthase) did not affect BAL cells, but did restore bronchial responsiveness; administration of 48/80 to deplete mast cells blunted FA-induced effects on both BAL cell counts and bronchial response	Not Informative [formalin ((MeOH controls); unquantified high levels; small sample size; short duration and periodicity; comparisons reported to naïve rats rather than MeOH controls; some ex vivo)] NOTE: if a relevant MOA is identified from more informative studies, pharmacological intervention endpoints might be reconsidered
(Lino-Dos-Santos-Franco et al., 2011a)	Female Wistar rats (n = 5)	Formalin 1% or naïve for 3 d (90 min/d), with or without ovariectomy	BAL counts and mast cell degranulation	FA increased total BAL cell counts, mononuclear cells and neutrophils, but not eosinophils Decreased lung mast cell number and increased degranulation	Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity; impact of sham surgery/ FA alone untested; naïve not chamber exposed]
(Lino-Dos-Santos-Franco et al., 2010)	Male Wistar rats (n = 5–6)	Formalin 1% for 3 d (90 min/d)	Pulmonary vascular permeability (Evans blue) BAL cell counts Ex vivo cultured BAL cells factors/cytokines	Increased BAL mononuclear cells and neutrophils, but N/C in eosinophils or in lung ICAM-1 Increased vascular permeability ($\pm$ OVA) FA increased ex vivo LTB ₄ ; FA+OVA increased BAL LTB ₄ , TXB ₂ , IL-1 β , IL-6, VEGF	Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity; some ex vivo]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
			Phagocytosis (flow)	N/C in phagocytosis;	Note: vitamin C and E blunted effects
		Sensitization: immediately post-FA, s.c. 10 µg OVA; boost 1 wk later with s.c. 10 µg OVA injection Challenge: 1 wk later with 1% aerosol OVA (15 min)			
(Kita et al., 2003)	Male Hartley guinea pigs (n = 10+/group)	Nasal Instillation of saline or Formalin 0.1 or 1.0%; 3x/wk for 6 wk s	BAL cell counts	N/C in BAL fluid cell counts by FA with passive or active sensitization (not measured for FA alone)	Not Informative [formalin; high, unknown levels; short periodicity; exposure route; effect of FA alone not measured]
		Sensitization: intradermal anti-OVA serum on day 38 (passive) or i.p. 2 mg OVA on Day 3 (active) with boost i.p. 10 mg OVA day 24 Challenge: 1 mg/mL nebulized OVA 15 min after last FA exposure on day 45			
(Kita and Oomichi, 1974)	In/Ex vitro: trachea from guinea pigs (n = 3)	Formalin gas: 39.4 or 67.7 mg/m³ for <30 min	In vitro ciliary beat frequency	FA decreased CBF 50% in 11.5 min (39.4 mg/m³) or 4.5 min (67.7 mg/m³)	Not Informative [formalin; excessively high levels; short duration; ex vitro; small sample size]
(Lino dos Santos Franco et al., 2009)	Male Wistar rats (n=5)	Formalin 0, 1% for 3 d (90 min/d)	BAL cell counts Lung mast cell degranulation	Increased Total BAL cells, mononuclear cells, and neutrophils (eosinophils undetected); FA inhibited OVA-induced increases in all cell counts FA increased mast cell degranulation; FA inhibited OVA induced degranulation FA induced PECAM expression; FA inhibited OVA induced increases	Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity]
		Sensitization: immediately post-FA, i.p. 10ug OVA; boost 1 wk later with s.c. injection Challenge: 1 wk later 1% aerosol OVA for 15 min			

Changes in pulmonary function involving provocation

Table B-44. Changes in pulmonary function involving provocation (e.g., bronchoconstrictors; allergens; etc.)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
<u>Observational Epidemiology Studies</u>					

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Górski and Krakowiak, 1991)	Human textile and shoemakers (n = 367)	Not exceeding 0.5 mg/m ³ (duration at least 1 year (average= ≈12 years)	Bronchial hyper-reactivity to histamine	Bronchial hyperreactivity in 11 nonbronchitic patients (14 bronchitic/2 asthmatic ppl)	Low Confidence [incomplete and confusing methods and results; comparisons unclear]
<i>Controlled-Exposure Studies in Humans or Primary Human Cells</i>					
(Krakowiak et al., 1998)	Human workers with bronchial asthma or healthy subjects (n = 10 each)	Formalin (assumed: test article NR): 0.5 mg/m ³ for 2 hr with follow-up out to 24 hr	Bronchial provocation responses (histamine)	N/C in Bronchial reactivity to histamine (Note: scoring measures of nasal symptoms were elevated)	Low Confidence [formalin; short duration; small sample size] NOTE: ACUTE; no effect on FEV ₁ , etc.
(Casset et al., 2006)	Human (n = 19 with mild asthma and allergy to mite allergen)	Formalin ≈0.1 mg/m ³ for 30 min; placebo at ≈0.03 mg/m ³ double-blind randomized; restricted to mouth breathing only	Airway response to mite allergen (Note: large allergen size chosen to deposit in large airways)	A lower level of allergen was necessary to induce bronchoconstriction following FA exposure and FA exposure: both immediate and late-phase responses; note: N/C in pulmonary function tests with FA exposure alone prior to allergen challenge	Low Confidence [formalin; short duration; not clear that restriction to mouth breathing is realistic for typical inhalation] NOTE: ACUTE; within-subjects comparison between air and FA
(Ezratty et al., 2007)	Human (n = 12 intermittent asthmatics with allergy to pollen)	Formalin 0.5 mg/m ³ for 60 min; randomized to air or FA first (no nonexposed controls)	Allergen (pollen)-induced changes in airway FEV ₁ and MCh responses (note: did not appear to test MCh w/o allergen) 8 hr later	N/C in pulmonary function by allergen (a borderline decreased response, <i>p</i> = 0.06, was observed) or to MCh responsiveness after allergen challenge; note: N/C in pulmonary function by FA	Low Confidence [formalin; short duration] NOTE: ACUTE; within subjects comparison between air and FA
<i>Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells</i>					
(Riedel et al., 1996)	Female Dunkin-Hartley guinea pigs (n=12)	Formaldehyde (bottled pressurized gas) 0, 0.16, 0.31 mg/m ³ for 5 d (8 hr/d) Sensitization: 0.5% inhaled OVA; OVA boost at 2wk Challenge: 1% inhaled OVA 1 wk later	Airway response to OVA	Increased OVA challenge-induced airway obstruction by 0.31 mg/m ³ (3, 7, and 10 animals exhibited airway obstruction across groups)	High or Medium Confidence [no comparison group with FA without OVA] NOTE: guinea pigs have been shown to be more sensitive to airway constriction from toxicants than other animals]
(Leikauf, 1992) [considered same cohort as (Swiecichows	Male Hartley guinea pigs (n = 5–7)	PFA 0, 0.12, 0.37, 1.23, 3.69, 12.3, or 36.9 mg/m ³ for up to 8 hr	Bronchial reactivity to i.v. acetylcholine	Increased specific resistance at ≥12.3 mg/m ³ with 2 hr; Increased at ≥1.23 mg/m ³ with 8 hr (i.e., duration > concentration); with 8 hr, hyperreactivity persisted >24 hr postexposure	See Swiecichowski et al., 1993 NOTE: ACUTE

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
ki et al., 1993]					
(Swiecichows ki et al., 1993)	Male Hartley guinea pigs (n = 5–7/group)	PFA from 0.12–123 mg/m ³ , for 2 or 8 hrs (multiple experiments)	Airway reactivity Ex vivo airway reactivity (trachea)	Increased pulmonary resistance (reversible bronchoconstriction) and airway reactivity to acetylcholine at ≥1.23 mg/m ³ (not at 0.36 mg/m ³) for 8 hr; at ≥ 12.3 mg/m ³ (not at ≤3.6 mg/m ³) for 2 hr Increased ex vivo reactivity (smooth muscle contraction) at 4.18 mg/m ³ for 8 hr	High or Medium Confidence at 1.23 mg/m³ and above [short duration] Low Confidence below 1.23 mg/m³ and ex vivo [ex vivo; sample size of 5 at 1 or more levels below 1ppm] NOTE: ACUTE; duration appeared to be more important than FA level for pulmonary resistance
(Larsen et al., 2013)	Male BALB/cA mice (n = 10)	PFA 0.49, 2.21, or 4.9–7.0 (dry vs. humid air) mg/m ³ ; 60 min Sensitization: pre-FA i.p. 1 µg OVA, with 0.1 µg OVA boosts i.p. on days 14 and 21 (note: FA on day 31) Challenge: 0.2% OVA aerosol- 20 min on day 29 and 30	Airway reactivity	Increased airway reactivity (decreased expiratory flow rate) in humid air in OVA-sensitized mice at 7 mg/m ³ Increased bronchoconstriction in a dry environment without OVA sensitization at 4.92–7.0 mg/m ³ (with OVA sensitization reducing the response to formaldehyde)	High or Medium Confidence [short duration] NOTE: ACUTE; suggests that environmental humidity may affect acute airway reactivity induced by formaldehyde; experiments on inflammatory markers (below) considered less informative
(Liu et al., 2011)	Male Balb/c mice (n = 6/ group)	Formalin 0, 0.5, or 3 mg/m ³ for 21 d (6 hr/d) Sensitization: i.v. 20 mg OVA on Days 10 and 21 Challenge: 1% OVA aerosol for 30 min/d for 7 d	Airway reactivity	Slightly increased responsivity to MCh compared to saline controls; robust amplification in 3mg/m ³ FA+OVA group	Low Confidence [formalin]
(Qiao et al., 2009)	Male Wistar rats (n = 8/group)	Formalin 0, 0.51 or 3.08 mg/m ³ for 3 wk (6 hr/d) Sensitization: i.p. OVA on Days 10 and 18 Challenge: 1% OVA 30 min/d for 7 d	Airway response to methylcholine	3.08 mg/m ³ FA alone increased hyperresponsiveness to MCh, which was amplified with OVA administration at ≥ 0.51 mg/m ³	Low Confidence [formalin]
(Wu et al., 2013)	Male Balb/c mice (n = 8/group)	Formalin 0, 3 mg/m ³ for 4 wk (6 hr/d, 5 d/wk) Sensitization: s.c. 80 µg OVA on Days 10, 18, and 25 Challenge: 1% OVA aerosol 30 min/d on Days 29–35	Airway responsivity to Methylcholine (MCh)	Airway was slightly hyperresponsive to MCh by FA alone, but severely so in FA+OVA groups TRPA1 and TRPV1 antagonists reduced FA+OVA-induced airway responsiveness	Low Confidence [formalin; pharmacological interventions did not include effects of FA alone]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Biagini et al., 1989)	Male cynomolgus monkeys (n = 9)	Formalin 3.08 mg/m ³ for 10 min (challenge experiment)	Bronchoreactivity to methylcholine (all with MCh)	Increased bronchoconstriction by FA challenge at 2, 5, and 10 min postchallenge	Low Confidence [formalin; short duration; FA without methylcholine untested]
(Maiellaro et al., 2014)	Pregnant Wistar rats (n = 5)	Formalin 0.92 mg/m ³ from GDs 1–21: 1 hr/d, 5 d/wk Sensitization: s.c. 10 µg OVA with sc boost after 7 d Challenge: 7 d later, 1% OVA aerosol 15 min/d, 3 d	Tracheal response to MCh	24 hr after OVA challenge, offspring have: decreased tracheal response to MCh Note: Decreased birth weight in offspring. Nonmanipulated group exhibits large, unexplained differences from vehicle control (and has reporting limitations)	Not Informative [formalin; short periodicity; offspring comparisons do not include FA alone; unclear comparability for some groups; small sample size]
(Silva Ibrahim et al., 2015)	Pregnant Wistar rats (n = 5 dams; 10 pups/ group for experiments)	Formalin 0.92 mg/m ³ from GDs 1–21: 1 hr/d, 5 d/wk Randomly assigned pups all received 5 mg/kg lipopolysaccharide (LPS) injections at PND 30	Response to MCh	24 hr after LPS challenge, offspring exposed to formaldehyde have decreased MCh response	Not Informative [formalin; short periodicity; offspring comparisons do not include FA without LPS; small sample size]
(Kita et al., 2003)	Male Hartley guinea pigs (n = 5–7/group)	Nasal Instillation of saline or Formalin 0.1 or 1.0%; 3×/wk for 6 wk Sensitization: intradermal anti-OVA serum on day 38 (passive) or i.p. 2 mg OVA on day 3 (active) with boost i.p. 10 mg OVA Day 24 Challenge: 1 mg/mL nebulized OVA 15 min after last FA exposure on Day 45	Bronchoconstriction to methylcholine	N/C in airway response to MCh by FA or FA with passive sensitization, but induced by FA with active sensitization	Not Informative [formalin; high, unknown levels; short periodicity; exposure route]
(Lee et al., 1984)	Male English guinea pigs (n = 4)	Formalin: 7.38 or 12.3 mg/m ³ for 5 d FA challenge with 2.46 or 4.9 mg/m ³ for 1 or 4 hr, respectively on Days 7, 22, and 29 Respiratory rate change from prechallenge baseline		N/C in pulmonary sensitivity (either immediate or delayed-onset) to formaldehyde challenge Note: 2/4 animals exhibited dermal sensitivity (likely contact-mediated) to topical FA; 12.3 mg/m ³ caused 40–50% respiratory rate decrease for ≥5 hr (later time points NR)	Not Informative [formalin; small sample size; high exposure levels; no comparison to controls with no prior formaldehyde exposure (unclear if this, by itself, caused effects); unclear reporting]
(Lino-Dos-Santos-Franco et al., 2013a)	Female Wistar rats (n = 5)	Formalin 1% or methanol vehicle for 3 d (90 min/d), ± ovariectomy	Lung oxidative stress, microvascular leakage and mast cell degranulation; ex vivo tracheal reactivity	1 d after OVA challenge: FA/OVA versus OVA alone: Reduced MPO and vascular permeability; decreased mast cell degranulation Decreased tracheal reactivity	Not Informative [formalin (MeOH controls), naïve not chamber exposed; high, unquantified levels, FA alone untested; small sample size]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
		Sensitization: After FA, s.c. 10 µg OVA, with s.c. boost 7 d later Challenge: After 7 d, 1% OVA aerosol for 15 min			
(Lino-Dos-Santos-Franco et al., 2011a)	Female Wistar rats (n = 5)	Formalin 1% or naïve for 3 d (90 min/d), with or without ovariectomy	Ex vivo trachea response	N/C in ex vivo tracheal response to methacholine	Not Informative [formalin, naïve not chamber exposed; ex vivo; high, unquantified levels, FA alone untested; small sample]
(Lino dos Santos Franco et al., 2006)	Male Wistar (n = 5–6)	Formalin 1% or methanol vehicle for 4 d (30, 60, or 90 min/d)	Ex vivo airway responsivity	Decreased ex vivo bronchial, but not tracheal, response to methacholine Note: number of cells recovered in BAL was significantly reduced by capsaicin (depletes neuropeptides from sensory nerve endings), but bronchial hyporesponsiveness not altered; conversely L-NAME (inhibits NO synthase) did not affect BAL cells, but did restore bronchial responsiveness; administration of 48/80 to deplete mast cells blunted FA-induced effects on both BAL cell counts and bronchial response	Not Informative [formalin (MeOH controls); naïve not chamber exposed; high, unquantified levels, comparisons to naïve rats rather than MeOH controls; small sample size] NOTE: if a relevant MOA is identified from more informative studies, pharmacological intervention endpoints might be reconsidered
(Lino-Dos-Santos-Franco et al., 2013b)	Male Wistar rats (n = 5–8)	Formalin 1% or naïve for 3 d (90 min/d), with or without subsequent OVA Sensitization: after FA inhalation, s.c. 10 µg OVA with same boost 7 d later Challenge: after 1 wk, 1% OVA aerosol for 15 min	Ex vivo bronchial response to MCh	Prior FA exposure reduced OVA-induced ex vivo bronchial hyperresponsiveness Note: N/C in respiratory resistance or elastance with FA alone	Not Informative [formalin; naïve not chamber exposed; high, unquantified levels; short duration and periodicity; comparisons did not include all relevant controls (e.g., FA alone; air alone); small sample size]

Evaluation of Mechanistic Evidence Related to Changes in the Blood or Lymphoid Organs

Changes in serum (primarily) antibody responses

Table B-45. Serum (primarily) antibody responses

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
<i>Observational Epidemiology Studies</i>					
(Wantke et al., 1996a)	Human children in schools (n = 62) vs. control (n = 19)	Particleboard schools: 0.053, 0.085, or 0.092 mg/m ³ (n = 18, 22, 22); brick schools: 0.036, 0.028, or 0.032 mg/m ³ (n=18, 22, 22); unclear duration (<2.5 year)	Serum FA-specific IgE	Before switching schools, 40% of students had elevated FA-specific IgE, which significantly decreased 3 mos after switch to low-FA schools ($p < 0.002$) Note: while symptoms correlated to FA levels, FA-specific IgE did not	High or Medium Confidence [no blinding, but not clearly an issue] Note: Natural experiment (pre- and postschool switch) with limited exposure contrast and assays
(Kim et al., 1999)	Human medical students (n = 167) and nonexposed controls (n = 67)	3.74 ± 3.48 mg/m ³ for up to 4 years of school (periodicity NR)	Serum FA-specific IgG and IgE (antibodies to FA-human serum albumin conjugate)	14 (8.4%) students had FA-specific IgG, which was not related to duration of schooling (No relationship to symptoms) N/C in FA-specific IgE	High or Medium Confidence Note: Limited assays
(Aydın et al., 2013)	Human male fiberboard workers	0.25 ± 0.074 mg/m ³ (average 7.3 year employed; n = 46) vs. nonexposed controls	Serum Antibodies	Decreased IgG and IgM N/C in IgA	High or Medium Confidence
(Wantke et al., 1996b)	Human medical students (n = 45)	0.153± 0.062 mg/m ³ for 4 wk (Total: 17 d; 51 hr); phenol co-exposure	Serum FA-specific IgE Total IgE	N/C in FA-specific IgE; N/C in total IgE	Low Confidence [37% participation; phenol co-exposure; limited periodicity] Note: limited assays
(Wantke et al., 2000)	Human medical students (n = 27); 23 controls	0.265± 0.07 mg/m ³ for 5 or 10 wks (intermittent—not specified, but assumed ≈3 hr/d)	Serum Antibodies and FA-specific Antibodies	After 5 wk: N/C FA-IgE or Total IgE After 10 wk: 4/27 students developed IgE against FA-albumin, but 0/23 developed IgG; N/C in Total IgE	Low Confidence [no reporting of % participation or population demographics; limited, unclear periodicity; phenol co-exposure] Note: 1 of 4 positive was a smoker (4 smokers in study); limited assays
(Erdei et al., 2003)	Human (sex NR) symptomatic students (9–11 yo	0.006–0.057 mg/m ³ (average = 0.018 mg/m ³); duration unknown [co-exposure: NO ₂ , benzene,	Serum Antibodies	N/C total IgG, IgA, IgM, or IgE (data NR) Increased airway pathogen bacteria-specific IgG (not IgA or IgM) with FA	Low Confidence [comparisons to “normal” range rather than to control group; co-exposure; limited reporting]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
	w/ respiratory issues) (n = 176)	toluene, xylene, and dust mite allergen]			Note: symptomatic only; authors hypothesized increased bacterial-specific IgG may represent increased B cell response (maybe more infections)
(Zhou et al., 2005)	Human anatomy students (n = 8)	0.74 ± .11 mg/m ³ (4-wk course—intermittent)	Serum FA-specific IgE antibodies	No students had FA-specific IgE after exposure	Low Confidence [small sample (n=8); limited, unclear periodicity; reporting as yes/no rather than analytical results, and no clear comparison to preexposure]
(Ohmichi et al., 2006)	Human anatomy students (n = 8 measured for FA; n = 6 for FA-specific IgE)	0.41–1.81 mg/m ³ (20 laboratory sessions over 10 wks; laboratory sessions ranged from 1.1–10 hrs, averaging 3hr)	Serum IgE and FA-specific IgE (threshold of 0.34 UA/mL)	No significant changes in IgE, and no positive result for FA-specific IgE (data presented was highly variable), as compared to measure 90 min before 1 st session of laboratory course	Low Confidence [small sample (n=6–8); limited and variable periodicity]
(Thrasher et al., 1987)	Human symptomatic exposed subjects, controls (n = 8/ group)	Exposed (mobile home measures): 0.086–0.68 mg/m ³ (residency ≈6–7 year); nonexposed: not measured (authors assume: <0.037)	Serum FA-specific IgG and IgE	No detection of FA-specific IgE Increased FA-specific IgG in all 8 exposed subjects, but only in 1/8 controls (had PD)	Low Confidence [small sample; symptomatic vs. nonsymptomatic comparison; reporting limitations]
(Dykewicz et al., 1991)	Human medical volunteers (n = 55; 31 F, 24 M)	Generally, 0.25–0.79 mg/m ³ (1 subject up to 13.5 mg/m ³); duration 4.53 ± 1.09 year	Serum FA-specific IgG and IgE	N/C in incidence of FA-HSA- specific IgG or IgE (3 subjects had FA-specific IgG and IgE, and 2 more had FA-specific IgG only)	Low Confidence [periodicity unspecified; unclear exposure comparison- control levels NR and variable range in exposed]
(Thrasher et al., 1990)	Human various exposed groups of patients, and asymptomatic controls	“controls”—chiropractic students (n = 28): assumed ≥ 0.53 mg/m ³ for 28 wk (13 hr/wk); mobile home residents (n = 19): 0.05–0.62 mg/m ³ for 2–7 year; office workers (n = 21): assumed 0.012–0.95 mg/m ³ , duration N/R; occupational (n = 8): levels/ duration N/R; removed from exposure	Serum FA-specific IgG, IgM, and IgE Blood autoantibodies	Proportion of pooled titers (IgG, IgM, and IgE) of FA-specific antibodies (i.e. % at ≥ 1:8) was greater in all patient groups than in controls (Note: most apparent for IgG, but others also appear elevated; FA-specific IgE was not found in any of the patients “removed” from exposure) Mobile home residents and office workers had increased autoantibodies vs. controls (i.e., antismooth muscle or antiparietal cell)	Low Confidence [controls not unexposed; patients to nonpatients comparisons questionable] Note: authors argue only real difference between asymptomatic control students and patients is one of duration of exposure

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
		for ≥ 1 year: 0.17–1.0 mg/m ³			
(Górski and Krakowiak, 1991)	Human textile and shoe makers (n = 367)	Not exceeding 0.5 mg/m ³ (duration at least 1 year (average $\approx$ 12 years)	Serum FA-specific IgE Antibodies	No FA-specific IgE in patients tested (seems to be testing in a small subset of all subjects)	Low Confidence [incomplete and confusing methods and results; comparisons unclear]
(Palczynski et al., 1999)	Human apartment residents (n = 465 total, $\approx$ 40% children)	3 categories of exposure: <0.025, 0.025–0.05, and >0.0501 mg/m ³ ; duration unclear, periodicity assumed to be constant	Total serum IgE Note: N = 1–2 at high HCHO levels; N = 27–38 at mid, low levels Serum antibodies to FA	Total IgE was not changed at 0.025–0.5 as compared to <0.025 in children or adults (n size at >0.05 was too small to compare); No FA-specific antibodies were detected (details NR); note: children exposed to 0.025–0.05 mg/m ³ and tobacco smoke had elevated IgE	Low Confidence: IgE [small sample size; subsampling for IgE not reported; minimal exposure differential; results not stratified by sex or smoking status] Not Informative: FA antibodies [methods NR; data NR]
(Madison et al., 1991)	Human residents, spill-exposed (n = 41) or unexposed controls (n = 29)	Formaldehyde (PFA): >2.46 mg/m ³ for first 48 hr, then average dropped to 0.028 mg/m ³ , but urea and methylamines unmeasured/not corrected	FA-specific serum antibodies and autoantibodies	N/C in FA-specific IgE Increased FA-specific IgM and IgG Increased odds ratio of having 1+ autoantibodies (although higher, no sig. increase in any one auto-antibody)	Not Informative [mixture exposure; co-exposures not corrected for; FA in controls unmeasured]
(Grammer et al., 1990)	Human workers (Boeing; n = 37); details N/R	0.0037–0.090 mg/m ³ (not stratified by exposure; all exposed; duration N/R)	Serum FA-specific IgG and IgE	0/37 had FA-specific IgG 5/37 had elevated IgE (vs. control sera) that was not specific to FA-HSA or HSA	Not Informative [details on population N/R; details on exposure NR; no specific comparison to FA levels]
<i>Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells</i>					
(Fujimaki et al., 2004b)	Female C3H mice (n = 5–6 per group)	PFA 0, 0.098, 0.49, or 2.46 mg/m ³ ; 12 wks (16 hr/d, 5 d/wk) Sensitization: i.p. 10 μ g OVA prior to FA exposure; aerosol OVA boost for 6 min on wks 3, 6, 9, and 11	Serum Antibodies and Antibodies to Antigen	No change in anti-OVA IgE (variable) or IgG _{2a} or Total IgE Decreased anti-OVA IgG ₁ (at 0.49 mg/m ³ only) and IgG ₃ (at 0.098–0.49 mg/m ³) Body weight decreased 20% at 0.49 mg/m ³	High or Medium Confidence [slightly small sample size]
(Riedel et al., 1996)	Female Dunkin-Hartley guinea pigs (n = 12)	Formaldehyde (bottled pressurized gas) 0, 0.13, 0.31 mg/m ³ for 5 d (8 hr/d); Sensitization: 0.5% inhaled OVA; OVA boost at 2 wk Challenge: 1% inhaled OVA 1 wk later	Serum OVA-specific IgG1	Increased OVA-specific IgG1 by 0.31 mg/m ³	High or Medium Confidence [no comparison group with FA without OVA]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Sapmaz et al., 2015)	Male SD rats (n = 5–7)	PFA 0, 6.15, 12.3 mg/m ³ ; 4 wks (8 hr/d, 5 d/wk)	Serum Antibodies	Increased IgA, IgM, and complement 3 Decreased IgG	High or Medium Confidence [slightly small sample size; high formaldehyde levels]
(Tarkowski and Gorski, 1995)	Female Balb/c mice (n = 4/ group)	Formalin (assumed; test article N/R) 0 or 2 mg/m ³ for 10 d (6 hr/d) or 7 wk (6 hr/d, 1 d/wk) Sensitization: intranasal 25 µg OVA 1x/wk for 7 wk OR i.p. 1 µg OVA 1x/wk for 4 wk	Serum OVA-specific IgE	Increased OVA-specific IgE in mice exposed for 10 d, but not in those exposed 1x/ wk, as compared to controls Specific to nasal tissue, as OVA sensitization via i.p. injection caused N/C	Low Confidence [formalin; small sample size] Note: pinpoints issue of importance and interpretability of different sensitization methods
(Wu et al., 2013)	Male Balb/c mice (n = 8/group)	Formalin 0, 3 mg/m ³ for 4 wk (6 hr/d, 5 d/wk) Sensitization: s.c. 80 µg OVA on Days 10, 18, and 25 Challenge: 1% OVA aerosol 30 min/d on Day 29-35	Serum antibodies	FA alone increased total IgE, but not OVA-IgG or OVA-IgE; FA+OVA increased IgE compared to OVA alone, but did not further elevate OVA-IgG or OVA-IgE (slight, NS increases) compared to OVA TRPA1 and TRPV1 antagonists reduced FA+OVA-induced serum antibodies	Low Confidence [formalin; pharmacological interventions did not include effects of FA alone]
(Kim et al., 2013b)	Female NC/Nga (atopic-prone) mice (n = 5–7/group)	Formalin (assumed; test article NR) 0, 0.25, 1.23 mg/m ³ for 4 wk (6 hr/d, 5 d/wk) Sensitization: topical house dust mite (HDM; ear) stimulation (25 mg Df ointment) 1x/wk for 4 wk	Plasma Antibodies and Antigen-specific Abs	Plasma IgG1 increased by FA alone (0.25 mg/m ³ only), but N/C in total IgE or IgG2a FA exacerbates HDM-induced IgE (≥0.25 mg/m ³) and IgG2a (0.25 mg/m ³ only), but not IgG1 HDM-specific IgE not changed	Low Confidence [formalin; small sample size] Note: multiple supplementary files; HDM-specific IgE data NR
(Gu et al., 2008)	Female Balb/c mice (n = 5–6/ group)	Formalin (assumed; test article NR) 0.12 or 0.98 mg/m ³ for 5 wk (24 hr/d, 5 d/wk) Sensitization: i.p. 10 mg OVA on day 0 and 7 pre-FA	Serum Antibodies and OVA-specific Antibodies	N/C in total serum IgG or IgE Increased OVA-specific IgE in allergen primed host, only at 5 wks (not ≤ 4 wk) and only at 0.98 mg/m ³ ; N/C in OVA-IgG	Low Confidence [formalin; small sample size]
(Jung et al., 2007)	Female C57BL/6 mice (n≥5/ group)	Formalin (assumed; test article NR) 0, 6.15, 12.3 mg/m ³ for 2 wk (6 hr/d, 5 d/wk)	Serum Antibodies	Increased Total IgG1, IgG3, IgA, and IgE Decreased Total IgG2a and 2b; N/C IgM Note: body wt decreased ≈10%	Low Confidence [formalin; high exposure levels; small sample size]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Holmstrom et al., 1989a)	Female SD rats (n = 8–9 treated rats; n = 6 control)	Formalin (assumed; test article NR) 15.5 ± 2.3 mg/m ³ for 22 mos (6 hr/d, 5 d/wk); all rats vaccinated: anti-tetanus and Pneumovax	Serum antibody response to vaccination	N/C in IgM response to vaccine-related antigens Variable increases in IgG against specific antigens were not statistically significant (Note: IgE not measured)	Low Confidence [formalin; excessively high exposure level; no unvaccinated comparison group] Note: authors indicate B cell function unchanged
(Lee et al., 1984)	Male English guinea pigs (n = 4)	Formalin: 7.38 or 12.3 mg/m ³ for 5 d, with FA challenge with 2.46 or 4.9 mg/m ³ for 1 or 4 hr, respectively	Serum antibody to formaldehyde (isotype not specified) measured 9 or 17 d (i.e., Days 14 or 22) after exposure	N/C antibody response to 2.46 or 4.9 mg/m ³ (data NR) Note: 2/4 animals exhibited dermal sensitivity (likely contact-mediated) to topical FA	Low Confidence [formalin; small sample size; high exposure levels] Note: although there was no comparison to controls with no prior formaldehyde exposure, this is not expected to affect this measure
(Sadakane et al., 2002)	Male ICR mice (n = 9 or 18)	Formalin 0.5% for 4 wk (15 min/wk) ± sensitization of house dust mite allergen (Der f) Sensitization: i.p. with 3 mg/mL Der f (house dust mite allergen) prior to FA Challenge: intratracheal 10 µg Der f 3 hr after last exposure (note: measures 3 d later)	Blood Der f-specific IgG1 and IgE	N/C in Der f-specific IgG1 or IgE (latter appears to have been lower than detection limit)	Low Confidence [formalin; high, unknown exposure levels; short periodicity]
(Kita et al., 2003)	Male Hartley guinea pigs (n = 5–7/group)	Nasal Instillation of saline or Formalin 0.1 or 1.0%; 3×/wk for 6 wk Sensitization: i.p. anti-OVA serum on after 5 wk FA (passive) or i.p. 2 mg OVA on day 3 (active) prior to FA exposure with boost i.p. 10 mg OVA Day 24 Challenge: 1 mg/mL nebulized OVA 15 min after last FA exposure on Day 45	PCA reaction of naïve animals to injected serum of exposed animals	Increased anti-OVA IgG at ≥0.1% FA (at 4 hr, but not 7 d after OVA challenge) in naïve animals injected with serum	Not Informative [exposure route; formalin; high, unknown exposure levels; short periodicity; small sample size (for some endpoints/groups)]
(Lino dos Santos Franco et al., 2009)	Male Wistar rats (n = 5)	Formalin 0, 1% for 3 d (90 min/ d) Sensitization: immediately post-FA, i.p. 10 µg OVA; boost 1 wk later with s.c. injection Challenge: 1 wk later with aerosolized OVA	Skin Antibodies	N/C in skin IgE	Not Informative [formalin; unquantified high exposure levels; small sample size; short duration and periodicity] Note: unclear endpoint relevance

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Lino-Dos-Santos-Franco et al., 2013a)	Female Wistar rats (n = 5)	Formalin 1% or methanol vehicle for 3 d (90min/d), ± ovariectomy	Skin IgE	1 d after OVA challenge: FA/OVA vs. OVA alone: N/C in cutaneous OVA-specific IgE	Not Informative [formalin (MeOH controls); unquantified high exposure levels; small sample size; short duration and periodicity; naïve not chamber exposed] Note: unclear endpoint relevance
		Sensitization: After FA, s.c. 10 µg OVA, with s.c. boost 7 d later Challenge: After 7 d, 1% OVA aerosol for 15 min			

Serum markers of immune response (other than antibodies), inflammation, or oxidative stress

Table B-46. Serum markers of immune response (other than antibodies), inflammation, or oxidative stress

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
<i>Observational Epidemiology Studies</i>					
(Aydin et al., 2013)	Human male fiberboard workers	0.25 ± 0.074 mg/m ³ (average 7.3 year employed; n = 46) vs. nonexposed controls	Serum cell counts, cytokines, and related factors	N/C in # hematologic cells, WBC, RBC, Hb, neutrophils, or monocytes; N/C in helper T, suppressor T, or B lymphocytes Increased % of lymphocytes, and numbers and % of T cell (CD3+) and NK cell (CD56+) Increased TNFα, but N/C in Complement 3 or 4; TNFα increased more significantly in those not using protective measures	High or Medium Confidence Note: annex reviews immune data
(Bassig et al., 2016) (same cohort as (Zhang et al., 2010))	Human melamine workers (n = 43) or n = 51 age- and sex-matched unexposed from different factories in the same region of China	1.6 mg/m ³ (10% and 90% = 0.74 and 3.08 mg/m ³); unclear exposure duration (sampling over a 3-wk period)	Serum cell counts and soluble markers	Decreased total WBC, Granulocytes, Monocytes, Platelets, and Lymphocytes Decreased CD8+ cells (CD8 effector memory cells most affected) and NK cells N/C in Monocytes, CD4+ cells, CD4/CD8 ratio, or B cells; N/C in soluble CD27 or CD30	High or Medium Confidence
(Costa et al., 2013)	Human pathology anatomists (n = 35) or administrative controls (n = 35)	0.44 ± 0.037 mg/m ³ (as high as 0.85 mg/m ³ in peaks); duration of employment ≥ 1year	Serum lymphocyte subtypes	Decreased B cells (% CD19+) in exposed N/C in T cells or NK cells in exposed Within the exposed workers: FA exposure level correlated with Increased % T cells (CD3+) and % T helper cells (CD4+), and decreased % NK cells	High or Medium Confidence Note: authors suggest immunosuppression

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Costa et al., 2019)	Human anatomy-pathology lab workers (n = 85) or administrative controls (n = 87)	8 hr TWA=0.47 ± 0.037 mg/m ³ (range = 0.098–1.71 mg/m ³ ; as high as 3.94 mg/m ³ in peaks); duration of employment average ≈12 year	Serum lymphocyte subtypes	Increased Cytotoxic (CD8+) T cells and NK cells; Decreased B cells and CD4/CD8 ratio; N/C in total T cells or Helper (CD4+) T cells	High or Medium Confidence Note: authors suggest immunostimulation
(Zhang et al., 2010)	Human formaldehyde melamine workers	51 Controls: <0.037 mg/m ³ ; 43 Exposed: 1.8 (0.42–6.9) mg/m ³ ; Duration at least 3 mos (41/43 exposed > 1 year)	Serum immune markers	22/38 immune/inflammation markers that were detectable were decreased Stringent FDR cutoff (10%): significantly decreased CXCL11 and CCL17 (both ≈25%) FDR at 20%: significantly decreased CRP, TRAIL, SAP, IL-10, sCD40L, and Insulin N/C in TNF-α; other markers below LOD	High or Medium Confidence [Note: the strongest correlation of marker changes was with monocyte levels (p = 0.05), but overall the results suggest that cell counts do not explain the marker changes]
(Zhang et al., 2010)	Human formaldehyde melamine workers	51 Controls: <0.037 mg/m ³ ; 43 Exposed: 1.57 (0.77–6.9) mg/m ³ ; Duration at least 3 mos (41/43 exposed > 1 year)	Serum cell counts Proliferation of serum hematopoietic progenitor cells	Decreased WBC, lymphocytes, granulocytes, platelets, and RBC Increased mean corpuscular volume N/C in monocytes, hemoglobin Decreased colony formation in cultured hematopoietic progenitors from subjects	High or Medium Confidence [one ex vivo endpoint: possible influence of culturing- still expected to be due to exposure, but could involve in vitro amplification of phenomena]
(Jia et al., 2014)	Human plywood workers (n = 118) and controls (n = 79)	[High] workers: 0.77 (0.44–1.88) mg/m ³ (n = 70); [Low] workers: 0.18 (0.086–0.23) mg/m ³ (n = 48); duration ≥6 mos; controls <0.01 mg/m ³	Serum lymphocyte subtypes and cytokines	Dose-dependent increased % CD19+ B cells at ≥ 0.18 mg/m ³ ; increased CD56+ NK cells at 0.18 mg/m ³ only N/C in %CD3+, CD4+ or CD8+ T cells Increased IL-10 and decreased IL-8 at ≥ 0.18 mg/m ³ ; Increased IL-4 and decreased IFNγ at 0.77 mg/m ³	High or Medium Confidence
(Hosgood et al., 2013) Note: Same cohort as (Zhang et al., 2010)	Human formaldehyde melamine workers	51 Controls: 0.032 (0.01–0.032) mg/m ³ ; 43 Exposed: 1.57 (0.77–3.09) mg/m ³ ; Duration at least 3 mos (41/43 exposed >1 year)	Serum counts and analyses of lymphocyte subsets.	Decreased lymphocytes, NK cells, T cells, and CD8+ T cells N/C in B cells, or CD4+ T cells (overall; note: CD4+/FoxP3+ decreased) T cells subset analyses showed decreased CD8+ effector T cells and regulatory T cells	High or Medium Confidence Note: Authors hypothesized decreased effector T cells (which circulate to inflamed tissues) may reflect decreased response to antigenic-related inflammation, and decreased regulatory cells as decreased immunosuppression (which may lead to autoimmunity)
(Ye et al., 2005)	Human students (n = 23), waiters (n =	[High] Manufacturers: 0.98 ± 0.286 mg/m ³ (8.5 year,	Blood lymphocyte subset analysis	N/C in waiters exposed to low levels.	High or Medium Confidence [data not adjusted for age or gender]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
	16), or FA manufacturers (n = 18)	8 hr/d; 1.69 maximum); [Low] waiters: 0.107 ± 0.067 mg/m ³ (12 wk, 5 hr/d); Controls: 0.015 mg/m ³		Increased % B cells and ratio of T helper to T cytotoxic T cells (CD4/CD8 ratio), and decreased total T cells and CD8+ T cells in workers exposed to high levels	
(Bono et al., 2010)	Human pathologists (n = 44) and controls (n = 32)	Controls: 0.028 ± 0.0025 mg/m ³ ; Pathologists: 0.032 ± 0.006 or 0.21 ± 0.047 mg/m ³ (in "reduction room"); duration unclear	Serum lymphocyte ROS (MDA-dG adducts)	Increased MDA-dG at > 0.066 mg/m ³ ; N/C in MDA-dG at <0.022 mg/m ³ or 0.023–0.066 mg/m ³ (significant association with air-FA levels)	High or Medium Confidence (unknown duration)
(Romanazzi et al., 2013)	Human Laminate workers (males, years employed NR)	0.21 ± 0.10 mg/m ³ exposed (n = 51); 0.04 ± 0.02 mg/m ³ nonexposed (n = 54)	15-F _{2t} Isoprostanes in urine (also measured cotinine and smoking)	Smoking and air-formaldehyde exposure were independently associated with increased IsoP	High or Medium Confidence - indirect [accuracy of single measure questionable] Note: serum and urine isoprostanes are correlated (Rodrigo et al., 2007); thus, this finding is indirect for serum ROS
(Lyapina et al., 2004)	Human workers with carbamide-FA glue (n = 29)	Exposed workers: 0.87 ± 0.39 mg/m ³ (n = 21 nonexposed); duration mean: 12.7 ± 9.6 years	Blood neutrophil oxidative burst Routine hematology Assessment of chronic URT inflammation	Significant decreases in neutrophil function/ oxidative burst were only detected when comparing the 12 workers with evidence of URT inflammation (N/C across full groups) Decreased erythrocyte count and hematocrit levels correlated with duration of exposure (no other changes)	High or Medium Confidence [mixture exposure] Note: Authors hypothesized that decreases in erythrocyte and hematocrit counts might indicate FA toxicity on bone marrow hematopoiesis
(Jakab et al., 2010)	Human female pathologists or controls (n = 37)	0.9 mg/m ³ (8 hr-TWA exposure); mean duration >17 years; slightly more (not significant) smokers and drinkers in exposed	Serum lymphocyte parameters: CD71 in fresh cells; apoptosis/ proliferation in cells cultured with PHA	N/C in T cell activation marker, CD71 Exposure to FA alone increased apoptosis and 1 out of 3 measures of cell proliferation in PBLs; N/C % in S phase	High or Medium Confidence - CD71 [limited precision of exposure assessment - sampling 1–3 years from study] Low Confidence -other measures [ex vivo; limited exposure assess]
(Bellisario et al., 2016)	Human nurses (Italian females, years employed NR)	0.034 ± 0.038 mg/m ³ using formalin (n = 64); 0.015 ± 0.005 mg/m ³ not using formalin (n=30), but noting that they did receive some exposure; 8-hr workshift	15-F _{2t} Isoprostanes and malondialdehyde in urine, normalized to creatinine (also measured cotinine)	Smoking and air-formaldehyde exposure were independently (positively) associated with increased oxidative stress biomarkers by pairwise comparisons and regression (note: in nurses who used vacuum sealing techniques, which reduce	Low Confidence - indirect [accuracy of single measure questionable]; small exposure differential; formalin test article Note: serum and urine isoprostanes are correlated

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
		measures on 2 separate days		formaldehyde exposure, also exhibited reduced biomarkers).	(Rodrigo et al., 2007); thus, this finding is indirect for serum ROS
(Erdei et al., 2003)	Human (sex NR) symptomatic students (9–11 yo w/ respiratory issues) (n=176)	0.006–0.057 mg/m ³ (average= 0.018); duration unknown [co-exposure: NO ₂ , benzene, toluene, xylene, and dust mite allergen]	Serum Cell Counts	Increased serum monocyte counts by linear regression; N/C in RBCs, WBCs, platelets, lymphocytes, neutrophils (mostly), or eosinophils (all data NR)	Low Confidence [comparisons to “normal” range rather than to control group; co-exposure; limited reporting] Note: symptomatic only
(Kuo et al., 1997)	Human dialysis nurses (n = 51) or ward nurses controls (n = 71)	Personal sampling ranged from 0.018–0.11 mg/m ³ ; area sampling was as high as 3.44 mg/m ³ (duration average= 3 year; ≈1/3 employed <1 year and ≈40% > 3 year); control area levels N/R	Blood cell counts	WBC decreased in 2 nd blood test (1 year after the first test at study onset- N/C): associated with FA concentration and symptoms, but not work duration (correlated, but N/S) N/C RBC, Ht, MCV, MCH, MCHC, Plt, neutrophil, lymphocyte, monocyte, eosinophil, or basophils	Low Confidence [not clear that controls are appropriately unexposed nor what co-exposures exist] (Note: 2 nd blood test, presumably, would involve an extra 1 year of exposure duration)
(Thrasher et al., 1987)	Human symptomatic exposed subjects, controls (n = 8/ group)	Exposed (mobile home measures): 0.086–0.68 mg/m ³ (residency ≈6–7 year); nonexposed: not measured (authors assume: <0.037)	Serum cell counts Ex vivo T and B cell blastogenesis (PHA or PWM stimulation)	T cell number decreased; B cell counts were not significantly changed T cell blastogenesis with PHA (not PWM: p>0.05, authors call significant) impaired	Low Confidence [small sample; symptomatic vs. nonsymptomatic comparison; questionable reporting]
(Thrasher et al., 1990)	Human various exposed groups of patients, and asymptomatic controls	“controls”- chiropractic students (n = 28): assumed ≥ 0.53 mg/m ³ for 28 wk (13 hr/wk); mobile home residents (n = 19): 0.062–0.62 mg/m ³ for 2–7 year; office workers (n = 21): assumed 0.012–0.95 mg/m ³ , duration N/R; occupational (n=8): levels/ duration N/R; removed from exposure for ≥ 1 year: 0.17–1.0 mg/m ³	Blood cell counts	Decreased WBCs in office workers; N/C in all T cells, T helper or T suppressor cells, or T cell H/S ratio Ta1+ lymphocytes (antigenic stimulation) elevated in all exposed patient groups B cells increased in office workers and removed patients IL2R+ lymphocytes increased in mobile home residents and removed patients	Low Confidence [limited exposure contrast- authors suggest the only real difference between asymptomatic control students and patients is one of duration of exposure; patients to nonpatients comparisons questionable]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Ying et al., 1999)	Human anatomy students (n = 23)	0.508 ± 0.3 mg/m ³ for 8 wks (3 hr/d, 3 d/ wk); in dormitories: 0.012 ± 0.003	Serum lymphocyte subsets Ex vivo lymphocyte proliferation (culture lymphoblast counts)	After exposure compared to before exposure: Increased % B cells (CD19), decreased Total T cells (CD3), T helper (CD4) and T cyto. (CD8) cells; N/C in ex vivo lymphocyte proliferation rate	Low Confidence [limited periodicity; some experiments ex vivo] Note: internally controlled
(Madison et al., 1991)	Human residents, spill-exposed (n = 41) or unexposed controls (n=29)	Formaldehyde (PFA): >2.46 mg/m ³ for first 48 hr, then average dropped to 0.028 mg/m ³ , but urea and methylamines not measured or corrected for	Serum cell counts	N/C in WBC, lymphocyte, CD8, CD8/CD4 ratio, CD19, or CD25 cells Decreased % CD5+ and % CD4+, although total counts of these were unchanged Increased CD26+ counts and %	Not Informative [mixture exposure; co-exposures not corrected for; FA in controls unmeasured]
(Vargová et al., 1992)	Human Woodworkers (Czechoslovakia)	Formaldehyde 0.55–10.36 mg/m ³ and other, unquantified exposures	Serum IgG, IgA, IgM, IgE Complement and other factors Lymphocyte proliferation	Increased lymphocyte proliferation to concanavalin A and decreased proliferation to phytohaemagglutinin “no significant differences in natural cellular and specific humoral immunity”	Not Informative [mixture exposure; co-exposures not corrected for; FA in controls unmeasured; no description of recruitment or how referents were matched- reporting limited]
(Zhang et al., 2010)	Human formaldehyde melamine workers	51 Controls: <0.037 mg/m ³ ; 43 Exposed: 1.57 (0.77–3.09) mg/m ³ ; Duration at least 3 mos (41/43 exposed >1 year)	In vitro proliferation of a volunteer’s cells	Decreased colony formation in cultured progenitors with in vitro FA treatment	Not Informative [formalin treatment- assumed; single donor, in vitro; nongaseous exposure, levels relevance]
<i>Controlled-Exposure Studies in Humans or Primary Human Cells</i>					
(Dietrich et al., 1996)	In vitro human leukocytes (single donor): not further described	Formalin (assumed; test article N/R) gas at 0.62 mg/m ³ for 1 hr	Heat shock protein 70 levels (Westerns)	FA, but not heat (42°C) stress, caused a significant increase in HSP70 levels	Not Informative [formalin; in vitro; short duration; exposure level relevance unknown; sample size NR; poor reporting]
<i>Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells</i>					
(Sorg et al., 2001a)	Male SD rats (n = 6–9/ group)	PFA (inferred from citation) 0, 0.86, or 2.95 mg/m ³ for 20–60 min, 2 or 4 wk	Serum corticosterone	N/C with acute exposure Increased CORT at 2.95 mg/m ³ at 2 or 4 wk	High or Medium Confidence Note: unclear utility of endpoint for respiratory effects interpretation
(Rager et al., 2014)	Male fischer rats (n = 3)	PFA 0 or 2.46 mg/m ³ for 7 d, 28 d or 28 d with 7 d recovery (6 hr/d)	miRNA microarray of blood WBCs	WBCs miRNAs were changed after 7 d or 28 d or 28 d with recovery (31 or 8 or 3	High or Medium Confidence [small sample size]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
				transcripts); associated primarily with inflammation and immunity	Note: unclear/indirect interpretation of endpoints
(Morgan et al., 2017)	Male B6.Trp53 ^{tm1Brd} and C3B6.129F1-Trp53 ^{tm1Brd} mice (heterozygote P53 allele); n = 25/group	PFA 0, 9.23, or 18.45 mg/m ³ for 8 wks (6 hr/d, 5 d/wk) with measures at approximately 1 year	Whole blood counts	N/C in hematological parameters, including RBC, WBC, neutrophils, monocytes, eosinophils, platelets, lymphocytes, reticulocytes, hemoglobin, hematocrit, MCV, MCH, or MCHC	High or Medium Confidence
(Dean et al., 1984)	Female B6C3F1 mice (n 10/ group)	PFA 0 or 18.5 mg/m ³ for 3 wk (6 hr/d, 5 d/wk)	Serum cell counts	N/C peripheral blood cell counts, including WBC differentials, except: Decreased number of monocytes (from 43 to 4)	Low Confidence [excessively high levels: 60-70% RB inferred at these levels] Note: monocyte decrease speculated as peripheral response to nasal inflammation and healing
(Aydin et al., 2014)	Male SD rats (n = 6/ group)	Test article unclear, but appears to be formalin in this experiment at 0, 6.48 (low), 12.3 (moderate), or 18.7 mg/m ³ for 4 wk (8 hr/d, 5 d/wk)	Serum total antioxidant and total oxidant levels (TAS and TOS; kit uses vitamin E and H ₂ O ₂ as reference, respectively Serum oxidative stress index (OSI: TOS/TAS) Serum irisin (hormone may regulate obesity)	Increased TOS, and decreased TAS and irisin, at ≥ 12.3 mg/m ³ formaldehyde Increased OSI at ≥ 6.48 mg/m ³ Note: serum biochemical parameters (e.g., cholesterol) are not included here, but were unchanged. Carnosine supplementation reduced changes.	Low Confidence [formalin; high levels]
(Zhang et al., 2013)	Male Balb/c mice (n = 9)	Formalin 0, 0.5, or 3 mg/m ³ for 2 wk (8 hr/d, 5 d/wk)	Serum cell counts	D/D Decreased serum WBC, RBC, and lymphocytes, and increased platelets, at ≥ 0.5 FA; decreased intermediate cells at 0.5 FA; N/C in neutrophils	Low Confidence [formalin]
(Ye et al., 2013)	Male Balb/c mice (n ≥ 9/ group/ endpoint)	Formalin 0, 0.5, 1, or 3 mg/m ³ for 7 d (8 hr/d)	ROS (dichlorohydro-flourescein and MDA) blood mononuclear cells (PBMC)	Dose-dependent decrease in GSH levels in PBMC at ≥ 1 Dose-dependent increase in DCFH and MDA in PBMC at 3 Co-administered GSH attenuated effects	Low Confidence [formalin]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Im et al., 2006)	Male SD rats (n = 10)	Formalin (assumed; test article not specified) 0, 6.15, 12.3 mg/m ³ for 2 wks (6 hr/d; 5 d/wk)	Plasma ROS, cytokines, and proteomic analysis	Increased MDA & protein carbonyls at 12.3 mg/m ³ (note: similar increases in liver) D/D Increased IL-4 and decreased IFN γ Other protein changes (e.g. increased GSTs and ApoE; decreased heme oxygenase, fibrinogen, ApoA1, SNAP-25)	Low Confidence [formalin; high levels]
(Matsuoka et al., 2010)	Male ICR mice (n $\geq$ 7)	Formalin at 0.12 mg/m ³ for up to 24 hr; also, a single experiment at 3.69 mg/m ³ for 24 hr	Plasma ROS (8OHdG) and NO (nitrates/nitrites); NO response to LPS injection: 3.69 mg/m ³	Increased plasma ROS at 0.12 mg/m ³ for $\geq$ 8 hr and NO at 24 hr Increased plasma SOD activity at 3.69 mg/m ³ ; N/C in plasma IL-6 at 0.12 mg/m ³ Decreased NO ₃ with LPS stimulation	Low Confidence [formalin; short duration] NOTE: ACUTE
(Sandikci et al., 2007b)	SD rats (n = 6/ group) at GD1 [I], PND1 [II], PND28 [III] or adults [IV]	Formalin (assumed: test article NR): 0 or 7.38 mg/m ³ for 6 wk (8 hr/d, 7 d/wk)	Blood T lymphocyte counts	Increased blood T lymphocytes (ANAE+ as marker) in all groups by FA	Low Confidence [formalin; high exposure levels; use of ANAE as T lymphocyte marker under all conditions has been debated]
(Katsnelson et al., 2013)	Rat “white” females (n = 12–15)	Formalin (assumed; test article NR) 12.8 $\pm$ 0.69 mg/m ³ for 10 wk (4 hr/d, 5 d/wk)	Blood cell counts and immune markers (other markers N/C or not inflammation)	Increased % lymphocytes and albumin; Decreased % segmented neutrophils, MDA, GSH, and lymphocyte SDH activity; some decreased serum amino acids	Low Confidence [formalin; excessively high levels; short periodicity]
(Yu et al., 2014b)	Male ICR mice (n = 6)	Formalin 20, 40, 80 mg/m ³ for 15 d (2 hr/d)	Blood cell counts	Decreased blood WBCs and platelets at $\geq$ 40 mg/m ³	Low Confidence [formalin; excessively high levels; short periodicity]
(Brondeau et al., 1990)	Male SD rats (n = 10)	Formalin (assumed; test article NR) 35.7–75 mg/m ³ for 4 hr, with or without adrenalectomy	Serum cell counts	Decreased WBCs at $\geq$ 52.9 mg/m ³ , not at 35.7 mg/m ³ ; N/C in RBCs Adrenalectomized rats did not show decreased WBCs at 60.3 mg/m ³	Low Confidence [formalin; excessively high levels; short periodicity] NOTE: ACUTE
(Zhao et al., 2020)	Male Balb/c mice (n = 3, pooled into single sample for nose and lung samples); 2 experiments by different researchers	Formalin 0, 3 mg/m ³ for 2 wks (8 hr/d, 5 d/wk)	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies in nose, lung, spleen, and bone marrow	Bone marrow results: Decreased formation of CFU-GM and BFU-E in both experiment I and II	Low Confidence [formalin; small sample size] Not Informative: ex vivo results

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Wei et al., 2014)	Male C57BL/6 mice (n = 6)	Methanol-free formalin at 0, 0.5, or 2 mg/kg/d for 1 wk or 1 mo (5 d/wk)	Serum cytokines for Th1, Th2, and Th17	Increased Th1-related cytokines (IFN- γ , TNF, and IL-2), TH2-related cytokines (IL-4, IL-6, and IL-10), and Th17-related cytokine (IL-17A) at 2 mg/kg/d for 1 or 4 wks; specific statistically significant increases only noted for 1 wk IL-2 and IL-4 levels (note: magnitude of change was equal or greater at 1 mo and for all tested cytokines in all comparisons; in general, small decreased levels noted at 0.5 mg/kg)	Not Informative [levels of unknown relevance; i.p. injection] Note: Kruskal-wallis test
(Ibrahim et al., 2016)	Pregnant Wistar rats (n = 5 dams; 10 pups/ group for experiments; note: individual pup data for n = 10 pups did not appear to account for litters)	Formalin 0.92 mg/m ³ from GDs 1–21: 1 hr/d, 5 d/wk Randomly assigned pups all received 5 mg/kg lipopolysaccharide (LPS) injections at PND 30	Blood cell counts and Myeloperoxidase activity	Increases in total cells and granulocytes (lymphocytes and monocytes were unchanged) by LPS were reduced in offspring exposed to formaldehyde, as were increases in myeloperoxidase activity	Not Informative [formalin; short periodicity; offspring comparisons do not include FA without LPS; small sample size; did not appear to account for litter effects] Note: effects rescued by vitamin C
(Maiellaro et al., 2014)	Pregnant Wistar rats (n = 5)	Formalin 0.92 mg/m ³ from GDs 1–21: 1 hr/d, 5 d/wk Sensitization: s.c. 10 μ g OVA with sc boost after 7d Challenge: 7 d later, 1% OVA aerosol 15 min/d, 3 d	Blood cell counts	N/C in parental blood total cells, monocytes, lymphocytes, or granulocytes Decreased birth weight in offspring 24 hr after OVA challenge, offspring have: decreased blood total cells, mononuclear cells, neutrophils, and eosinophils	Not Informative [formalin, short periodicity, offspring comparisons do not include FA alone; small sample size]
(Kum et al., 2007b)	Female SD rats (n = 6)	Formalin (assumed: test article NR): 0 or 7.38 mg/m ³ for 6 wks (8 hr/d, 7 d/wk)	Serum biochemistry (proteins and factors)	Increased serum urea, but N/C in total protein, albumin, or creatinine Note: experiments with FA + xylene not considered	Not Informative [formalin; high levels; tests not considered relevant to inflammation or respiratory effects]
(Ciftci et al., 2015)	Male Wistar albino rats (n = 10)	Formalin i.p. injection at 9 mg/kg-d every other day for 2 wks	Serum markers for ROS, antioxidants, as well as beta amyloid and tumor protein 53 levels	Increased MDA (ROS marker) Decreased total antioxidants, TP53, and A-beta1-40 (not 1–42)	Not Informative [formalin; high levels of unknown relevance; i.p. injection]
(Murta et al., 2016)	Male Fischer rats (n = 7)	Formalin (assumed) 1%, 5%, or 10% for 5 d (3 $\times$ 20 min/d)	Blood cell counts, chemokine levels, and ROS indicators	FA increased total leukocyte, lymphocytes at 5%, and decreased platelets at 10%; N/C in other cell types; 1% caused	Not Informative [formalin; unquantified high levels; static

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
				increased catalase and other ROS indicators were observed; increased CCL2 at 10%, CCL3 at 1–5%, and CCL5 at 1%	exposure chamber; short periodicity]
(da Silva et al., 2015)	Male Wistar rats (n = 6/ group)	Formalin 1% for 3 d (90 min/d); rats exposed in static chambers 5 rats/time	Blood cell counts	FA increased total cells, monocytes, lymphocytes, and neutrophils Note: while reduced effects were reported as reduced with laser therapy, laser therapy-only controls were not used	Not Informative [formalin; unquantified high levels; static exposure chamber and group exposure; short duration and periodicity]
(Lino dos Santos Franco et al., 2006)	Male Wistar rats (n = 5–6)	Formalin 1% or methanol vehicle for 4 d (30, 60, or 90 min/d)	Serum cell counts	Increased serum leukocytes and mononuclear cells, but not neutrophils	Not Informative [formalin (MeOH controls); unquantified high levels; short periodicity; small sample size; presented comparisons to naïve rats rather than MeOH controls]
(Lino-Dos-Santos-Franco et al., 2011a)	Female Wistar rats (n = 5)	Formalin 1% or naïve for 3 d (90 min/d), with or without ovariectomy	Serum cell counts and factors	Increased total serum leukocytes Increased serum corticosterone	Not Informative [formalin; impact of sham surgery NR; short periodicity and duration; unquantified high level; FA alone untested; naïve not chamber exposed; small sample size]
(Lino dos Santos Franco et al., 2009)	Male Wistar rats (n = 5)	Formalin 0, 1% for 3 d (90 min/d) Sensitization: immediately post-FA, i.p. 10 µg OVA; boost 1 wk later with s.c. injection Challenge: 1 wk later with aerosolized OVA	Serum cell counts	Increased Total serum leukocytes and mononuclear cells, not neutrophils; FA inhibited OVA-induced increases	Not Informative [formalin; unquantified high level; small sample size; short duration and periodicity]

Effects on other immune system-related tissues (e.g., bone marrow, spleen, thymus, lymph)

Table B-47. Effects on other immune system-related tissues (e.g., bone marrow, spleen, thymus, lymph nodes, etc.)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
<i>Controlled-Exposure Studies in Animals, Animal Cells, or Immortalized Human Cells</i>					

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Fujimaki et al., 2004b)	Female C3H mice (n=5–6 per group)	PFA 0, 0.098, 0.49, or 2.46 mg/m ³ ; 12 wks	Splenic Cell counts Ex vivo splenic cells	No significant change in counts of splenic CD3 T cells, CD19 B cells, or CD4/CD8 ratio D/D Increased IFN γ with LPS stimulation of cells at 2.46 mg/m ³ D/D Increased MCP-1 at $\geq$ 0.49 mg/m ³ in cells of OVA-stimulated mice; N/C in IFN γ , MIP-1 α or IL-5 Body weight decreased at $\geq$ 0.49 mg/m ³	High or Medium Confidence [small sample size]: cell counts Low Confidence [small sample size; ex vivo]: cytokine measures
		Sensitization: i.p. 10 μ g OVA prior to FA exposure; aerosol OVA boost for 6 min on wks 3, 6, 9, and 11			
(Rager et al., 2014)	Male Fischer rats (n=3)	PFA 0 or 2.46 mg/m ³ for 7 d, 28 d or 28 d with 7 d recovery (6 hr/d)	miRNA microarray of femur BM cells	N/C in BM miRNAs at any time	High or Medium Confidence [small sample size] NOTE: indirect interpretation of endpoints
(Ma et al., 2020)	Male BALB/c mice (n=8)	Methanol-free formalin 0 or 2 mg/m ³ for 8 wks (8 hr/d, 7 d/w)	T cells in the spleen (mature) and thymus (immature)	Spleen: Decreased CD8+ and increased CD4/CD8 ratio; N/C in organ weight and CD4+ cells Thymus: Increased CD4/CD8 ratio ; Decreased organ weight and CD8SP cells; N/C in CD4SP cells	High or Medium Confidence: counts NOTE: experiments in directly treated cells considered <i>Not informative</i> for these endpoints (not extracted)
(Park et al., 2020)	Female BALB/c mice (n=10)	Fresh formaldehyde solution (methanol-free) 0, 1.38, 5.36 mg/m ³ for 2 wks (4 hr/d, 5 d/wk)	Splenic cytokines, T cell populations and Th1/Th2 balance, differentiation markers	Spleen: N/C in CD4+ T helper cells, D/D increased T reg cells (CD4+CD25+Foxp3+) subset of CD4+ cells; Increased calcinurin and NFAT1 (regulatory and inhibitory functions), N/C in NFAT2 Spleen (ex vivo production): D/D decreased IL-4, IL-5, IL-13, IFN-g, IL-17A, and IL-22 with similar changes in mRNA for same; [also, N/C in relative spleen wt. and increased rel. lung wt. at 5.36 mg/m ³]	High or Medium Confidence [small sample size]
(Dean et al., 1984)	Female B6C3F1 mice (n=6–10/ group/ endpoint, except n=5 for splenocyte assays)	PFA 18.5 mg/m ³ for 21 d (6 hr/d, 5 d/wk)	Lymphoid organ weights/ cellularity Host immunity response	N/C in thymus or spleen weight; N/C in BM cells/ femur or spleen cell counts; N/C in CFU in spleen or BM; N/C in splenic lymphocyte proliferation or splenic B cell IgM production N/C in cell-mediated immunity (response of spleen lymphocytes to mitogens, splenocyte cell surface markers, NK cell cytotoxicity) or humoral immunity	Low Confidence [excessively high levels small sample size; some experiments ex vivo] NOTE: 60–70% RB inferred

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
				(number of IgM Ab-producing B cells for 3 separate antigens) Decreased host susceptibility to bacteria challenge, but not tumor challenge; N/C in hypersensitivity or NK cytotoxicity	
(Liu et al., 2017)	Male ICR mice (n=10/group)	Unspecified test article 0, 1, 10 mg/m ³ for 20 wk (2 hr/d)	Bone marrow (BM) polychromatic erythrocytes (PCE)/normochromatic erythrocyte (NCE) ratio	Dose-dependent decrease in BM PCE/NCE ratio (markers of immature/mature RBCs), significant at ≥1 mg/m ³	Low Confidence [presumed formalin]
(Ye et al., 2013)	Male Balb/c mice (n≥9/ group/ endpoint)	Formalin 0, 0.5, 1, or 3 mg/m ³ for 7 d (8 hr/d)	ROS (dichlorohydro-flourescein and MDA) and GSH in BM and Spleen	Dose-dependent decrease in GSH levels in BM and spleen at ≥1 Dose-dependent increase in DCFH and MDA in BM and spleen at ≥1 Co-administered GSH attenuated effects on GSH, DCFH and MDA in all tissues	Low Confidence [formalin]
(Zhang et al., 2013)	Male Balb/c mice (n=9)	Formalin 0, 0.5, or 3 mg/m ³ for 2 wk (8 hr/d, 5 d/wk)	BM ROS and cytokines/ factors	BM increased megakaryocytes at ≥0.5 FA BM ROS (DCFH-DA) D/D increased at ≥0.5 FA; GSH decreased, and caspase-3 increased, at 3 FA; BM NFκB, TNFα, and IL-1β increased at 3 FA	Low Confidence [formalin]
(Zhao et al., 2020)	Male Balb/c mice (n=3, pooled into single sample for nose and lung samples); 2 experiments by different researchers	Formalin 0, 3 mg/m ³ for 2 wks (8 hr/d, 5 d/wk)	Burst-forming unit-erythroid (BFU-E), and colony-forming unit-granulocyte macrophage (CFU-GM) colonies in nose, lung, spleen, and bone marrow	Spleen results: Decreased formation of CFU-GM in both experiment I and II Decreased formation of BFU-E in experiment II; N/C in experiment I	Low Confidence [formalin; small sample size] Not Informative: ex vivo results
(Gu et al., 2008)	Female Balb/c and C3H/He mice (n=10 for in vivo; n=3 ex vivo experiments)	Formalin (assumed; test article NR) 0.098 mg/m ³ for 5 wk (in vivo); 0.12, or 0.98 mg/m ³ for 5 wk (ex vivo); both 24 hr/d, 5 d/wk	Splenic cell phenotypes Ex vivo cytotoxicity	N/C in T cell or B cell subtypes at 0.08 Increased NK1 cells (NK1.1 expression) at 0.098 mg/m ³ Increased ex vivo NK1 cell cytotoxicity at ≥0.12 mg/m ³	Low Confidence [formalin] Not Informative [small sample size; ex vivo; unclear reporting: ex vivo cytotoxicity]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Dallas et al., 1987)	Male SD rats (n=2/ time point; unclear reporting)	PFA 0, 0.62, 3.69, or 18.5 mg/m ³ for 1 wk to 24 wk (6 h/d, 5 d/wk)	Flow cytometry DNA/RNA analysis of BM cell proliferation/ health	N/C in RNA or DNA measures (e.g., % S phase) in BM cells	Low Confidence [small sample size; unclear reporting] NOTE: indirect utility for evaluating respiratory effects or inflammation
(Kim et al., 2013b)	Female NC/Nga (atopic-prone) mice (n=5–6/group)	Formalin (assumed; test article NR) 0, 0.25, 1.23 mg/m ³ for 4 wk (6 hr/d, 5 d/wk) Sensitization: topical house dust mite (HDM; ear) stimulation (25 mg Df ointment) 1×/wk for 4 wk	Cytokine mRNA for spleen	Spleen mRNA: FA D/D increase IL-13 only With HDM, FA exacerbated IL-4 (0.2), IL-5 (1.23 mg/m ³), IL-13 and IL-17A (≥0.25 mg/m ³), but caused D/D decreased IFN γ (≥0.25 mg/m ³)	Low Confidence [small sample size; unclear reporting] NOTE: indirect utility for evaluating respiratory effects or inflammation
(Kim et al., 2013a)	Female C57BL/6 mice (n=5 “experiments”; number of mice/ group unclear)	Formalin (assumed; test article NR) 0, 6.15, or 12.3 mg/m ³ 2–3 wk (6 hr/d, 5 d/wk)	Spleen and bone marrow cell counts Ex vivo cellular functional assays	N/C in absolute cell number or T cell or B cells subtypes in spleen or BM; No significant changes in %CD8 or % B cells in spleen Decreased NK1 cells in spleen, including reduced function, which was inhibited at 12.3 mg/m ³ : duration-dependent	Low Confidence [formalin; unclear, low sample size; high levels] Not Informative: ex vivo function
(Yu et al., 2014b)	Male ICR mice (n=6)	Formalin 20, 40, 80 mg/m ³ for 15 d (2 hr/d)	BM histology, cell counts and ROS	Decreased BM cells observed by pathology and GSH-Px activity at ≥40 FA Increased MPO activity and protein and decreased Prx2 protein at ≥20 FA Decreased BM cells (karyocytes) and CFUs and MMP levels at 80 mg/m ³ D/D increased BM oxidative stress (MDA increased and SOD decreased) ≥20 FA Increased BM apoptosis markers ≥40 FA	Low Confidence [formalin; excessively high levels; short periodicity]
(Yu et al., 2015a)	Male mice (strain NR; n=6/ group)	Formalin 0, 20, 40, 80 mg/m ³ for 15 d (2 hr/d)	BM H ₂ O ₂ production, caspase and antioxidant enzyme levels/ activity, and apoptosis	Increased ex vivo caspase-3 activity, peroxiredoxin levels and H ₂ O ₂ production at ≥20 mg/m ³ Increased apoptosis at ≥40 mg/m ³	Low Confidence [formalin-excessively high levels; short periodicity]
(De Jong et al., 2009)	Male Balb/c mice (n=6)	Formalin 3.6 mg/m ³ nose-only (up to 360 min/d for 3 d)	Ex vivo cytokine production from isolated lymph nodes	No cell proliferation in LNs N/C in IL-4, IL-10, or IFN γ production from isolated cells by FA alone, but FA with sensitization results in increased IL-4 and	Low Confidence [formalin; short duration and periodicity; ex vivo]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
				IL-10 (and slight increase in IL-12), but N/C in IFN γ	
(Zhang et al., 2014a)	Balb/c mice (n=3/sex/group)	Formalin 0, 4, 8 mg/m ³ for 7 d (6 hr/d)	Spleen and thymus weights Ex vivo spleen cell lymphocyte proliferation and ROS Urine metabolomics	Decreased relative spleen and thymus weights (only statistically significant for thymus at 8 mg/m ³) Decreased ex vivo lymphocyte proliferation and SOD activity at ≥ 4 mg/m ³ and increased ex vivo ROS at 8 mg/m ³	Low Confidence [formalin; ex vivo; no chamber control exposure; lowest tested exposure of 4 mg/m³] Note: some ex vivo assays after in vivo exposure; n=6 (pooled sexes assumed- not explicit in reporting)
(Fujii et al., 2005)	Female Balb/c mice (n=6–10)	Formalin (assumed; test article NR) 0, 0.25 mg/m ³ ; exposed during elicitation (reporting unclear) or sensitization (4 wk) or w/ chronic hypersensitivity	Ex vivo lymph node cells all w/ epicutaneous trinitrochlorobenzene TNCB	During elicitation: FA increased CD4+ T cells (IL-4+: Th2, not IFN γ +: Th1), not CD8+, in draining lymph node (LN) During sensitization (and in CH model): FA increased LN CD8+ T cells (N/C CD4+; CD4+CD25+/CD4+ decrease)	Not Informative [formalin; ex vivo; reporting for some experiments unclear; No FA-only controls; short duration]
(da Silva et al., 2015)	Male Wistar rats (n=6/ group)	Formalin 1% for 3 d (90 min/d); rats exposed in static chambers 5 rats/ time	Bone marrow cell counts	FA caused N/C in total bone marrow cells Note: while reduced effects were reported as reduced with laser therapy, laser therapy-only controls were not used	Not Informative [formalin; unquantified high levels; static exposure chamber and group exposure; short duration and periodicity]
(Ibrahim et al., 2016)	Pregnant Wistar rats (n=5 dams; 10 pups/group for experiments; design did not appear to account for potential litter effects)	Formalin 0.92 mg/m ³ from GDs 1–21: 1 hr/d, 5 d/wk	Total cells in femur lavage	Decreases in total cells by LPS were further reduced in offspring exposed to formaldehyde	Not Informative [formalin; short periodicity; offspring comparisons do not include FA without LPS; small sample size; did not appear to account for litter effects] Note: effects rescued by vitamin C; effects on dam uterine tissue not included in these tables
		Randomly assigned pups all received 5mg/kg lipopolysaccharide (LPS) injections at PND 30			
(Lino dos Santos Franco et al., 2009)	Male Wistar rats (n=5)	Formalin 0, 1% for 3 d (90 min/ d)	BM cell counts	N/C in total BM cells; FA inhibited OVA-induced increases)	Not Informative [formalin; unquantified high levels; small sample size; short duration and periodicity]
		Sensitization: immediately post-FA, i.p. 10 μ g OVA; boost 1 wk later with s.c. injection Challenge: 1 wk later with aerosolized OVA			

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Lino-Dos-Santos-Franco et al., 2011a)	Female Wistar rats (n=5)	Formalin 1% or naïve for 3 d (90 min/d), with or without ovariectomy	Bone marrow cell counts	Decreased total bone marrow cells	Not Informative [formalin; impact of sham surgery; unquantified high levels; FA alone untested; naïve not chamber exposed; small sample size; short duration & periodicity]
(Lino dos Santos Franco et al., 2006)	Male Wistar (n=5–6)	Formalin 1% or methanol vehicle for 4 d (30, 60, or 90 min/d)	Splenic and bone marrow cell counts	Increased total splenic cells, but total bone marrow cells unchanged	Not Informative [formalin (MeOH controls); unquantified high levels; small sample size; short duration and periodicity; comparisons to naïve rats rather than MeOH controls]
(Golalipour et al., 2008)	Wistar albino rats (n=7; sex N/R)	Mixture (dissection room vapor of undocumented composition) $\approx 1.85 \text{ mg/m}^3$ for 18 wk: 2 hr/d for 2 d/wk, 4 d/wk, or 4 hr/d for 4 d/wk	Spleen morphometry	Frequency-dependent increases in white pulp diameter and marginal zone diameter	Not Informative [mixture exposure; short periodicity; poor reporting; controls do not account for co-exposures; quantitative comparisons for results NR]

Evaluation of Mechanistic Evidence for Effects on Tissues Other than those of Interest to the Current Analysis

Table B-48. Effects on other tissues (data extracted for possible future consideration, but not included in this assessment)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
(Fujimaki et al., 1992)	In vitro male SD Rat peritoneal mast cells (n = 3+ experiments)	PFA 0, 1.23, 6.15, 12.3, 61.5 mg/m^3 for 30 min; stimuli: substance P, A23187 (increases cellular Ca^{2+} and NO production), and ant-rat IgE (in sensitized cells)	Peritoneal mast cell Histamine release	Enhanced histamine release stimulated by A23187 and anti-IgE at $\geq 6.15 \text{ mg/m}^3$; enhanced release by substance P at 61.5 mg/m^3 (note: release was inhibited by PLA2 inhibition, but not by antioxidant or dexamethasone)	Excluded (not tissues of interest) [In vitro; questionable relevance of peritoneal cells and exposure levels]
(Fujii et al., 2005)	Female Balb/c mice (n = 6–10)	Formalin (assumed; test article NR) 0, 0.25 mg/m^3 ; exposed during elicitation (reporting unclear) or	Ear swelling, skin histopathology	During elicitation: FA suppressed contact hypersensitivity (i.e., decreased ear swelling and edema)	Excluded (not tissues of interest) [Formalin; reporting for some experiments unclear; No FA-only]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
		sensitization (4 wk) or w/ chronic hypersensitivity (CH)—all w/ epicutaneous trinitrochlorobenzene		During sensitization (and in CH model): FA increased swelling, edema, and mast cell infiltration	controls; endpoint relevance unclear]
(Dean et al., 1984)	Female B6C3F1 mice (n=5–10/group/endpoint)	PFA 18.5 mg/m ³ for 21 d (6 hr/d, 5 d/wk)	Peritoneal macrophage function	N/C in peritoneal macrophage function, except: FA-increased H2O2 production by macrophages isolated after injection with MVE-2 and stimulation with PMA	Excluded (not tissues of interest) [Excessively high exposure levels; small sample size]
(Adams et al., 1987)	Female B6C3F1 mice (n=10)	PFA 18.5 mg/m ³ for 3 wk (6 hr/d, 5 d/wk)	Peritoneal macrophage counts and function (some in ex vivo cultures)	N/C in macrophage number or phagocytosis of antibody-covered erythrocytes; FA decreased leucine aminopeptidase expression FA increased release of ROS in response to external challenge (MVE-2 priming and PMA stimulus); N/C w/o challenge	Excluded (not tissues of interest) [Excessively high levels]
(Kim et al., 2013b)	Female NC/Nga (atopic-prone) mice	Formalin (assumed; test article NR) 0, 0.25, 1.23 mg/m ³ for 4 wk (6 hr/d, 5 d/wk)	Atopic dermatitis/ Ear skin Inflammation Cytokine mRNA for ear skin	FA increased AD-like clinical skin inflammation by HDM, but not FA alone Mast cell infiltration in dermis by FA alone, exacerbates HDM eosinophil & mast cell	Excluded (not tissues of interest) [Formalin; small sample size] Note: unclear utility for evaluating respiratory effects or inflammation; multiple supplementary files; eosinophil data not reported
		Sensitization: topical house dust mite (HDM; ear) stimulation (25 mg Df ointment) 1x/wk for 4 wk		Skin mRNA: 0.25 mg/m ³ increased IL-13, IL-17A, COX-2; with HDM, FA exacerbated these and IFN γ , IL-4, and TSLP; N/C IL-5	
(Maiellaro et al., 2014)	Pregnant Wistar rats (n=5)	Formalin 0.92 mg/m ³ from GD1–GD21: 1 hr/ d, 5 d/wk	Uterine factors	Decreased uterine IL-10, SOD2, and cNOS, and increased COX-1, at birth (N/C in IL-6, IL-4, IFN γ , COX-2, iNOS, SOD1, or catalase) Decreased birth weight in offspring	Excluded (not tissues of interest) [Formalin, short duration, offspring comparisons do not include FA alone]
		Sensitization: s.c. 10ug OVA with sc boost after 7 d Challenge: 7 d later, 1% OVA aerosol 15 min/d, 3 d			
(Aydin et al., 2014)	Male SD rats (n=6/group)	Test article unclear, but appears to be formalin in this experiment at 0, 6.48 (low), 12.3 (moderate), or 18.7 mg/m ³ for 4 wk (8 hr/d, 5 d/wk)	Liver tissue total antioxidant and total oxidant levels (TAS and TOS; kit uses vitamin E and H2O2 as reference, respectively Liver tissue apoptotic index and oxidative	Increased TOS and decreased TAS, at $\geq$ 12.3 mg/m ³ formaldehyde Decreased irisin and increased OSI at $\geq$ 6.48 mg/m ³ Note: Carnosine supplementation reduced changes.	Excluded (not tissues of interest) [Formalin; high levels]

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
			stress index (OSI: TOS/TAS)		
(Bakar et al., 2015)	Male Wistar albino rats (n=7)	i.p. Formalin every other day at 1 mg/kg-d for 14 d	Kidney biochemistry, immunoreactivity for Bcl-2 and Bax, ROS and antioxidant markers, and electron microscopy	Increased Bcl-2 and Bax immunostaining, and increased ROS markers and altered antioxidant enzyme activities; kidney damage and inflammation noted	Excluded (not tissues of interest) [Formalin; high levels of unknown comparability to inhaled levels; i.p. injection]
(Matsuoka et al., 2010)	Male ICR mice (n≥ 7)	Formalin at 0.12 mg/m ³ for up to 24 hr; also, a single experiment at 3.69 mg/m ³ for 24 hr with LPS	Urine, liver, brain ROS (8OHdG) and NO metabolites (nitrates/nitrites)	Decreased ROS in urine and liver; N/C in brain; decreased NO in urine, liver and brain at 0.12 mg/m ³ at 24 hr Increased urinary SOD activity: 3.69 mg/m ³	Excluded (not tissues of interest) [Formalin; short duration]
(Kum et al., 2007a)	Female SD rats (n=6/group) at GD1 [I], PND1 [II], PND28 [III] or adults [IV]	Formalin (assumed: test article NR): 0 or 7.38 mg/m ³ for 6 wk (8 hr/d, 7 d/wk)	Liver oxidative stress (i.e., SOT, CAT, GSH, MDA)	CAT activity and MDA levels increased [I] GSH decreased in [II] SOD activity decreased [III] N/C in adult [IV] oxidative stress markers Note: body and liver weight decreased in I and II; liver weight increased in III	Excluded (not tissues of interest) [Formalin, high levels; limited assays]
(Kum et al., 2007b)	Female SD rats (n=6/ group)	Formalin (assumed: test article NR): 0 or 7.38 mg/m ³ for 6 wk (8 hr/d, 7 d/wk);	Renal oxidative stress	N/C in renal SOD, CAT, GSH-Px, GSH, or MDA by FA alone	Excluded (not tissues of interest) [Formalin, high levels; limited assays]
(Ciftci et al., 2015)	Male Wistar albino rats (n=10)	Formalin i.p. injection at 9 mg/kg-d every other day for 2 wks	Brain and urine oxidative DNA damage Beta amyloid in brain	Increased A-beta1-42 in brain Increased brain DNA 8-OHdG damage; slightly increased (nonsignificant-assumed) DNA damage in urine	Excluded (not tissues of interest) [high levels of unknown relevance; i.p. injection; formalin]
(Ye et al., 2013)	Male Balb/c mice (n≥9/ group/ endpoint)	Formalin 0, 0.5, 1, or 3 mg/m ³ for 7 d (8 hr/d)	ROS (dichlorohydro-flourescein and MDA) and GSH in Liver and Testes	D/D decrease in GSH levels in liver at ≥0.5 mg/m ³ ; decreased in testes at 3 mg/m ³ D/D increase in DCFH and MDA in liver at ≥0.5 mg/m ³ ; in testes at ≥1 mg/m ³ ; co-administered GSH attenuated effects on GSH, DCFH and MDA in all tissues	Excluded (not tissues of interest) [Formalin]
(Jiang et al., 2015)	In vitro PC12 (immortalized neuronal) cells	Formalin (assumed; test article NR)—in vitro levels of unknown relevance	Viability, neurotrophic factor, and ROS markers	Decreased BDNF and viability Increased MDA and other ROS markers	Excluded (not tissues of interest)

Supplemental Information—Formaldehyde (Inhalation)

Study	System	Exposure	Endpoint(s)	Results	Utility and notes
	(n=3 technical replicates)				[Formalin, high levels of unknown relevance; in vitro; small sample size]
(Kim et al., 2013a)	Female C57BL/6 mice (n=5 “experiments”; number of mice/group unclear)	Formalin (assumed; test article NR) 0, 6.15, or 12.3 mg/m ³ 2–3 wk (6 hr/d, 5 d/wk)	liver cell counts Ex vivo cellular functional assays	N/C in absolute cell number or T cell or B cells subtypes in liver	Excluded (not tissues of interest) [Formalin; unclear sample size]
(Güleç et al., 2006)	Wistar albino rats (n=10; sex NR)	PFA 0, 12.3 or 24.6 mg/m ³ (8 hr/d, 5 d/wk) for 4 or 13 wk	Heart oxidative stress (i.e., SOD, CAT, TBARS, NO)	Increased SOD at ≥ 12.3 mg/m ³ (4 or 13 wk); Decreased CAT at ≥ 12.3 mg/m ³ at 4 wks, but not 13 wk; N/C in TBARS or NO	Excluded (not tissues of interest) [excessively high levels; limited assays]
(Xin et al., 2015)	HepG2 (liver) cells; n=3 technical replicates	Formalin; in vitro (unknown relevance)	Heat shock protein reporter assays	Increased promotion of HSPA1, correlated with oxidative stress and cellular damage	Excluded (not tissues of interest) [in vitro; high levels; formalin; small sample size]

B.3.7. Nervous System Effects

The details of the study evaluation approach methods are provided in Section 2.3.7 of the Toxicological Review. Within Table B-49 (epidemiology studies of ALS and neurobehavior), Table B-50 (controlled exposure studies in humans), Table B-51 (animal studies), and Table B-52 (mechanistic studies of inhalation exposure) below documenting the results of the study evaluations for nervous system effects, studies are arranged alphabetically. Gray shading is indicative of aspects of the study design or analysis that decreased EPA's confidence rating.

Observational Epidemiology Studies

Table B-49. Evaluation of observational epidemiology studies of formaldehyde—neurological effects

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
<i>Amyotrophic Lateral Sclerosis (ALS)</i>							
Bellavia et al. (2021) ,	ALS cases, 1982–2009, from Seals et al. (2017) with	Occupational histories obtained from Danish	Danish National Patient Register,	Evaluated diabetes, obesity,	Selected joint predictors and	N = 1086 incident	ALS incidence <i>Low</i>

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
(Denmark) Population-based nested case-control	data for several health factors and environmental risk factors previously linked with ALS. Controls, 100 per case matched on being alive on index date for case diagnosis, same birth year and sex. Excluded individuals with less than 5 years work experience.	Pension Fund databases. Used NOCCA (Nordic Occupational Cancer Study)- Danish JEM for periods 1960–74, 1975–84, and 1985 and after. Formaldehyde exposure metric was ever/never exposed. Anticipate exposure misclassification and large variation in prevalence and intensity of exposure across individuals. In men, correlations between formaldehyde, diesel exhaust and solvents were 0.22 and 0.41, respectively (Phi coefficients)	discharge diagnosis ICD-8 348.0 OR ICD-10 G12.2. Case definition was 1 st diagnoses on or after 1/1/1982–12/31/2009.	physical/ stress trauma, CVD (1977–2009) and lead, diesel exhaust and solvents	interactions using boosted regression trees and Logic regression, which were included in a logistic regression model adjusting for age, SES, and geography. Model used a 3 year lag.	ALS cases, 677 exposed; 111,507 controls	Large uncertainty regarding exposure assessment (ever/never exposed). Adequacy of 3 year lag is unknown.
Seals et al. (2017) (Denmark) Population-based case-control	Registry-based case identification using the Danish National Patient Register, 1982–2009 (3,650 incident cases). Controls, 4 per case matched on sex, age, and no ALS diagnosis in Hospital Register as of index date obtained from Central Person Registry (All Denmark residents since 1968).	Occupational histories obtained from Danish Pension Fund databases. Used NOCCA (Nordic Occupational Cancer Study)- Danish JEM for periods 1960–74, 1975–84, and 1985 and after. Inputs year and industry code and outputs prevalence of exposure for each job along with expected	Danish National Patient Register, discharge diagnosis ICD-8 348.0 OR icd-10 G12.2. Case definition was 1 st diagnoses on or after 1/1/1982–12/31/2009.	Controls were matched to cases by age, sex and calendar date. Assessed SES (highest attained, 5 groups based on job title), marital status and residence. Other covariates were relative to 4 th year before index year: whether worked	Conditional logistic regression adjusted for age, sex, index date, SES, marital status and residence. In secondary analyses included other work variables, # hospital diagnoses, plus Charlson	N = 3650 incident cases, 1,068 exposed; 14,600 controls	ALS incidence <i>Medium</i> Uncertainty regarding exposure assessment. JEM not validated to predict formaldehyde exposure level.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		exposure level (ppm) in exposed. The JEM has not been validated to estimate levels of formaldehyde. Cumulative expected exposure calculated (prevalence multiplied by expected level) summed over jobs and time (3 & 5 year lags). Exposure misclassification expected.		on that year, years worked prior, hospital admission, # times admitted prior, # admissions, prior diagnoses used to construct Charlson Comorbidity Index. No information on smoking status	Comorbidity Index. Exposure metrics were dichotomous (ever exposed lagged 3 years), quantiles, and continuous		
Fang et al. (2009) (United States) General population (case-control)	Sequential ALS cases recruited, 1993–1996, from 2 major referral centers in New England; eligibility criteria cases & controls: lived in New England at least 50% of year, mentally competent, English speakers; 71% of eligible cases participated; controls by random telephone screening, frequency matched on sex, age (3 groups), & region; 76% of eligible (256 of 270 completed questionnaire).	Occupational history by structured questionnaire; industry, occupation, frequency and duration; jobs held before ALS diagnosis or 2 years before interview (controls); formaldehyde- exposed occupations identified <i>a priori</i> by industrial hygienist; calculated life-time hours of exposure weighted by probability in specific jobs	Diagnoses by board-certified specialists in motor neuron disease using World Federation of Neurology El Escorial criteria	Adjusted for age, sex, area of residence, smoking (ever/never), & education; no additional workplace exposures associated with ALS	Unconditional logistic regression models; linear trend with lifetime exposure days, probability, & weighted exposure duration (4 categories); effect modification by smoking; missing occupational data for 2/111 cases & 3/256 controls	N = 109 ALS cases (N = 20 exposed) 253 controls	ALS incidence <i>Medium</i> Uncertainty regarding exposure assessment; small number of exposed cases
Peters et al. (2017) (Sweden) Nested case-control study	All Swedish births (1901–1970) and included in 1990 Swedish Population and Household census, N=5,763,437. Controls randomly selected (5 per	Occupational history obtained from 1970, 1980, and 1990 census; included occupations listed ≥ 10 years prior to index date;	Linkages to National Patient Register, primary or secondary diagnosis, ICD-9	Addressed age and sex via matching, adjusted for education and evaluated 12 of	Conditional logistic regression, OR and 95% CI, adjusted for education and	2,647 cases (n=323 exposed), 13,378 controls	ALS incidence <i>Medium</i> Uncertainty regarding exposure assessment

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	case) from population alive on date of diagnosis, matched on birth year and sex. 25,100 controls.	occupational exposures assessed using Swedish version of JEM (Nordic Occupational Cancer Study), prevalence and level of exposure at specific calendar time. Exposure metric for dose response, prevalence multiplied by annual mean level for each occupation at time of census (mg/m ³), averaged across all censuses; dichotomized at median in controls	335C or ICD-10 G12.2 (inpatient visits 1991-2010 and outpatient visits 2001–2010); follow-up to date of first visit, migration, death, or 12/31/2010. 5,010 cases	>20 agents possibly associated with ALS. No adjustment for smoking status although restriction to blue collar workers and farmers may have partially addressed potential confounding	other 11 chemicals; restricted analyses to cases and controls with at least one occupation listed in any census and to blue-collar workers or farmers; sensitivity analysis restricting to < 65 years at index date, age of retirement		
Pinkerton et al. (2013) (United States) Garment workers (cohort)	Cohort of garment workers (N=11,098) exposed for ≥ 3 mos at 3 facilities (late 1950s to early 1980s).	Monitoring in 1980s, geometric mean 0.15 ppm (GSD 1.9 ppm), constant levels across departments and facilities, year of first exposure (42% before 1963), time since 1 st exposure (median 39.4 years) and exposure duration (median 3.3 years). Exposure levels prior to 1980 not assessed.	Vital status ascertained through 2008, ICD-10 G12.2, ICD-9 335.2, ICD-8 348.0, and ICD-7 356.1; ALS mortality is a good surrogate for ALS incidence	Adjusted for age, calendar time, sex, race; no information on smoking. Mortality for COPD and lung cancer in cohort was similar or greater than national rates suggesting possible bias away from null.	Life table analysis, excluded missing birth date (n=55), deaths (n=8), loss to follow-up prior to rate file begin date (n=13); SMRs and 95% CI	N = 11, 022 414,313 person-years at risk; 8 ALS deaths	ALS mortality <i>Medium</i> Small number of cases. Confounding by smoking away from null not of concern because effect estimates were null.
Roberts et al. (2016) (United States) National Longitudinal	794,541 men and 674,694 women (recruitment date unclear, but study from 1973–2011) aged 25+ at recruitment (national).	Self-reported at enrollment based on survey regarding last or most recent job. Exposure matrix	ALS Mortality (National Death Index from 1979–2011) as underlying	Adjusted for education, race/ethnicity, and income (participants	Data handling and analysis as in Weisskopf et al. (2009)	N = 472 deaths in men (100 exposed); 285 deaths	ALS mortality <i>Medium</i> Uncertainty regarding exposure assessment, including the influence of

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Mortality Study. Occupational (cohort) Note: same laboratory and data handling procedures as Weisskopf et al. (2009)	Follow-up time provided by participants. Internal comparison, participation unlikely to be influenced by knowledge of exposure and disease.	constructed by industrial hygienists at the National Cancer Institute. Metrics included intensity and probability of exposure. No information about duration in job. Information on other exposures not collected/reported.	cause; ICD-9 code 335.3 (specific for ALS) or ICD-10 code G12.2 (for all motor neuron diseases, of which ALS comprises the overwhelming majority)	tended to be poorer, less educated, and less frequently non-Hispanic white. One sensitivity analysis among high probability, high exposure group (all funeral directors) included adjustment for smoking and military service.	HRs provided for each exposure intensity and probability for men and women separately. Additional sensitivity analyses to evaluate validity of exposure and outcome assignments and selection bias, included follow up restricted to 75 years or excluding first 5 years, age restricted to 35–75 or 50–75 years at enrollment, or restricted to those employed at enrollment. Did not provide or incorporate any data on duration.	in women (61 exposed). N = 2 in jobs with high probability and intensity of formaldehyde exposure	duration, no information about job history prior to most recent job; very small number of exposed cases (n=2 in jobs with high probability and intensity of formaldehyde exposure)
Weisskopf et al. (2009) (United States) American Cancer Society Cancer Prevention Study II.	987,229 (414,493 men, 572,736 women) enrolled in 1982. National recruitment; no major illness at baseline, not missing age or sex data. Follow-up from 1989 through 2004.	Self-reported, mailed questionnaire in 1982. Current or past regular exposure to formaldehyde and duration (years) (not specified, but likely in occupational settings).	Mortality (National Death Index), underlying or contributing cause; ICD-9 (1989–1998) code 335.3 or	Adjusted for age, sex, smoking, military service, education, alcohol, occupation (farmer, lab technician,	Cox proportional hazards modeling, analyzed with and without approximately 1/3 who reported exposure but did	N = 1,156 ALS deaths (36 exposed)	ALS mortality <i>Medium</i> Uncertainty regarding exposure assessment

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
General population (cohort)	Internal comparison, participation unlikely to be influenced by knowledge of exposure and disease.	Data on 10 other types of chemicals and X-ray exposure also collected.	ICD-10 (1999–2004) G12.2. (ALS represents > 98% of these categories)	machine assembler, programmer), vitamin E use, and the other chemical (and X-rays) exposures assessed at baseline.	not provide duration data (i.e., less likely to be truly exposed).		
<i>Neurobehavioral tests and olfactory detection</i>							
Broder et al. (1988c) (Canada; Toronto) Residences (household survey) Additional reference: Broder et al. (1988b) ; Broder et al. (1988a)	Homes with UFFI insulation, within 60 miles of Toronto. 4,400 of 8,200 agreed to be contacted; 95% participated. Control homes randomly selected from streets adjacent to UFFI homes, 20% participated. Some demographic and symptom data allowed comparison with nonparticipants; similar neighborhood, demographics.	2-day samples in homes, 5 hr/d Median ppm Control 0.031 UFFI 0.038 No exposure contrast	Sense of smell threshold for pyridine; three control bottles (mineral oil only) plus 3 bottles with 0.00005, 0.008, and 0.012% pyridine. Replicate tests conducted. Variability and stability of test kits assessed. Participant blinded.	Detailed demographic data collected	Prevalence by group and Chi-square test.	N = 1,726 from UFFI homes, 720 from control homes	Sense of smell <i>Not informative</i> No appreciable difference in median exposure between groups
(Wilmer et al., 1989) ; Kilburn et al. (1985b) ; Kilburn et al. (1987) ; Kilburn et al. (1989b) (United States)	Recruited from attendees (female) at annual histology technician conferences, 1982 and 1983. Participation rate not reported. Potential for participation based on knowledge of exposure and symptoms.	Self-reported hours per day (based on detection of odor)	Neuro-behavioral test battery (memory, cognition, spatial relation integration, dexterity, conceptual	Adjusted for age, number of cover slipped slides (for other solvent exposure), duration of smoking	Multiple regression. Coefficients and designation if $p < 0.05$ (no standard errors)	N = 305	Neurobehavioral tests <i>Low</i> Potential selection bias (could be influenced by perceived exposure and effects), limited detail presented in results

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Workers: histology technicians (survey)			motor speed, balance, reaction time); 1 hour				
Kilburn and Warshaw (1992) (United States) Workers: histology technicians (survey, multiple time points)	Recruited from attendees (female) at annual histology technician conferences, 1982, 1983, 1985, 1986, 1989. Participation rates not reported. Potential for participation based on knowledge of exposure and symptoms	No information on intensity or frequency of exposure	Neuro-behavioral test battery (memory, cognition, pattern recognition, dexterity, decision making, motor speed, balance); 2–3 hrs	Considered age, sex, number of cover-slipped slides (for other solvent exposure), years of exposure	For analysis of single (first) test per subject (n=350), reported as “not statistically significant.” For longitudinal analysis (n=19), no decline in performance noted (formaldehyde exposure not explicitly analyzed).	N = 19 with 4 tests, 299 with 2 or 3 tests, 350 with one test	Neurobehavioral tests <i>Low</i> Potential selection bias, limited detail presented in results. Longitudinal analysis limited by sample size and did not specifically address formaldehyde exposure
Kilburn (2000) (United States, 6 states). Home or office exposure (survey)	Exposed (e.g., new mobile homes or renovated offices), experienced “adverse effects almost daily”; referent group randomly selected from voter registration rolls in 4 cities (location and participation rate not reported).	No exposure measures.	Neuro-behavioral test battery	Frequency matched by age and education	Mean ± SD percent prediction	20 exposed, 202 referents	Neurobehavioral tests <i>Not informative</i> Likely selection of exposed based on symptoms; no exposure measures, limited covariate data.
Schenker et al. (1982) (United States) Residences (survey)	People self-referred to occupational and environmental health clinic regarding health effects of formaldehyde insulation. No comparison group.	Measured in 4 homes (protocol not described), ranged from 0.03 to 0.23 ppm	Neurobehavioral battery	Not addressed	Prevalence	18 adults, 6 children (from 6 homes)	Neurobehavioral tests <i>Not informative</i> Likely selection of exposed based on symptoms; limited exposure measures, no comparison group

Controlled Exposure Studies in Humans

Table B-50. Evaluation of human controlled exposure studies of formaldehyde – nervous system effects

Reference, setting, and design	Exposure assessment (quality descriptor and exposures; see Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure) and confounding	Analysis and completeness of results	Size	Confidence
Andersen and Molhave (1983)^a	<i>Chamber type and analytical concentrations not provided; testing during exposure (distractibility likely contributes)</i> 4 d of exposure	<i>Endpoints limited: sparse methods on conduct of partial neurobehavioral test battery</i>	Exposure order by Latin square design; blinding not indicated	Comparisons appear to represent pooled sexes; results data NR	n=16	<i>Low</i> Test batteries performed during exposure (irritation anticipated to result in distracted responses), an incomplete set of tests, blinding not reported, and reporting deficiencies.
Bach et al. (1990)	<i>Test article not defined</i> (inferred from related study, (Andersen and Molhave, 1983)^c ; testing during exposure (distractibility likely contributes); acute (5.5 hr) exposure	<i>Endpoints limited: sparse methods on conduct of partial neurobehavioral test battery</i>	Occupation exposure group and controls from population registry (attempted matching by age, education, smoking prevalence but workers had higher smoking and lower education; details not reported); <i>Exposure order by balanced Latin square design; blinding not indicated</i>	Results reporting incomplete & difficult to decipher	n=61 males only	<i>Low</i> Test batteries performed during exposure (irritation anticipated to result in distracted responses), incomplete set of tests, blinding not

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Exposure assessment (quality descriptor and exposures; see Appendix B.3.1)	Outcome classification	Consideration of possible bias (randomized exposure order, blinding to exposure) and confounding	Analysis and completeness of results	Size	Confidence
						reported, and reporting deficiencies.
Lang et al. (2008) ^a	<i>“Quasi-static” exposure chambers; testing immediately after exposure; study focus on irritation; no indication of acclimation; recovery not examined (reaction time); 10 d of exposure</i>	<i>Endpoints limited: decision reaction time</i>	Exposure order randomly assigned double blinded	Data= combined sexes; high variability in reaction time data	n=21 ≈20% attrition	<i>Medium</i> Limited set of tests; high attrition rate

^aAt the request of EPA’s Office of Chemical Safety and Pollution Prevention, EPA’s Human Studies Review Board review of four intentional exposure studies for potential use in assessing acute formaldehyde inhalation determined the ethical conduct of this study to be adequate.

Animal Toxicology Studies

Animal studies in Table B-51 are grouped by endpoint type, and then organized alphabetically by first author. Specific study details (or lack thereof) that highlight a limitation or uncertainty in answering each of the domain criteria are noted in the table cells. For those identified as having a substantial limitation likely to influence the study results (major limitations), the relevant study details are bolded. Italics are used to highlight other limitations not viewed as substantial in the context of nervous system effects (minor limitations). A 'Good' domain rating is denoted by '++'; an 'Adequate' rating by '+'; and a 'Deficient' rating by gray shading.

Table B-51. Evaluation of controlled inhalation exposure studies examining nervous system in animals

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
<i>Odorant or Irritant Detection/Effects</i>						
(Apfelbach and Weiler, 1991)	+ <i>Chamber type not specified</i>	+ <i>N = 5 (exposed) or 10 (controls); males only</i>	Testing during exposure; controls not air-exposed in exposure chamber; possible continuous exposure Note: 130 d exposure	Training started 30d after exposures began (not clear if training ability prior to endpoint testing was affected)	++	<i>Not informative</i> [Tested during exposure; missing controls; training during exposure]
(Wood and Coleman, 1995)	++	+ <i>N = 8; males only</i>	Testing during exposure; each animal served as its own control (multiple exposures/animal); <i>acute exposure (60 seconds on/off for ≈1hr)</i>	++ Note: endpoint is not adverse (irritant detection)	++ Note: statistical comparisons not possible	N/A * Olfactory detection/irritation response [Tested during acute exposure]
<i>Cursory Examinations in Long-Term Toxicity & Carcinogenicity Studies</i>						
(Appelman et al., 1988)	++	+ <i>N ≥ 10; males only</i>	Behaviors tested during exposure; study focus not nervous system-specific; 1 year study	+ <i>Endpoints limited to cursory cage-side observations, gross pathology, & weight</i>	Results data NR; behavioral effects not quantified	** [Tested during exposure; study focus not CNS; data NR]
(Coon et al., 1970)	+	+	Behaviors tested during exposure; study focus on	Endpoints limited: cursory cage-side	Results data NR; behavioral effects not	<i>Not informative</i>

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
	<i>Multiple species exposed simultaneously</i>	<i>N = 15 (rats); age & sex ratio/group not given</i> <i>Dogs (N = 2) deficient</i>	overt toxicity and inflammation; 90 d study	observations & brain sections "retained" (not clear if examined)	quantified; one death noted, but no cause provided	[Tested during exposure; limited endpoints; data NR]
(DHGC, 2010)	Formalin (high concentration: methanol may drive responses)	<i>N = 3-6</i>	Behaviors tested during exposure; acute exposure	Endpoints limited: cursory observations of behavior during exposure	Effects not quantified	<i>Not informative</i> [Tested during exposure; limited endpoints; data NR; High formalin levels; small N]
(Kerns et al., 1983) ^b See also (Battelle, 1982) and (Swenberg et al., 1980b)	++	++ <i>N = 10</i>	Behaviors appear to have been tested immediately after exposure; study focus on carcinogenicity Note: based on a 2 year GLP-compliant study	+ <i>Endpoints limited</i> to simple neurofunctional observations & gross pathology; methods provided in the full GLP report indicate <i>lack of observer blinding</i>	Results data NR in published article; latency NR; data in the full GLP report is qualitative (normal vs. abnormal) & is pooled across test battery endpoints	** [Tested immediately after exposure; study focus not CNS; data NR]
(Maronpot et al., 1986)	Formalin	++ <i>N=10</i>	Behaviors tested during exposure; study design not nervous system-specific; 13 wk study	+ <i>Endpoints limited</i> to cursory cage-side observations & gross pathology	Results data NR; behavioral effects not quantified	<i>Not informative</i> [Formalin; tested during exposure; study focus not CNS]
(Morgan et al., 1986a)	+ <i>Analytical concentrations not provided</i>	<i>N = 3–6; males only</i>	Behaviors tested during exposure; study design not nervous system-specific; <i>acute exposure</i>	Endpoints limited: cursory observations of distress during exposure	No quantified neurological effects	<i>Not informative</i> [Formalin; small sample size; tested during exposure; data NR; small N]
(Tobe et al., 1985b ; Tobe et al., 1985a)	Formalin (Note: methanol control group included in the chronic study)	+ <i>N = 3–20 (depending on the experiment, endpoint & exposure group); males only</i>	Behaviors tested during exposure; study design not nervous system-specific Note: studies of variable duration (up to 28 mos)	+ <i>Endpoints limited</i> to cursory cage-side observations; gross pathology, brain wt. weight also performed in 28-month study	Results details NR for many experiments & animals; behavioral effects not quantified; multiple dead animals could not be examined for	** [Formalin: controlled for some endpoints; tested during exposure; data NR]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
					comparisons due to decomposition	
(Woutersen et al., 1987)	+ <i>Animals were housed in the inhalation chambers</i>	++ N=40	Behaviors tested during exposure; study design not nervous system-specific Note: 13 wk study	+ <i>Endpoints limited to cursory cage-side observations, brain wt.</i>	Results data NR; behavioral effects not quantified	** [Tested during exposure; data NR]
Neuropathology						
(Aslan et al., 2006)	++	N= 3 litters (5 pups); males only; dam health during lactation & pup health not presented Note: possible subset of Songur et al. (2003) study; same animals as Sarsilmaz et al. (2007) study ^e	+ <i>Unclear if potential litter bias was corrected</i> (although randomized treatment groups); dams seemed to be co-exposed with pups from PND 1–14 Note: 30 d of exposure	++ Note: regional or hemisphere volume changes not verified by immunostaining, leaving interpretations unclear; sensitive stereology methods; random sampling indicated	As presented, data do not account for potential litter effects (pup means presented)	Medium [Small sample size; potential for litter bias]
(Bian et al., 2012)	Formalin (high concentration: methanol may drive responses)	N= 3/endpoint/time point; males only; mild toxicity: decreased food intake (effect not quantified)	Controls not air-exposed in exposure chamber; all groups had anesthesia & antibiotic injections; exposures = 1 hr/d Note: 90 d exposure; single exposure level	+ Number of slides/animal not provided; relatively insensitive method for cell count quantification Note: blinding & other methods appropriate	++	Not informative [High formalin levels; small N; insufficient controls]
(Liu et al., 2010)	Formalin (high concentration: methanol may drive effects)/static chamber	+ Group size for staining not clear; males only; groups determined by preexposure probe trial performance	+ Exposures only 30 min twice daily; 28 d	Potential sampling bias: details on blinding, slides/animal, etc. not provided; imaging specifics not provided and qualitative only	+ Hippocampal Nissl staining not quantified	Not informative [High formalin levels; potential sampling bias]
(Mei et al., 2016)	Formalin	+ N = 8; males only	+	Potential sampling bias: details on blinding, slides/animal, etc. not	No quantitative results (e.g., counts; severity scores; etc.)	Not informative

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
			<i>No comparisons to chamber or air exposure alone; 8 hr/d for 7 consecutive days</i>	provided; qualitative only		[formalin; potential sampling bias; no results quantification]
(Pitten et al., 2000)	Formalin/static chamber	+ N = 5–8 Note: no changes in body weight were observed	+ <i>Exposures only 10 min/d for 90 days</i>	Potential sampling bias: details on blinding, slides/animal, etc. not provided; qualitative only	Results data NR	** [Formalin; potential sampling bias; data NR]
(Sarsilmaz et al., 2007)	++	N= 3 litters (5 pups); dam health during lactation & pup health not presented; males only ^c Note: possible subset of Songur (2003) study; same animals as Aslan et al. (2006) study ^c	+ <i>Unclear if potential litter bias was corrected</i> (although randomized treatment groups); <i>dams seemed to be co-exposed with pups</i> from PND 1–14; 30 d of exposure	++ Note: regional or hemisphere volume changes not verified by immunostaining, leaving interpretations unclear; sensitive stereology methods; random sampling indicated	As presented, data do not account for potential litter effects (pup means presented)	Medium [Small sample size; potential for litter bias]
(Songur et al., 2003)	+ <i>Analytical concentrations not provided</i>	N= 6 pups (likely 3 litters); mild toxicity (body weight changes at 30 & 60 d, but not 90 d ^d); males only	+ <i>Unclear if potential litter bias corrected</i> (& not indicated as randomized); 30 d of exposure	Cell counting methods do not detail how many slides/animal were examined (may be a single slide)	as presented, data do not account for potential litter effects (pup means presented)	Low [Small sample size; potential for sampling and litter bias]
(Wang et al., 2014a)	Mixture (formalin, benzene, toluene and xylene)/static chamber	++ N = 12 males/group Note: no changes in body weight were observed	++ 2 hr/d exposure for subchronic (90 d)	Relative, but not absolute (preferred), brain weights were reported; number of H&E samples NR Note: both insensitive	++	Not Informative [Mixture exposure only; non-preferred results presentation]
Neural Sensitization-Related Responses						
(Sheveleva, 1971) (translation)	Test article not defined (assumed to be formalin)	+ Use of mongrel white rats; N = 7 dams or 6 offspring/sex evaluated	+ <i>Latency between dam exposure and testing not provided</i> ; unclear if reflex	"Neuromuscular excitability" protocol specifics not provided	+ <i>Statistical methods used were not specified; data appear</i>	Low [Formalin; endpoint methods NR]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
		from 6 litters, so assumed 1 pup/sex/litter examined, but not specified; unclear why 7 dams vs. 6 offspring	bradypnea can influence these measures (e.g., reduced respiration leading to transiently reduced O ₂ content in muscle tissue, causing reduced excitability); 4 hr/d exposures from GD1–19	(e.g., blinding; how assessed)	<i>to account for possible litter effects, but not clearly described</i>	
(Sorg et al., 1996)	Formalin (high concentration: methanol may drive responses)	+ <i>N ≥ 4; females only</i>	Potential high concentration irritation-related responses (that may affect odor discrimination in tasks involving exploration) were not measured; <i>exposure 1 hr/d for 7 d</i> ; Note: single exposure level	+ <i>Overall plus maze activity not provided</i> ; Note: questionable human relevance of rodent sensitization responses	+ <i>Groups divided into high & low responders for presentation of most endpoints & statistical comparisons; statistical comparisons NR for 1-month recovery data</i>	<i>Not informative</i> [High formalin levels; possible confounding by irritation]
(Sorg et al., 1998)	+ <i>Chamber type not provided</i> ; declining HCHO exposures across days	+ <i>N= 15–24; females only</i>	+ <i>Imprecise timing of assessment; unclear effect of prior cocaine exposure/handling on nociception (assumed to be minimal)</i> Note: 1 or 4 wk exposure; single exposure level	Experimenter blinding not indicated ; methods for measuring vertical activity NR in cited reference Note: questionable human relevance	++	<i>Medium</i> [Blinding NR; limited methods description] Note: relevance of inescapable stress unclear
(Sorg and Hochstatter, 1999)	+ <i>Chamber type and analytical concentrations not provided</i>	+ <i>N = 4; females only (conditioned fear) OR N= 8; males only (approach/avoidance)</i>	Possible effects on olfactory detection of conditioned odors by HCHO nasal effects; Approach/avoidance tested during exposure to formalin vapors Note: 4 wk exposure; single exposure level	++ Note: questionable human relevance of rodent sensitization responses	Effects without cocaine NR : (unclear influence of prior cocaine exposure in conditioned fear responses)	<i>Low</i> [Unclear influence of changes in olfactory detection or prior cocaine exposure]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
(Sorg et al., 2001b)	+ <i>Chamber type and analytical concentrations not provided</i>	+ <i>N = 7–8</i>	Testing during exposure; exposures ≤ 4 wk Note: single exposure level	+ <i>Methods for measuring vertical activity NR in cited reference (but automated using photocell counts)</i>	++	Low [Tested during exposure; limited methods reporting]
(Sorg et al., 2002)	Formalin (likely high concentration- not quantified: methanol may drive responses); HCHO levels NR	+ <i>N = 6–12</i>	Formalin used as an aversive stimulus- results more specific to cocaine; behaviors evaluated coincident with exposures; <i>acute exposure</i>	Tests involve odor detection & irritation-specific responses: could confound results Note: questionable human relevance	Specific effects of formaldehyde alone on behaviors NR; some data presented with groups divided into high & low responders for statistical comparisons	Not informative [High formalin levels; likely confounding by irritation and odor; missing controls]
(Sorg et al., 2004)	+ <i>Chamber type not specified</i>	+ <i>N = 7–8</i>	Possible effect on olfactory detection of conditioned odor by HCHO nasal effects; context testing prior to conditioned fear tests may cause order effects Note: single exposure level; 4 wk exposure	+ <i>Possible contribution of change in footshock sensitivity not examined</i> Note: questionable human relevance of rodent sensitization responses	++	Low [Unclear influence of changes in olfactory detection]
(Usanmaz et al., 2002)	++	+ <i>N = 6; unexplained overt toxicity (body weight decrease) with multiple exposures</i>	Observations immediately after exposure; <i>acute (3 hr) or short-term (1–3 wk) exposure</i>	Observations not blinded; 5 min test duration; peripheral vs. central square crossings not measured, limiting interpretability	++	Low [Tested immediately after exposure; no blinding]
Motor Activity, Habituation, and Anxiety (& Aggression)						
(Boja et al., 1985) ^e	+ <i>Analytical concentrations not provided</i>	+ <i>N = 8; males only</i>	Behaviors tested during exposure; <i>acute exposure (3 hr/d for 1–2 d)</i> ; timing of exposures (9–12 pm vs. 12–3	Appropriateness of protocol for adult animals is questionable (methods designed for	+ <i>Statistical comparisons to air-only exposure groups</i>	Low [Tested immediately after acute exposure;

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
			pm) may not have been same across groups Note: single exposure level	pups); "active" vs. "nonactive" endpoint readout is nonspecific	NR for all treatment groups; higher exposure groups data NR and text suggests results are somewhat inconsistent	endpoint methods may be inappropriate]
(Katsnelson et al., 2013)	Test article not defined (assumed to be formalin; high concentration: methanol may drive effects)	++ N= 12–15 females/group	Testing indicated as immediately after exposure; Note: subchronic (10 wk) exposure	Protocols not specified, although hole board test methods assumed to be conducted in a standard manner; blinding not indicated	++	Not informative [High levels of test article assumed to be formalin; irritation effects likely]
(Li et al., 2016)	Formalin; static chambers	+ N = 15 (inferred); males only	+ Testing began ≈2 hr postexposure Note: exposure 2 hr/d for 7 d	Blinding not indicated for all tests except forced swim: of particular concern for nonautomated novel object testing; unclear impact of multiple tests in same animals (chosen test order may reduce impact); % open time in EPM does not include % closed time; note: slight body weight loss 2.46 mg/m ³	++	Low [Formalin; endpoint evaluations fail to control for several important variables]
(Liu et al., 2009a)	Formalin (high concentration: methanol may drive effects)/static chamber	+ N = 8; males only	+ 14 d exposure Note: tested >24 hr after exposure;	Spontaneous locomotor activity was assessed subsequent to aggression tests, which may influence anxiety-related responses; blinding not indicated	++	Not informative [High formalin levels; test order concerns]
(Malek et al., 2003a)	Formalin	++ N= 15/sex	+	+	+	Low [Formalin]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
			2 and 26 hr postexposure; acute: 2 hr	3 min test duration; manual scoring (blinded); <i>peripheral vs. central square crossings not quantified</i> , limiting interpretability	Assuming data is SE, some statistical significance calls are questionable; <i>variability unclear</i> : SE reported is higher than SD for same parameters in 2003 ^b	
(Malek et al., 2003b)	Formalin	++ N= 10/sex	+ 2 hr postexposure; acute: 2 hr	+ 3 min test duration; manual scoring (blinded); <i>peripheral vs. central square crossings not quantified</i> , limiting interpretability	++	Low [Formalin]
(Malek et al., 2004)	Formalin	+ N = 20; males only	+ 2 and 26 hr postexposure; acute; 2 hr	+ 3 min test duration; manual scoring (blinded)	++	Low [Formalin]
(Senichenkova, 1991a) (translation); (Senichenkova, 1991b)	Test article not defined (assumed to be formalin)	Sex, N, & strain NR; could not be evaluated due to lack of reporting	+ <i>Unclear if litter bias corrected</i> Note: 4 hr/d exposures from GD 1–19; single exposure level	Open field protocol specifics not provided (e.g., blinding; manual vs. automated assessment of activity)	+ <i>Statistical methods NR</i>	<i>Not informative</i> [Test article assumed to be formalin; test animal and endpoint protocol details NR].
(Sheveleva, 1971) (translation)	Test article not defined (assumed to be formalin)	+ <i>Mongrel white rats</i> ; N = 6 offspring/sex evaluated from 6 litters, so assumed 1 pup/sex/litter examined, but this was NR	++ 4 hr/d exposures from GD 1–19	"Spontaneous mobility" protocol specifics not provided (e.g., blinding; manual vs. automated assessment of activity)	+ <i>Statistical methods NR</i>	Low [Test article assumed to be formalin; missing endpoint protocol details].
(Sorg et al., 1998)	+ <i>Chamber type not provided; declining</i>	+ N = 15–24; females only	+ <i>Imprecise timing of assessment & unclear effect</i>	<i>Experimenter blinding not indicated</i> (note: activity measures	++	Activity Medium

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
	<i>HCHO exposures across days</i>		of prior exposure to cocaine/handling on plus maze endpoints (assumed to be significant) Note: 1 or 4 wk exposures; single exposure level	automated); overall plus maze activity not provided; unclear impact of saline injection, handling; methods for measuring vertical activity NR in cited reference		[Blinding NR; limited methods description; unclear impact of prior manipulations] Plus maze <i>Low</i> [Blinding NR; limited methods description; overall activity NR; test order concerns]
(Sorg et al., 2001b)	+ <i>Chamber type and analytical concentrations not provided</i>	+ <i>N = 6; males only</i>	+ No EEG/EMG sham controls and influence of 37% formalin irritation responses NR; exposures ≤ 4 wk Note: single exposure level	No pre-formaldehyde sleep measures; sleep pattern methods NR Note: questionable adversity of endpoints	++	<i>Low</i> [limited methods reporting; pre-formaldehyde comparisons NR] Note: questionable adversity
(Usanmaz et al., 2002)	++	+ <i>Unexplained overt toxicity</i> (body weight decrease) with multiple exposures; <i>N = 6</i>	Observations immediately after exposure; acute (3 hr) or short-term (1–3 wk) exposures	Observations not blinded; 5 min test duration; peripheral vs. central square crossings not measured, limiting interpretability	++	<i>Low</i> [Tested immediately after exposure; lack of blinding]
Learning and Memory						
(Chonglei et al., 2012)	Mixture (formalin, benzene, toluene and xylene)/static chamber	+ <i>N = 5 males/group</i>	+ Testing 30 min after exposure; 2 hr/d exposure for short term (10 d)	Path length or similar NR (contribution of motor effects not tested); visual cues NR; no blinding indicated	++	Not informative [Mixture exposure; endpoint protocol deficiencies]
(Liao et al., 2010) (translation)	Formalin/static chamber	<i>N = 8</i> : pooled sexes (<i>N = 4/sex</i>); overt toxicity during exposure (e.g., listlessness; up to ≈30%)	Latency not provided (assumed that observations made immediately after exposure); no indication of	Path length or similar NR (contribution of motor effects not tested); pool temperature, pool	+ Data= combined sexes (test often displays sex differences)	Not informative [Formalin; overt toxicity; endpoint

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
		decreased body weight gain), most likely from deficient exposure quality, as only 0.5 mg/m ³ HCHO	correction for possible litter bias Note: exposures 2 hr/d for 28 d	<i>diameter, & platform size NR; recovery time between escape latency trials not indicated; no blinding indicated</i>		timing and protocol deficiencies]
(Liu et al., 2010)	Formalin (high concentration: methanol may drive effects)/ static chamber	+ <i>Males only</i> ; treatment groups determined by performance in preexposure probe trials, but <i>unclear exactly how groups were matched</i> ; Note: <i>N</i> = 8–11	+ <i>Latency for all assessed time points unclear</i> , but appears that most had ≥24 hr habituation period between exposure and training/testing; <i>exposures only 30 min twice daily</i> ; 28 d exposure	++ Note: probe trials preexposure were comparable; cued trials conducted to rule out HCHO effects on vision	++	<i>Not informative</i> [High formalin levels; static chamber]
(LICM, 2008)	Unspecified wood (possible co-exposures not tested)	+ <i>N</i> = 5; <i>males only</i>	Training behaviors assessed 30 min postexposure and possible indirect effects of irritation on training may influence performance in the probe trial test; 7 d exposure	+ <i>Path length or similar NR</i> (contribution of motor effects not tested); <i>no blinding indicated</i>	+ <i>Comparisons across treatment groups NR for probe trial test</i>	<i>Low</i> [Likely mixture exposure; possible impact of irritation]
(Mei et al., 2016)	Formalin	+ <i>N</i> = 8; <i>males only</i>	+ <i>No comparisons to chamber or air exposure alone</i> ; testing 3 hr after exposure during training; Note: 8 hr/d for 7 consecutive d	<i>Path length or similar NR</i> (contribution of motor effects not tested); <i>pool temperature, pool diameter, start positions & platform size NR</i> ; <i>no blinding indicated (of concern, as not automated</i> ; note: cited references did not contain these details)	++	<i>Low</i> [formalin; endpoint protocol reporting deficiencies; lack of blinding]
(Malek et al., 2003c)	Formalin/static chamber	++ <i>N</i> = 15/sex/group; no changes in body weight were observed	+ <i>Latency 2 hr postexposure</i> ; <i>exposures for 2 hr/d for 10 d</i>	Motor effects appear to drive some responses & were not tested (<i>path length or similar NR</i>);	+ <i>No ANOVA or trend tests performed</i>	<i>Low</i> [Formalin; endpoint protocol deficiencies; no blinding]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
				<i>possible influence of changes in olfaction and/or vision not tested; blinding not indicated</i>	across the 4 groups (only pair-wise tests)	
(Pitten et al., 2000)	Formalin/static chamber	+ N = 5–8 Note: no changes in body weight were observed	+ 22 hr postexposure; exposures only 10 min/d Note: 90 d exposure	+ Possible influence of changes in olfaction and/or vision not tested; path length or similar NR	+ Data= combined sexes (test often displays sex differences)	Low [Formalin]
(Wang et al., 2014a)	Mixture (formalin, benzene, toluene and xylene)/static chamber	+ N = 6 males/group Note: no changes in body weight were observed	+ Testing 30 min after exposure; Note: 2 hr/d exposure for 49–90 d	Path length or similar NR (contribution of motor effects not tested); visual cues NR; no blinding indicated	++	Not informative [Mixture exposure; endpoint protocol deficiencies]
Nociception						
(Sorg et al., 1998)	+ Chamber type NR; declining HCHO exposures across days	+ N= 15–24; females only	Imprecise timing of assessment following exposure ; unclear if cocaine or saline challenged Note: single exposure level; 1 or 4 wk exposures	+ Experimenter blinding not indicated	++	Medium [Unclear exposure to testing latency]
Functional Observational Battery or Grip Strength						
(Chonglei et al., 2012)	Mixture (formalin, benzene, toluene and xylene)/static chamber	+ N = 5 males/group	+ Unclear exposure to testing latency; 2 hr/d exposure for short term (10 d)	No description of grip strength protocol provided	++	Not informative [Mixture exposure; endpoint protocol NR]
(Tepper et al., 1995)	Carpet emission exposures: formaldehyde not primary exposure (BHT, toluene, etc.)	N = 2 (nonexposed controls) or 4; males only	Behaviors tested immediately after exposure	++	Quantitative data NR for the majority of measures; some measures presented as compared to preexposure or	Not informative [Mixture exposure; small sample; likely confounding; selective reporting]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
					summarized qualitatively	
(Wang et al., 2014a)	Mixture (formalin, benzene, toluene and xylene)/static chamber	+ N = 6 males/group Note: no changes in body weight were observed	+ Unclear exposure to testing latency; Note: 2 hr/d for 49–90 d	+ No blinding indicated; Note: 5 s inter-trial delay and 3 trials/d	++	Not informative [Mixture exposure]
<i>Electrophysiology (for Hazard; see below for MOA)</i>						
(Bokina et al., 1976)	Details of exposure were not provided	Details on test subjects were not provided	Details of study design were not provided	Details of endpoint measures were not provided	No quantitative comparisons to controls were performed	Not informative [Experimental details NR]
(Katsnelson et al., 2013)	Test article not defined (assumed to be formalin; high concentration: methanol may drive effects)	+ N = 12–15/group; females only	+ Testing indicated as immediately after exposure: unclear if RB-related effects could affect these impulses Note: subchronic (10 wk) exposure	++ Note: Citation for temporal summation of impulses protocol was provided	++	Not informative [High levels of test article assumed to be formalin]
<i>Autonomic Effects (for Hazard; see below for usefulness for MOA)</i>						
(Nalivaiko et al., 2003)	Unregulated exposure without reporting of levels; no chamber Note: paraformaldehyde	+ N = 6–13; males only	No nonexposed groups indicated (internal comparisons); acute exposure; All animals implanted with electrodes (duration before tests NR)	+ ECG implantation procedures NR Note: endpoint not considered adverse	++	Not informative [Exposure levels NR and unregulated; missing controls]
(Tani et al., 1986)	Formalin (high concentration: methanol may drive responses)	+ N = 4–5; males only	No nonexposed groups indicated (internal comparisons); acute exposure; all animals received anesthesia, surgery, and anticoagulants (no recovery before exposure)	Blocker experiments may be influenced by prior exposure to formaldehyde Note: endpoint not considered adverse	+ Effects of blocker experiments without prior HCHO exposure NR	Not informative [High formalin levels; missing controls; test order concerns]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence Classification
(Yu and Blessing, 1997)	Formalin (likely high concentration- not quantified; methanol may drive responses); HCHO concentrations NR	+ <i>N = 5–16; males only</i>	No nonexposed groups indicated (internal comparisons); <i>acute exposure</i> ; all animals received surgery, anesthesia, and catheterization 1 wk prior to exposure	++ Note: Endpoint not adverse	+ Data were pooled across groups for some measures Note: all comparisons to preexposure measures	<i>Not informative</i> [Formalin levels NR; missing controls]
(Yu and Blessing, 1999)	Test article not defined (assumed to be formalin); levels not quantified (likely high: methanol may drive responses)	+ <i>N = 4; males only</i>	No nonexposed groups indicated (internal comparisons); other alerting & noxious stimuli administered pre-HCHO; 2 surgeries- <i>only 1 d recovery after cannulation before exposure; acute exposure</i>	++ Note: Endpoint not adverse	+ Justification for selection of resting periods used for comparison unclear; <i>data qualitative only</i>	<i>Not informative</i> [Test article assumed to be formalin; exposure levels NR; missing controls]

NR = not reported; N/A = not applicable.

* Three studies examined an endpoint that is not adverse and has no MOA relevance. These are briefly mentioned in the assessment, as they inform the irritant/odorant threshold of rodents, but these studies were not used to characterize the potential neurotoxicity hazard.

** Five animal studies sufficient for hazard characterization were not categorized using confidence ratings, and they are not included in the exposure-response array, as they represent cursory observations with none or minimal data reporting; however, these studies were used to help describe the potential neurotoxicity hazard.

^a Although some notes on exposure levels are included, considerations and interpretations related to the tested exposure levels are discussed as part of the evidence synthesis in the Toxicological Review, with one exception for tracking “high exposures” when co-exposure to methanol is expected to be a driver of observed responses. For details, see the draft Methanol Toxicological Review (http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=233771), which proposes an RfC of ≈ 2 mg/m³. Assuming methanol is present in the breathing zone somewhere in the range of 1/10–1/3 the levels of formaldehyde when stabilized formalin solutions are used as the test article (determination of the exact ratio of exposure is not currently available), exposures > 10 mg/m³ are interpreted as “high exposure” and assumed to have at least some methanol-driven effects.

^b Kerns is a report of a GLP study by CIIT ([Battelle, 1982](#)), which was not identified in the literature search [Note: use of GLP or guideline study protocols is provided to identify the most stringent studies but did not factor into the confidence ratings or sufficiency evaluations for this database].

^c Communication with the study author detailed those male rats (2 per litter from 3 separate dams per dose group) were used in the ([Sarsilmaz et al., 2007](#)) study. A review from this same laboratory ([Songur et al., 2010](#)) indicated that the stereological studies of the hippocampus were conducted to confirm previous observations ([Songur et al., 2003](#)); thus, the separate reports of stereological changes in the CA and DG regions of the hippocampus ([Sarsilmaz et al., 2007](#)) and ([Aslan et al., 2006](#)), respectively) are assumed to represent the same cohort of animals (note: it is possible that these two stereological studies report effects on a subset of the same animals used in the ([Songur et al., 2003](#)) study, but this inference is less clear and is not assumed).

d Note: although pup body weight changes would be of concern as potential confounders for behavioral analyses, endpoints such as neuropathology and brain weight are unlikely to be secondary to these changes: at least for brain weight, the current literature does not support a consistent causal relationship. In (Songur et al., 2003), body weight decreases were $\approx 10\%$ and 20% at 30 d (low and high formaldehyde concentrations, respectively) & $\approx 10\%$ at 60 d (high concentration only).

e Because data for exposure groups other than 6.15 mg/m^3 were not reported by (Boja et al., 1985), the higher exposure groups were not included in the study quality analysis or the Toxicological Review hazard ID synthesis.

Mechanistic Studies Related to Nervous System Effects

This review constrained the mechanistic studies considered relevant to the potential nervous system effects of inhaled formaldehyde to experimental studies of inhalation exposure (see Table B-52). Thus, the methods and documentation applied paralleled those described above, with a few exceptions in application (i.e., acute exposure duration and the ability to infer the endpoint as adverse were not considered limitations, and within endpoint evaluation consideration was given to whether the endpoint evaluates a mechanism relevant to humans, although mechanisms are considered relevant to humans in the absence of evidence to the contrary).

Table B-52. Evaluation of studies pertaining to mechanistic events associated with nervous system effects

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	MOA Confidence Classification
(Ahmed et al., 2007)	++	+ <i>N = 4–5; females only</i>	Lack of OVA-free controls: inability to separate effects of OVA & formaldehyde; possible altered distribution/effectiveness of aerosolized OVA given after formaldehyde; Note: 12 wk exposure; single exposure level	++	++	Medium [Control group deficiencies]
(Bian et al., 2012)	Formalin (high concentration: methanol may drive effects)	<i>N = 3/ endpoint /timepoint (males); mild toxicity: decreased food intake (effect not quantified)</i>	Controls not air-exposed in exposure chamber; all groups had anesthesia & antibiotic injections Note: exposure 1 hr/d for 90 d; single exposure level	++	++	Not informative [High formalin levels; Small N; missing control]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	MOA Confidence Classification
(Boja et al., 1985)	+ Analytical concentrations NR	+ N = 8; males only; data from experiments with N = 1 (air-HCHO NE & DA levels) not included in the assessment	+ Timing of exposures (9–12 pm vs. 12–3 pm) may have varied across groups Note: single exposure level; acute exposure: 3 hr/d for 1–2 d	+ Molecular verification of regional "punches" not performed	+ Higher exposure groups data NR; inability to evaluate findings for exposures indicated as tested but NR	Medium [Selective reporting; some methods detail NR]
(Bokina et al., 1976)	Details of exposure were not provided	Details on test subjects were not provided	Details of study design were not provided Note: continuous exposure for 45d	Details of endpoint measures were not provided	No quantitative comparisons to controls were performed	Not informative [Experimental details NR]
(Fujimaki et al., 2004b)	+ Analytical concentrations NR	+ N = 5–6; females only; unclear influence of splenic effects (e.g., decreased weight)	+ For OVA groups: unclear if prior formaldehyde exposure had nasal effects influencing inhaled OVA booster distribution/effects; Note: 12 wk exposure	+ Methods for ELISA of plasma NR: assumed to be same as BAL fluid ELISA	++	Medium [Control group deficiencies; some methods detail NR]
(Fujimaki et al., 2004a)	+ Analytical concentrations NR	+ ELISA data: N = 5; males only RT-PCR data: N = 3; (considered major limitation)	+ for OVA groups: unclear if prior formaldehyde exposure had nasal effects influencing inhaled OVA booster distribution/effects; 12 wk exposure	Methods for brain dissection & homogenization, as well as gel quantification NR; ELISA and booster challenge methods NR	++	ELISA Medium RT-PCR Low [Control group deficiencies; small sample size; some methods detail NR]
(Gieroba et al., 1994)	Formalin (likely high concentration- not quantified:	N = 2 or 6	Unclear contribution of apnea & bradycardia; results may be specific to exposure combined with	+ Number of sections analyzed/animal NR	Immunostaining results were not quantified across groups; results are	Not informative [High formalin levels; small N; possible confounding]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	MOA Confidence Classification
	methanol may drive response)		restraint & anesthesia; strong irritation induced		qualitative only; TH ⁺ cell counts alone NR	
(Hayashi et al., 2004)	++	+ <i>N = 5; females only</i>	++ Exposures up to 12 wk	+ <i>Possible mild sampling bias</i> (3 sections, but selection methods NR); blinding indicated	++	<i>Medium</i> [Small N; limited sampling]
(Kimura et al., 2010)	Formalin	<i>N = 5–6; males only; systemic toxicity not evaluated (HCHO tested up to ~55 mg/m³)</i>	+ <i>Irritation-related effects probable, as tested near-simultaneous with exposures; acute exposure; unclear if anesthesia/dye injection influenced sensory nerve responses</i>	+ <i>Blinding not indicated</i> for cell type counts	++	<i>Low</i> [Formalin; possible overt toxicity; small N]
(Kulle and Cooper, 1975)	+ Analytical concentrations NR	<i>N = 3; males only; no air-only controls</i>	+ All animals underwent surgery prior to exposure (<i>no recovery prior to exposure</i>); <i>some exposures were complicated by amyl alcohol co-exposure; acute exposure</i>	++ Note: unclear relevance of these surgical preparations to human nerve responses	No quantitative comparisons to controls performed (extrapolated threshold only)	<i>Low</i> [small sample size; comparison group deficiencies]
(Chonglei et al., 2012)	Mixture (formalin, benzene, toluene, and xylene)/static chamber	+ <i>N = 5 males/group</i>	++ 2 hr/d exposure for short term (10 d)	No description of hippocampal MDA and GSH protocols provided	++	<i>Not informative</i> [Mixture exposure; protocols NR]
(Li et al., 2016)	Formalin; static chambers	+	++	+	++	<i>Low</i> [Formalin]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	MOA Confidence Classification
		<i>N</i> = 7 (inferred); <i>males only</i>	2 hr/d exposure for short term (7 d)	<i>Some sampling bias possible: 3 sections</i> Note: although not corrected for neuron number, location determined from atlas; slides were randomized and coded for blinded evaluation		
(Liao et al., 2010) (translation)	Formalin/static chamber	<i>N</i> = 8: pooled sexes (<i>N</i> = 4/sex); overt toxicity during exposure (e.g., listlessness; up to ≈30% decreased body weight gain), most likely from deficient exposure quality, as only 0.5 mg/m ³ HCHO	+ <i>No indication of correction for possible litter bias;</i> Note: 2 hr/d for 28 d	Potential sampling bias: <i>N</i> = 5 fields (assumed to be per animal), but number of slides not indicated (DAB amplification used) & no correction made to account for the number of neurons visible/field	+ <i>Data= combined sexes; CA3 cell number or viability measures NR</i>	<i>Not informative</i> [Formalin; endpoint protocol deficiencies; overt toxicity]
(Liu et al., 2009a)	Formalin (high concentration: methanol may drive effects)/static chamber	+ <i>N</i> = 5; <i>males only</i>	++ 28 d exposures	++	++	<i>Not informative</i> [High formalin levels]
(Liu et al., 2010)	Formalin (high concentration: methanol may drive	+ <i>N</i> =5; <i>males only</i> ; treatment groups determined by preexposure probe	++ 28 d exposures	Methods for quantification of western blots NR	++	<i>Not informative</i> [High formalin levels; protocols NR]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	MOA Confidence Classification
	effects)/static chamber	trial performance, but method for matching groups NR				
(LICM, 2008)	Unspecified wood	+ Sample sizes for MOA-related endpoints were NR, but assumed to be <i>N = 5; males only</i>	++ 7 d exposures	Regional brain dissections were nonspecific & methods incompletely described; RT-PCR analyses were semi-quantitative only	++	Low [Possible mixture exposure; endpoint protocol description insufficient]
(Matsuoka et al., 2010)	Formalin	+ <i>N = 7–9; males only</i>	+ <i>Did not appear that controls were air-exposed in chambers ("noninhalation controls"); acute exposure</i>	Methods for brain dissection/regions analyzed NR; assumed brain region-specific analyses were not conducted	+ High variability in measures, possibly due to lack of regional specificity	Low [Formalin; endpoint protocol description insufficient]
(Mei et al., 2016)	Formalin	+ <i>N = 8; males only</i>	+ <i>No comparisons to chamber or air exposure alone; 8 hr/d for 7 consecutive d</i>	No blinding for biochemical measures; no regional specificity (homogenates)	++	Low [formalin; some endpoint protocol limitations]
(Nalivaiko et al., 2003)	Unregulated exposure without reporting of levels; no chamber Note: paraformaldehyde	+ <i>N = 6–13; males only</i>	+ No nonexposed groups indicated (internal comparisons); all animals were implanted with electrodes, but duration prior to testing not provided; acute exposure	+ ECG implantation procedures NR	++	Not informative [Exposure levels NR and unregulated; etc.]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	MOA Confidence Classification
(Ozen et al., 2003b)	+ <i>Analytical concentrations NR</i>	Unclear contribution of unexplained overt toxicity (robust effects on body weight); males only; <i>N</i> = 7	++ 4 wk or 13 wk exposures	Methods for analyses of brain tissue were not clearly described, even in cited reference	++	<i>Not informative</i> [Overt toxicity; endpoint protocol description insufficient]
(Sari et al., 2004)	++	+ <i>N</i> = 5/endpoint; females only	++ 12 wk exposure	Cell counts were not reported as observer blinded, but were from serial sections; RT-PCR analyses were semi-quantitative only	++	Medium [possible experimenter bias- no blinding]
(Sari et al., 2005)	++	+ <i>N</i> = 5; females only	Nasal instillation of toluene may affect formaldehyde distribution	Cell counts were not reported as observer blinded, but were from serial sections	Data for exposures without toluene NR Note: 2004 paper data cited was not considered	<i>Not informative</i> [Data on formaldehyde exposure alone NR; no blinding; exposure paradigm concerns]
(Songur et al., 2003)	+ <i>Analytical concentrations NR</i>	<i>N</i> = 6 (assumed 3 litters); mild toxicity (body weight & food/water intake changes): HSP activation may be indirectly related to health/nutrition	+ <i>Litter assignments NR; unclear if litter bias corrected; 30 d of exposure</i>	Potential sampling bias: details on blinding, slides/animal, etc. not provided; nonblinded intensity ratings subject to observer bias	+ <i>No statistical comparisons for HSP staining</i>	Low [small sample size; possible litter and/or sampling bias]
(Songur et al., 2008)	++	Dam health during lactation & pup	+	++	++	Medium

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	MOA Confidence Classification
		health not presented; sex and litters/group unknown (likely males & 3 litters); body weights were indicated as measured, but NR; N = 7 pups	Unclear if litter bias corrected (& not indicated as randomized); dams exposed from PND 1-14; 30 d of exposure			[Small sample size; possibly litter effects]
(Sorg et al., 2001a)	++	+ N = 6–10; males only	+ Possible difference in harvest day (20 vs. 21) across groups may contribute to high variability noted in results; exposures ≤4 wk	+ Volume of trunk blood/animal and some other details (e.g., serum isolation) NR Note: chamber exposure itself (tested) had a large influence, so critical to rapidly remove rats after exposure (as indicated)	++ Note: sensitive endpoint, so high level of variability is as expected	Medium [Small sample size; some protocol details unclear]
(Sorg et al., 2002)	Formalin (likely high concentration; not quantified: methanol may drive response)	+ N = 6–12	Formalin used as an aversive stimulus- results more specific to cocaine; acute exposure to concentrated vapors	Tests involve odor detection & irritation-specific responses could be confounding results	+ Specific effects of formaldehyde alone not tested or NR	Not informative [Formalin (assumed high level) levels NR]
(Tani et al., 1986)	Formalin (high concentration: methanol may drive responses)	+ N = 4–5; males only	+ No nonexposed groups indicated (internal comparisons); animals received anesthesia,	+ Blocker experiments may be influenced by prior exposure to	++	Not informative [High formalin levels]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	MOA Confidence Classification
			<i>surgery, and drugs with no recovery before exposure; acute exposure</i>	<i>formaldehyde (not tested)</i>		
(Tsukahara et al., 2006)	++	+ <i>Females only; Western Blot data: N ≥ 6; Caspase data: N = 3; (considered major limitation)</i>	+ <i>For OVA groups: unclear if prior formaldehyde exposure had nasal effects influencing inhaled OVA booster distribution/effects; 60 d exposure</i>	++ <i>(for Western Blot data) Caspase data: likely sampling bias: number of slides/animal & neurons visible/field NR; counts were not reported as observer blinded</i>	++	Western blot <i>Medium</i> [small N; possible minor confounding] Caspase <i>Low</i> [Caspase data: small sample size; likely sampling bias]
(Wang et al., 2014a)	Mixture (formalin, benzene, toluene, and xylene)/static chamber	+ <i>N = 6–12; males only</i> Note: no changes in body weight were observed	++ 2 hr/d exposure for subchronic (90 d); tested 1 d postexposure	No description of grip strength protocol provided	++	<i>Not informative</i> [Mixture exposure; endpoint protocol NR]
(Yu and Blessing, 1997)	Formalin (likely high concentration; not quantified; methanol may drive responses)	+ <i>N = 5–16; males only</i>	Animals received surgery, anesthesia, & catheterization 1 wk prior to exposures; no nonexposed groups indicated (internal comparisons); acute exposure	++	+ <i>Data was pooled across groups for some measures. Note: all comparisons to preexposure measures</i>	<i>Not informative</i> [Formalin (assumed high level) levels NR; missing controls]
(Yu and Blessing, 1999)	Test article not defined (assumed to be formalin); levels not quantified (likely	+ <i>N = 4; males only</i>	No nonexposed groups indicated (internal comparisons); other alerting & noxious stimuli administered pre-HCHO;	++	+ Justification for selection of resting periods used for	<i>Not informative</i> [Unknown test article (assumed to be formalin) levels NR]

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	MOA Confidence Classification
	high: methanol may drive responses)		2 surgeries; only 1 d recovery after cannulation before exposure; acute exposure		comparison unclear; data qualitative only	(assumed high level); missing controls]
(Zitting et al., 1982)	Test article results in co-exposures to formic acid, acrolein, & possibly other chemicals	+ N = 4–5; males only	Formaldehyde levels >> 100 mg/m ³ are overtly toxic (rats gasped for air for hours after exposure); 6 hr or 3 d exposure	+ Evaluations are not brain-region-specific	+ Details on statistics NR (e.g., "Student's t test")	Not informative [Unknown test article (assumed to be formalin) at high level; overt toxicity]

^aMode-of-action study quality evaluations were conducted in a similar fashion as those described above for hazard identification, with minor adjustments to the types of experimental details considered for meeting sufficiency criteria (e.g., adversity of the endpoint was not considered).

^bA mechanism or mode of action is considered relevant to humans unless there is convincing evidence to the contrary.

B.3.8. Developmental and Reproductive Effects

Details of the study evaluation approach methods are provided in Section 2.3.8 of the Toxicological Review. As discussed in that section, these reviews focused on human epidemiology and experimental animal studies. Within Tables B-53 (epidemiology studies in residential and occupational settings) and Table B-54 (animal studies) below documenting the results of the study evaluations for developmental and reproductive effects, studies are arranged alphabetically. Gray shading is indicative of aspects of the study design or analysis that decreased EPA's confidence rating.

Epidemiology Studies

Table B-53. Evaluation of observational epidemiology studies of formaldehyde - reproductive and developmental outcomes

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Residential Studies							

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Amiri and Turner-Henson (2017) (Southeastern United States) Cross sectional study	Pregnant women in 2 nd trimester (convenience sample, n = 140) recruited from obstetrics and gynecology clinics with no history of chronic disease or high-risk pregnancy, 19–40 years old, Participation 63% (n = 88). Lower participation rate.	Participants wore vapor monitor badges, 24-hr period, detection limit 0.003 ppm. Mean (SD) 0.04 (0.06) ppm = 0.049 (0.074) mg/m ³ . This is a measure of total exposure from indoors and ambient air.	Ultrasonographic biometry during 2 nd trimester for head circumference, abdominal circumference, femur length, biparietal diameter, estimated fetal weight, and ratio of abdominal circumference to femur length. Measurements in mm converted to percentiles using gestational age and the Hadlock formulas. Sensitivity and specificity for IUGR are 67% and 93% for BPD, 42% and 100% for HC, 94% and 100% for AC and 46% and 90% for AC/FL ratio. Hadlock formulas are based on a sample of White women in the US with uncertain accuracy for other races. Over 50% of the participants were not White.	Urine cotinine adjusted for urinary creatinine (spot sample, methods and timing of collection were not described). Models adjusted for maternal demographics, obstetric history, and cotinine. Biometry measurements were not correlated with maternal age, education, marital status, yearly family income or employment status. No correlation with gravida, maternal smoking, or pregnancy intervals. BPD was lower among whites compared to African-Americans or other category. BPD and FL varied by sex.	Multiple linear regression for formaldehyde as dichotomous variable (cutoff at 0.03 ppm) adjusted for maternal age, fetal sex, and race. Mediation of tobacco smoke (urinary cotinine) on associations examined.	N = 88	Ultrasonographic biometry measurements <i>Low</i> Convenience sample, sampling frame not described. Lower participation rate. Small sample size. Reference population for BPD measure was not appropriate for >50% of participants.
Chang et al. (2017) (Birth cohort) South Korea	Pregnant women were selected from cohort (n = 383), originally	Personal formaldehyde measurements during mid- or late	Age-specific weights by gender using growth standard for Korean children.	Prenatal variables from questionnaire and medical records; postnatal via	Analyzed birth weight adjusted for maternal age, pre-	N = 360 singleton newborns	Birth weight; mean difference in weight at 6, 12, 24, and 36 months <i>Medium</i>

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Mother and Childrens Environmental Health Study	recruited from hospital; information on demographics and housing characteristics via questionnaire. Infants followed at 6 (n=262), 12 (n=234), 24 (n=199), and 36 months (n=92). Notable attrition from study.	pregnancy, 3 d. Categorized into two groups below and above the 75 th percentile and also continuous with log transformation. Mean (SD) 0.082 (0.052) mg/m ³ , geometric mean 0.067, 75 th percentile 0.106 mg/m ³ . Correlation between TVOCs and formaldehyde 0.22, <i>p</i> <0.01.		questionnaire and interview. ETS slightly higher in low formaldehyde group but was not associated with weight.	pregnancy body mass index, education level, parity, gender, gestational age at birth and residential factors. Analyzed postnatal weight at each visit using multiple linear mixed models adjusted for gender, birth order, breastfeeding and education.		Hospital-based cohort, notable attrition over time
Franklin et al. (2019) (Australia) Birth cohort	Pregnant women, all nonsmokers, recruited prior to 18 wks gestation. Recruited 373 women, 305 (81.7%) participated; 4 excluded because of smoking. Birth data available for 262 live births (70.2% of recruited).	Air monitoring in homes at 34 wks gestation, 7-d sampling duration using validated passive samplers in bedroom and living room. LOD 2.4 µg/m ³ ; used LOD/2 for values < LOD. House average Median (range) 2.81 (LOD – 17.33) µg/m ³ ; 23.3% < LOD. Uncertainties in exposure distribution due to proportion < LOD.	Gestational age, birth weight, birth length and head circumference from birth records.	Confounders were selected based on previous literature. Adjusted for maternal age, parity, maternal asthma, diabetes and blood pressure, season of birth. Distance from main road and ETS exposure were evaluated as potential confounders in models. Adjusted and unadjusted results presented.	Gestational age was normally distributed. Birth weight, birth length and head circumference were transformed to z-scores (accounting for sex and gestational age). General linear models.	N = 262	Gestational age, birth weight, birth length, head circumference <i>Medium</i> Uncertainties in exposure distribution due to proportion < LOD, and analysis as continuous variable.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
Occupational Studies							
(Axelsson et al., 1984) (case-cohort) laboratory work	University laboratory workers identified via payroll (born 1935 and after, worked in lab 1968–1979); 95% response; birth register records compared for respondents and nonrespondents.	Self-report (Y/N) during 1st trimester, open question; likely exposure misclassification, no information on intensity or frequency of exposure	Spontaneous abortion & birth defects; self-report & birth registry, 1968–1979. Spontaneous abortion verified using hospital records or via recall.	Miscarriage rate not associated with smoking before or during pregnancy (raises uncertainty about data quality); inverse association of solvent exposure with pregnancy number, age, and work shift	Unadjusted analyses for formaldehyde	Only N = 10 exposed pregnancies; potentially unstable risk estimates	Spontaneous abortion Birth defects <i>Not Informative</i> Open-ended question unreliable for exposure classification; uncertainty regarding data quality: miscarriage rate higher in nonresponders and not associated with smoking
(Ericson et al., 1984) (nested case control)	Controls (2 per case) selected from other infants in registry born in 1976 of laboratory worker; 50% of cases and 20% of controls responded about exposure	Lab work identified by occupational code in 1975 census; self-report on work during pregnancy & exposure to agents (open question); potential misclassification; no information on intensity or frequency of exposure	Perinatal deaths (< 7 d) & birth defects; National Birth Register, 1976	Controls selected randomly within same age (5-year categories) and parity stratum as case. No information on smoking or other risk factors.	Unadjusted analyses for formaldehyde	N = 3 exposed cases	Perinatal deaths Birth defects <i>Not Informative</i> Open-ended question unreliable for exposure classification; low response regarding exposure; very few exposed cases
(Hemminki et al., 1982) (cohort) hospital staff	Recruited from nursing staff working in sterilizing units (exposed to sterilizing agents, x-rays, or anesthetic gases) or auxiliary units (referent) in all	Exposure (Y/N) at beginning of pregnancy to specific agents assigned by supervising nurse, blind to case status, possible exposure misclassification, particularly for earlier years. No information	Spontaneous abortion: self report on pregnancies, 1951–1981; questionnaire & hospital discharge register	Regression adjusted for several risk factors and presented risk estimates for other sterilants (ethylene oxide, glutaraldehyde). Formaldehyde results not adjusted for other sterilants.	Binary logistic regression for exposure (yes/no) adjusted for age, parity, decade of pregnancy, smoking habits, alcohol, and	N = 50 exposed pregnancies (6 spontaneous abortions); 1,100 unexposed pregnancies (121	Spontaneous abortion <i>Low</i> Assumed sterilant use was same throughout period; no information on intensity and frequency of formaldehyde exposure (exposure misclassification—decreased sensitivity); no

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	general hospitals; Response > 90% for both exposed and referent; recall likely not related to exposure	on intensity and frequency.			coffee consumption	spontaneous abortions)	adjustment for other sterilants; adjustment for parity may introduce bias; small number exposed cases
Hemminki et al. (1985) (case control) nursing staff	Birth outcomes from national discharge register linked to national occupational register. Occupation identified for > 87% of exposed and referent. Selected hospital nurses.	Occupation during 1st trimester identified by head nurses at all general hospitals in Finland plus exposure (Y/N) to listed substances (used sterilizing agent or sterilized instruments; formaldehyde included in list); potential exposure misclassification; no information on intensity or frequency.	Spontaneous abortion & birth defects, 1973–1979; hospital discharge register linked to personnel register	Referent with healthy birth selected from same hospital as cases; matched on age; not adjusted for other risk factors or other workplace exposures	Conditional logistic regression. Unadjusted OR presented for FA; no statistical tests	N = 6 exposed cases for spontaneous abortion 3 exposed cases for birth defects	Spontaneous abortion and birth defects <i>Low</i> No information on intensity or frequency (exposure misclassification—decreased sensitivity); very small number of exposed cases
John et al. (1994) (case control) cosmetologists	Recruited from license registry (currently and formerly employed), 74% with eligible pregnancy, data obtained for 71.5% of cases, 74% live births; restricted analysis to full-time	Self-report; response to closed list (Y/N & frequency of use), no ambient measurements; relevant exposure period: 1st trimester; pregnancies while full-time cosmetologist	Spontaneous abortion, 1983 – 1988, most recent pregnancy (decreased sensitivity because of time-lapse bias). Self-report verified by positive pregnancy test or medical care	Regression adjusting for several risk factors plus other work exposures among full-time cosmetologists	Adjusted OR, 95% CI, unconditional logistic regression adjusting for previous pregnancy loss, mother's age at conception, & mother's cigarette	N = 67 cases, 351 controls	Spontaneous abortion <i>Medium</i> Selection of most recent eligible pregnancy (decreased sensitivity); no ambient measurements but exposure assessment was specific to formaldehyde and frequency of use; adjustment for previous

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	workers during 1 st trimester.				smoking during 1 st trimester		pregnancy loss may introduce bias
Lindbohm et al. (1991) (registry linkage) paternal occupation	Identified all pregnancies between 1/1/76–12/31/77 and 5/1/80–4/30/82, excluded maternal age < 12 and > 50 year and missing data on occupation, industry or SES	Industry/occupation code based on national census; assignments by industrial hygienist (IH) using database on chemical exposures and concentrations; potential misclassification into low and mod/hi, and exposure window during spermatogenesis for paternal exposure	Spontaneous abortion identified in hospital discharge register that occurred during a 2-year period close to census	Adjusted for age, SES, & maternal exposure	Linear logistic regression adjusted for age, SES, and maternal exposure to reproductive hazards; risk odds ratio comparing exposed to unexposed	N = 7,772 unexposed SA, 820 potential low, 139 moderate/high	Spontaneous abortion <i>Low</i> Industry/occupation coding has low specificity; potential exposure misclassification and imprecise assignment of exposure period to period of spermatogenesis relevant to identified pregnancy
Saurel-Cubizolles et al. (1993) (cohort, retrospective) operating room nurses	Recruited operating room nurses at 18 hospitals (exposed) and randomly from nurses in other departments from same hospital (unexposed); data collection in both groups conducted the same	Self-reported exposure (Y/N) to anesthetics, formol, & ionizing radiation during 1st trimester. No information on intensity and frequency. Potential for exposure misclassification.	Ectopic pregnancy: self-report by interview. Interviewed 1987–1988	Exposed and referent matched for age, duration of service, sex, occupation, and hospital. Formol exposure associated with exposure to anesthetics. No info on pelvic inflammatory disease but association with formaldehyde not likely.	Chi-square analysis for formol; no multivariate analyses	N = 15 ectopic pregnancies of 734 pregnancies; 1 exposed case	Ectopic pregnancy <i>Low</i> Small sample size and unadjusted analyses. No information on intensity and frequency of formaldehyde exposure (exposure misclassification—decreased sensitivity)
Saurel-Cubizolles et al. (1994) (cohort, retrospective)	Recruited operating room nurses at 18 hospitals (exposed) and	Self-reported exposure (Y/N) to anesthetics, formol, & ionizing radiation during 1st trimester.	Spontaneous abortion and birth defects (malformations ICD-9): self-report by questionnaire. First	Exposed and referent matched for age, duration of service, sex, occupation, and hospital.	Chi-square analysis for formol; no multivariate analyses	N = 72 spontaneous abortions (9.4%);	Spontaneous abortion and birth defects <i>Not Informative</i> No information on intensity and frequency of

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
operating room nurses	randomly from nurses in other departments from same hospital (unexposed); data collection in both groups conducted the same	No information on intensity and frequency. Potential for exposure misclassification.	pregnancy in or after 1970; interviewed 1987–1988	Formol exposure associated with exposure to anesthetics		22 pregnancies with birth defects (3.4%); 14 major malformations (2.2%)	formaldehyde exposure (exposure misclassification—decreased sensitivity). Possible confounding by other exposures and no adjustment (stronger associations observed for spontaneous abortion and anesthetics and ionizing radiation, but not all birth defects); no consideration of impact of gravidity on risk
Shumilina (1975) (cross sectional) cotton textile workers	Unable to assess; selection & response rate not reported	Range reported; sampling protocol not described; analyzed categories of textile finishers and sorted compared to saleswomen	Reproductive & pregnancy history including LBW. Gynecological exam and self-report; methods NR	Job demands among textile workers and referent (sales women) were different; shift work with standing and elevated ambient temperature for exposed	Prevalence & SD; incomplete		Reproductive disorders, and complications of pregnancy, low birth weight <i>Not informative</i> Reporting deficiencies; potential confounding by conditions in the workplace
Steele and Wilkins (1996) (cohort, retrospective) veterinarians	Recruited from graduation rolls; 85% of eligible graduates. Graduated 1970–1980; survey 1987	Self-reported exposure (Y/N) to specific agents for specific jobs, defined exposed pregnancy if estimated time of conception occurred during years of job where exposure also was reported. 81% reported exposure to formaldehyde; no	Spontaneous abortion occurring for pregnancy started after graduation from veterinary college, < 20-wk gestation, self-reported	Compared exposed pregnancies to employed women who reported no exposure to formaldehyde or not employed during pregnancy. Adjusted for other risk factors, but not other workplace exposures	Unconditional logistic regression adjusting for maternal age, gravidity, previous SA, alcohol, and smoking. Also evaluated height, previous stillbirth, and	1,757 exposed pregnancies, 482 not exposed	Spontaneous abortion <i>Low</i> No information on intensity and frequency of formaldehyde exposure which would likely be variable among veterinarians (exposure misclassification—decreased sensitivity). Adjustment for gravidity and previous spontaneous

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		information on intensity or frequency of exposure.			previous induced abortions.		abortion may introduce bias.
Seitz and Baron (1990) NIOSH Health Hazard Investigation (retrospective cohort) clothing manufacturer	Response: 98% of current employees, 18% of former employees employed 1984 or after. Possible survivor bias. Potential for selection bias for comparisons with pregnancy outcomes while at home (away from null); not a concern for comparisons with employment at other locations during pregnancy.	Air sampling 1987, full shift personal breathing zone for 5 task areas, 14 area samples full shift in several locations; perhaps not representative of earlier years; exposure range: TWA 0.17-0.57 mg/m ³ ; job status when pregnancy occurred.	Self-report, questionnaire, pregnancy while working at plant compared to employment at other locations or at home; miscarriage (not defined), birth outcomes, self-report (questionnaire). Former workers sent questionnaire in 1984.	Authors stated no differences among groups for other risk factors including smoking, alcohol, use of medications, and presence of diseases (diabetes)	Compared miscarriage and pregnancy outcomes by employment status when pregnancy occurred (employed at Rockcastle or other) or at home. RR (95% CI), Fisher's exact test	Pregnancies among current: N = 19 at Rockcastle, 71 other, 206 home	Miscarriage <i>Not Informative</i> No comparison group (compared pregnancy history during and not during job but could not account for gravidity in that kind of analysis). Limited exposure assessment for earlier years.
Stücker et al. (1993) (birth weight) (Stücker et al., 1990) (spontaneous abortion) (cohort, retrospective) nursing staff	Recruited all female daytime nursing staff, ≤45 year old and currently working in selected units. 87% response among all daytime nursing staff	Current and previous jobs; self-report by interview; dates of each prior pregnancy and dates of occupational exposure to cytostatic drugs, anesthetic agents, and formaldehyde. Exposure based on exposure during or before the pregnancy.	Self-report by interview (spontaneous abortion, birth weight, small for gestational age). Interview 1985–1986. Mean time since exposed and referent pregnancies, respectively, was 5 and 10 years (potential for	Exposed and referent were all female day time nursing staff	No analyses were presented for spontaneous abortion. Linear regression for birth weight & formaldehyde association, adjusted for gestational age; not adjusted for other work exposures; other	N = 348 births among formaldehyde exposed pregnancies; # of spontaneous abortions not reported	Birth weight spontaneous abortion <i>Not Informative</i> Inclusion of exposure before pregnancy of uncertain relevance for birth weight. No information on intensity and frequency of formaldehyde exposure (exposure misclassification—decreased sensitivity).

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		No information on intensity or frequency of exposure.	differential recall and misclassification?)		work exposures (quantitative results not reported, just reported as “not significant”)		Quantitative results not presented for formaldehyde for birth weight analysis; no results presented for spontaneous abortion analysis
Taskinen et al. (1994) (case-control) laboratory workers	Recruited from payrolls & union rolls, 82.4% response, reduced likelihood of selection bias; 2 referents per case with a live birth and no registered SA, 4 referents per congenital malformation case, study population restricted to age 20–34 years, referents matched to case for age (24 mos) at conception and year at end of pregnancy	Self-report, focus on 1 st trimester; exposed & frequency, reviewed by industrial hygienist; calculated exposure index based on reported quantity used, frequency (# hrs/d and # d/wk), and use of fume hood	Spontaneous abortion: hospital discharge register, 1973–1986	Smoking, alcohol, and employment status considered a priori, plus other factors (parity, previous miscarriages, febrile diseases during pregnancy and used contraception) with OR > 1.5 or <i>p</i> value < 0.05; no other work exposures; possible confounding by xylene exposure, majority of formalin exposed also exposed to xylene (OR 3.1)	Conditional logistic regression adjusted for factors listed in confounding column. Adjustment for parity and previous miscarriage may introduce bias	N = 206 SA cases, 329 referents; 36 malformation cases, 105 referents	Spontaneous abortion <i>Low</i> Adjustment for parity and previous miscarriage may introduce bias; lack of adjustment for xylene, an exposure associated with the spontaneous abortion and formalin exposure; evaluation of increasing frequency of use a strength.
Taskinen et al. (1999) (cohort, retrospective) woodworkers	Recruited from woodworker's union (not only current workers) reducing likelihood of	TWA assigned using measurements and reported time at task, sampling protocol not reported, JEM is a more robust exposure	Pregnancies identified from national birth register 1985–1996; live birth. Analysis limited to first pregnancy filling	FDR: Regression adjusting for several risk factors plus phenols, FDR for dusts & wood dusts were > 1 in low exposure	TTP: Discrete proportional hazards regression and likelihood ratio test, FDR (95%	Not exposed <i>N</i> = 367 Low <i>N</i> = 119 Medium <i>N</i> = 77 High <i>N</i> = 39	Time-to-pregnancy <i>Medium</i> Expect some error in individual exposure assignments

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	survivor bias, 64% returned questionnaire; evaluated exposure response trend; period of recall 1–11 years. Not an optimal design for spontaneous abortion: women with no live births but at risk for spontaneous abortion were not included.	assessment; focused on 6 mos prior to pregnancy for TTP relevant exposure window; evaluated risk by glove use in high exposure group; Exposure range: 0.01–1.23 mg/m ³ . Applied formaldehyde concentrations from a comparable workplace when data was missing (missing data was differential by exposure level; high 31%, moderate 61%, and low 46%)	criteria; TTP (FDR): self-report (question: did woman get pregnant during first menstrual cycle when not using contraception? Second? Or how many mos/years?) Left censoring: excluded 38 pregnancies as a result of contraception failure & 28 whose TTP started before the first job in the branch. any previous SA: self-report	category & equal to 1 (1.02 & 0.93) in middle & high categories; SA: reported that other exposures were not associated	CI), adjusted for employment, smoking and alcohol consumption, irregular menstrual cycles, and # of children. Spontaneous abortion: Unconditional logistic regression, odds ratios, adjusted for age, employment, smoking and alcohol, # exposed cases not reported	N = 52 spontaneous abortion cases (in women with same workplace as time-to-pregnancy analysis)	Spontaneous abortion <i>Medium</i> Exposures during critical exposure period(s) for spontaneous abortion were not estimated.; excluded women with no live birth (missing spontaneous abortions to women with no live births)
Wang et al. (2012) (cohort, retrospective) wood processing	100% of eligible recruited couples participated; did not describe recruitment or sampling frame; included if married males, Chinese Han ethnicity, had formaldehyde exposure for at least 24 mos; excluded couples with possible nonwork exposure	Measurements in factories, monitoring on 3 occasions during different periods; self-report of workplace, work tasks & hours/day exposed to formaldehyde; daily mean exposure = mean concentration multiplied by % of time exposed to formaldehyde (referenced Taskinen et al.	Prolonged time-to-pregnancy (> 12 mos), spontaneous abortion, birth outcomes (preterm birth, LBW, sex ratio, birth defects); semi-structured interview using questionnaire; data analysis for most recent pregnancy; potential under-ascertainment because interviewed male partners.	Exposed and referent matched on age, married men & from same area (salesmen and clerks); exposed and referent were of similar age, BMI, educational level, income, smoking, alcohol, frequency of intercourse. Confounding considered: age, BMI, education, income, smoking, alcohol, and	Logistic regression, paternal exposure risk; adjusted OR, 95% CI; compared low versus high formaldehyde exposed. Comparison of means (referent, low, and high) exposure, ANOVA; crude	302 exposed, 305 referents.	Time-to-pregnancy <i>Medium</i> Exposure levels not reported (but robust assessment method). Dichotomized time-to-pregnancy in analysis (low sensitivity). Spontaneous abortion birth defects <i>Medium</i> Exposure levels not reported (but robust

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	to formaldehyde (i.e., newly remodeled homes), or wives with other exposures to reproductive toxicants & pregnancies prior to formaldehyde exposure	1999). JEM is a more robust exposure assessment. Did not report formaldehyde estimates; relevant exposure period: gametogenesis	Left censoring: 106 excluded because wife's pregnancy began before exposed employment	frequency of intercourse. Adjusted for other risk factors but not for other work exposures (e.g., dust, phenols)	and adjusted regression coefficients and 95% CI; OR and 95% CI for abnormal sperm parameters; reported results of all analyses		assessment method). Other workplace exposures in woodworking industry (solvents) have been associated with the spontaneous abortion but not accounted for; analysis of most recent pregnancy: possible selection for live births (time-lapse bias) and possible impact of gravidity on spontaneous abortion risk
Wang et al. (2015) (cohort, retrospective) wood processing, 7 industrial sites	Recruited men aged 23–40 years of age, Chinese Han ethnicity, and formaldehyde exposed at least 24 mos. Excluded men who lived in newly built or recently decorated house, men with genital malformations or other chronic diseases. Comparison: age-matched male Han population volunteers living in same area (salesmen and	Referenced Wang et al. (2012) ; sampling: 25-min samples at 3 times on 1 workday, same day as investigation. Exposure information based on workplace, work tasks, work duration and time. Exposure index based on formaldehyde concentration (mean of 3 samples) times exposed work time during work day times exposure duration (years). Two categories with cutpoint at median.	Semi-structured interview using questionnaire; no change in lifestyle or environments 6 mo prior to semen collection; genital examination. Semen sample within 2 wks of exposure sampling, after a 2–7 d abstinence. Semen analysis within 60 min by 2 technicians using same apparatus (computer assisted semen analysis), blinded. Parameters: semen volume, sperm concentration, total sperm count, sperm	Addressed via design, sex, SES, education, age. Variables included in models: age, body mass index, education, income, smoking, drinking, and abstinence duration. No evaluation of other organic solvents such as phenol or wood preservatives.	Multiple linear regression of ln-transformed semen parameters and logistic regression of abnormal semen parameters; reported results for all parameters analyzed	N = 124 (62.3%) recruited, eligible and agreed to participate. 75 of 199 eligible refused to provide sample. No data for 10, N = 114 N = 81 referents (40.5% of eligible), no semen data for 5, N=76	<i>Medium</i> Other workplace exposures in woodworking industry (solvents) have been associated with sperm motility but not accounted for; however otherwise strong design and analysis, including evaluation of increasing exposure-response relationship.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
	clerks) not exposed to formaldehyde or other reproductive toxicants.	Concentrations: Exposed 0.22–2.91 mg/m ³ , exposure index 4.54–195.08, median 56.55; referent 0–0.02 mg/m ³ .	progressive motility and total sperm motility; kinematic parameters (WHO, 2010), velocity, linearity, displacement measures.				
Ward et al. (1984) (cross-sectional) autopsy service	Groups similar: exposed and referent all from university (exposed = autopsy service; referent = other medical branches)	Reported ranges for TWA and concentration; area and personal breathing zone. Exposure range: TWA 0.75–1.62 mg/m ³	Sperm abnormalities assessed every 2–3 mos (3 samples collected for standard sperm parameters); hand scoring of morphology (no QC data)	Matched on sex, age, tobacco, alcohol, and recreational drug use	No statistical analyses; EPA could compare prevalence	N = 11 men per exposure group	Sperm parameters <i>Low</i> Small sample size; uncertainty regarding reliability of morphology scoring
Zhu et al. (2005) (pregnancy cohort) laboratory work	Danish National Birth Cohort, 30–40% of all pregnancies, first pregnancy and laboratory technician (hospital, university, medical industry, food industry or public services), 77.5% initial cohort; referent teachers, 73.9% initial cohort; entered cohort at weeks 12–25 (median 17)	Self-report at gestational weeks 12–25 (median 17 wks), laboratory work processes during pregnancy and 3 mos before conception; JEM exposure index: exposure level (low or medium assigned to work process by study investigators) times frequency of contact. Formaldehyde: Low: processed human blood or tissues, worked with experimental animals or microorganisms;	Self-report of TTP (0–2 mos, 3–5 mos, 6–12 mos, >12 mos); fecundability ratio	Demographic characteristics of laboratory technicians and teachers comparable (maternal age, gravidity, history of spontaneous abortion, smoking, alcohol, BMI, paternal job). Possible confounding by other exposures in lab	Fecundability ratios analyzed within the exposed group (exposure index 1–5 vs. ≥6) using discrete-time survival analysis; adjusted for covariates listed in confounding column	Exposed N = 829, referent N = 6,250	Time-to-pregnancy <i>Low</i> Categorized time-to-pregnancy (decreased precision), missed pregnancies that ended before 1 st interview. Variation in probability or intensity of formaldehyde exposure possible for work processes across different types of labs, did not account for large proportion of participants who used protective measures to prevent inhalation exposure. JEM

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Consideration of participant selection and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and completeness of results	Size	Confidence
		Medium: prepared slides for microscopy. Exposure index did not include use of protective measures (40–64% used exhaust/flow bench). Exposure tool was not validated for formaldehyde. Potential for exposure misclassification					was not validated for formaldehyde.
Zhu et al. (2006) (cohort study) laboratory work	Members of the Danish National Birth Cohort, 30–40% of all pregnancies, first pregnancy and laboratory technician (hospital, university, medical industry, food industry or public services), 95% of eligible; referent teachers, 95% of eligible	Self-report at gestational weeks 12–25 (median 17 wks), laboratory work processes during pregnancy and 3 mos before conception; JEM exposure index: see (Zhu et al., 2005) above. Potential for exposure misclassification.	Birth outcomes: preterm birth, small for gestational age, major malformations	Demographic characteristics of laboratory technicians and teachers comparable (maternal age, gravidity, history SA, smoking, alcohol, BMI, paternal job). Possible confounding by other exposures in lab	Cox regression within the exposed group (exposure index 1–5 vs. ≥6), hazard ratios for fetal loss and malformations; logistic regression, odds ratios for other outcomes; adjusted for covariates listed in confounding column	Late fetal loss: exposed N = 9, unexposed N = 106; preterm birth: exposed N = 41, unexposed N = 317; SGA: exposed N = 80, unexposed N = 700; major malformations: exposed N = 56, unexposed N = 379	Preterm birth, small for gestational age, major malformations <i>Low</i> Variation in probability or intensity of formaldehyde exposure possible for work processes across different types of labs, did not account for large proportion of participants who used protective measures to prevent inhalation exposure. JEM was not validated for formaldehyde.

Animal Studies

Animal studies in Table B-54 are organized according to the general outcomes evaluated (i.e., gestation exposures and developmental outcomes and reproductive outcomes) and then listed alphabetically. Specific study details (or lack thereof) that highlight a limitation or uncertainty in answering each of the domain criteria are noted in the table cells. For those identified as having a substantial limitation likely to influence the study results (major limitations), the relevant study details are bolded. Italics are used to highlight other limitations not viewed as substantial in the context of respiratory tract pathology (minor limitations). A ‘Good’ domain rating is denoted by ‘++’; an ‘Adequate’ rating by ‘+’; and a ‘Deficient’ rating by gray shading. Some studies included both apical endpoints and endpoints informing potential MOAs. As was the case for nervous system effects, this review constrained the mechanistic endpoints relevant to the developmental and reproductive effects of inhaled formaldehyde to experimental studies of inhalation exposure. Thus, the methods and documentation applied paralleled those described above, with a few exceptions in application (i.e., acute exposure duration and the ability to infer the endpoint as adverse were not considered limitations, and the endpoint evaluation domain considered whether the endpoint evaluates a mechanism relevant to humans, although mechanisms are considered relevant to humans in the absence of evidence to the contrary).

Table B-54. Study quality evaluation of developmental and reproductive toxicity animal studies

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
Gestation Exposures and Developmental Outcomes ⁱ						
Al-Saraj (2009)	Test article = formalin; co-exposure with ivermectin (anthelmintic)	+ <i>N</i> = 7 controls and <i>N</i> = 26 FA-exposed; <i>strain NR</i>	Gestation day not standardized via cesarean section; detailed offspring evaluation methods not provided	Only external examination; no visceral or skeletal evaluation of newborn kits	Exposure during gestation not well-characterized; dose-dependent data in dams and offspring not shown. Litter incidences of external findings not provided; major confounding factor: co-exposure with ivermectin, a known developmental toxicant in rabbits	<i>Not informative</i> (Co-exposure to ivermectin)

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
Gofmekler (1968)	Test article NC; <i>generation method</i> , analytical method and concentrations, chamber type NR; <i>exposure regimen poorly characterized</i>	<i>N</i> = 3 males and 12 females/group; <i>source and strain NR</i>	+ <i>Limited study design</i> focused on offspring growth (body weight and organ weight)	+ <i>Methods were poorly described</i> but appeared appropriate for the evaluation of offspring growth	Mean body and organ weight data reported, but no variance provided; statistical methods not described although statistical analysis was conducted. Age at assessment of offspring NR; reproductive (maternal and litter) data not provided; overall limited data reporting.	<i>Low</i> (Test article NC, small <i>N</i> , exposure generation, animal strain/source NR; limited description of methods; limited reporting)
Gofmekler and Bonashevskaya (1969)	Test article NC; <i>generation method</i> , analytical method and concentrations, chamber type, exposure regimen NR	+ <i>N</i> = 12/group; <i>source and strain NR</i>	+ <i>Limited study design</i> focused on developmental anomalies, offspring reproductive organ weights, and histopathology	+ <i>Methods were poorly described</i> but appeared appropriate for the evaluation of.	Report contained only verbal summary of findings. No quantitative data were included in the paper.	<i>Not informative</i> (Test article NC, exposure generation, animal strain/source NR; limited description of methods; limited reporting)
Guseva (1973a)	Test article NC; <i>generation method</i> , analytical concentrations NR; <i>chamber type NC</i> ; co-exposure with formalin in drinking water	<i>N</i> = 4/group; <i>source and strain NR</i>	+ <i>Limited study design</i> focused on reproductive function, developmental anomalies, and postnatal maturation; gonadotropic response to pituitary emulsions, and testicular nucleic acids	+ <i>Methods were poorly described</i> but some appeared appropriate for the evaluation of reproductive function, developmental anomalies and postnatal maturation; gonadotropic response assay was not a standard testing paradigm	Only nucleic acid quantitative data (mean and variance) were reported; all other results were described verbally; statistical methods not described although statistical analysis was conducted.	<i>Not informative</i> (Test article NC; oral co-exposure with formalin; low <i>N</i> ; some experimental methods and data NR)
Kitaev et al. (1984)^d	Test article NC; <i>generation method</i> , analytical concentrations NR; chamber type NC	+ <i>N</i> = 5–9/group; <i>source NR</i>	+ <i>Limited study design</i> focused on early embryonic development, organ weights, and hormone measures; time	+ <i>Methods were poorly described</i> but appeared appropriate for the evaluation of early embryonic development,	+ Group mean data and variance presented; <i>statistical methods not described</i> although statistical analytical results	<i>Low</i> (Test article NC; limited description of methods)

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
			of day the hormone measures were taken NR	organ weights, and hormone measures	were described in text. <i>Statistical significance NR for some embryonic outcomes; relative organ weight and hormone measure graphs appeared to be hand-drawn</i>	
Kum et al. (2007a)	Test article = formalin; generation method, analytical concentrations NR	+ N = 6/group; source NR	+ <i>Limited study design</i> focused on embryonic and early postnatal body and liver weights and MOA (redox enzymes)	+ <i>Methods were poorly described</i> but appeared appropriate for the evaluation of embryonic and early postnatal body and liver weights	+ Group mean data and variance presented; <i>maternal toxicity not reported</i>	<i>Low</i> (Formalin; limited description of methods; maternal tox NR)
Martin (1990)	++ Test article = paraformaldehyde; well characterized exposure methods	+ N = 25 dams/group; source NR	+ Study design described as a “teratology study” although <i>few methods details were provided</i>	Methods were not described; endpoints listed in the statistical methods section appeared appropriate for a screening level evaluation of developmental toxicity	+ <i>Inadequate reporting of quantitative results. No group mean data were presented</i>	<i>Low</i> (Inadequate reporting of methods and quantitative results)
Monfared (2012)	Test article NC; generation method, analytical methods and concentrations NR	++ N = 10 dams/group; strain and source were reported	+ <i>Limited study design</i> focused on placental weight, histopathology, and ultrastructural pathology	++ Methods were appropriate for the evaluation of placental weight, histopathology, and ultrastructural pathology	+ Group mean placental weight data and variance presented; photomicrographs provided; <i>maternal toxicity not reported</i>	<i>Low</i> (Test article NC; maternal tox: NR)
Pushkina et al. (1968)	Test article NC; generation method, analytical method and concentrations, chamber type, exposure regimen NR	+ N = 10 females/group; strain NR	+ <i>Limited study design</i> focused on ascorbic acid levels in dams, fetuses, and placentas	Limited methodological information provided	+ Group mean ascorbic acid levels and variance presented; <i>statistical methods not described</i> although statistical analytical results were noted in table	<i>Not informative</i> (Experimental methods NR)

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
Saillenfait et al. (1989)	Test article = formalin with 10% methanol; well-characterized exposure methods	++ N = 25 dams/group; strain and source provided	++ Study design was equivalent to a guideline prenatal developmental toxicity study	++ Methods well described and appropriate for a screening level evaluation of developmental toxicity.	++ Group incidence and mean/variance data presented	<i>Low</i> (Formalin)
(Sanotskii et al., 1976)	Test article NC; generation method, analytical method and concentrations NR; chamber type NC	N = 334 total females (females/group NR); strain and source NR	Limited study design only evaluated pregnant vs. nonpregnant dams (did not evaluate reproductive or fetal parameters)	Limited methodological information provided	Inadequate reporting of methods and results (no primary or mean data presented); statistical methods not described although statistical analytical results were noted in text	<i>Not informative</i> (Experimental methods and data NR)
(Senichenkova, 1991a) (translation); (Senichenkova, 1991b)	Test article NC; generation method, analytical method and concentrations NR; chamber type NC	N = 137 total dams (dams/group NR); <i>strain and source NR</i>	++ Study design focused on in utero developmental outcomes (mortality, growth, visceral, skeletal outcomes), select open field neurotoxicity measurements in juveniles, and blood acid-base status	+ <i>Limited methodological information</i> provided for tests conducted; apparent methods appropriate for the evaluation of in utero developmental outcomes.	+ Group mean and variance data presented; <i>maternal toxicity not reported; statistical methods not described</i> although statistical analytical results were noted in tables	<i>Low</i> (Test article NC; exposure generation, animal strain/source, # dams/group, maternal tox NR; limited description of methods)
Senichenkova and Chebotar (1996a)	Test article NC; generation method, analytical method and concentrations NR; chamber type NC	N = 254 total dams (dams/group NR); <i>strain and source NR</i>	+ <i>Control group co-exposure to ethanol; limited study design</i> focused on in utero developmental outcomes (external anomalies and skeletal delays) and blood acid-base status	+ Limited methodological information provided for tests conducted; apparent methods appropriate for the evaluation of in utero developmental outcomes.	+ Group mean and variance data presented; statistical methods not described although statistical analytical results were noted in tables; <i>maternal toxicity not reported</i>	<i>Low</i> (Test article NC; exposure generation, animal strain/source, # dams/group, maternal tox NR; limited description of methods)
Sheveleva (1971)	Test article NC; generation method, analytical method NR	+ N = 15 dams/group for C-section, N = 6 dams/group for delivery; <i>strain and source NR</i>	+ <i>Limited study design</i> focused on developmental parameters, body weight	+ <i>Limited methodological information</i> provided for tests conducted; apparent methods appropriate for	+ Group mean and variance data presented; <i>statistical methods not described</i>	<i>Low</i> (Test article NC; exposure generation, animal strain/source NR;

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
			spontaneous mobility, temperature, and hematology parameters	the evaluation of developmental parameters.		limited description of methods)
Reproductive Outcomes						
(Appelman et al., 1988)	++ Test article = paraformaldehyde; well characterized exposure methods	++ N = 40 males/group; test animals adequately characterized	++ Study design focused on comparison of subchronic or chronic exposures to rats with undamaged or clinically damaged nasal mucosa; extensive tissue evaluation	No indication if histopathology was performed on male reproductive organs	Quantitative testes weight data were not presented in the study results. No histopathology findings for male reproductive organs were reported	Low (No indication if histopathology performed on male repro organs; quantitative testes weights not presented)
Golalipour et al. (2007)	Test article NC; generation method NR; open air exposures (i.e., not a controlled chamber study)	+ N = 4 males/group; test animals adequately characterized	+ <i>Limited study design</i> focused on testis toxicity	++ Methods were appropriate for the evaluation of testis toxicity.	++ Group mean data and variance presented	Low (Test article NC; open air exposures; N = 4/group)
Han et al. (2015)	Test article NC; <i>generation method, analytical method and concentrations NR, static chamber type</i>	++ N = 10 males/group; test animals adequately characterized	+ <i>Limited study design</i> focused on testis toxicity and MOA	++ Methods were appropriate for the evaluation of testis toxicity.	+ Group mean testis weight and seminiferous tubule diameter data reported but <i>variance not presented; quantitative microscopy findings not presented</i>	Low (Test article NC; exposure generation NR; static chamber used; limited reporting of study results and group data)
Maronpot et al. (1986)	Test article = formalin; well-characterized exposure methods	++ N = 10/sex/group; test animals adequately characterized	++ Subchronic study with limited in-life observations and extensive postmortem evaluation	++ Methods were appropriate for a screening level evaluation of general toxicity following subchronic exposure; no special emphasis on reproductive organs	+ <i>Selected incidence data presented (survival, histopathology); mean body weight data did not include variance; no indication of statistical data analysis</i>	Low (Formalin; limited reporting of methods and results)

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
Ozen et al. (2002)	++ Test article = paraformaldehyde; well characterized exposure methods	+ <i>N</i> = 7 males/group; test animals adequately characterized [Note: substantial weight gain decrease with exposure]	+ <i>Limited study design</i> focused on testis toxicity and MOA	++ Methods were appropriate for the evaluation of testis toxicity	+ Group mean data and variance presented; <i>preferred reporting is absolute, not relative, testes weight</i>	<i>Medium</i> (Small <i>N</i> ; non-preferred results reporting)
Ozen et al. (2005)	+ Test article = paraformaldehyde; analytical concentrations NR	+ <i>N</i> = 6 males/group; authors noted effects on food and water consumption but didn't report magnitude of effect, nor quantified changes in body weight	+ <i>Limited study design</i> focused on testis toxicity (includes Bouins fixation of testes)	++ Methods were appropriate for the evaluation of testis toxicity	+ Group mean data and variance presented; <i>histology results averaged by group not by animal</i>	<i>Medium</i> (Small <i>N</i> ; limited and incomplete reporting of some important results)
Sapmaz et al. (2018)	++ Test article = paraformaldehyde; well characterized exposure methods	<i>N</i> = 7 adult males; source not identified; strain provided	+ <i>Limited study design</i> focused on testis toxicity and biomarkers of oxidative stress	+ Methods generally appropriate for the evaluation of testis toxicity; however, <i>no indication of blinding and unclear sampling protocol for histopathology endpoints</i>	++ Group mean data and variance presented	<i>Medium</i> (Small <i>N</i> ; potential for bias in histopathology evaluations)
Sarsilmaz et al. (1999)	+ Test article = paraformaldehyde; analytical concentrations NR	++ <i>N</i> = 10 males/group; test animals adequately characterized	+ <i>Limited study design</i> focused on testis toxicity	++ Methods were appropriate for the evaluation of testis toxicity	+ Group mean data and variance presented; <i>unable to determine what the reported SD represents for Leydig cell numbers</i>	<i>Medium</i> (Inadequate information for quantitative analysis of histopathology data)
Vosoughi et al. (2012); Vosoughi et al. (2013)	++ Test article = paraformaldehyde; well characterized exposure methods; analytical concentrations reported	++ <i>N</i> = 12 males/group; test animals adequately characterized	+ <i>Limited study design</i> focused on testis toxicity, sperm measures, and hormone (LH, FSH, T) levels	++ Methods were appropriate for the evaluation of testis toxicity, sperm measures, and hormone levels (LH, FSH, T)	++ Group mean data and variance presented	<i>High</i> (No notable limitations)

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
Wang et al. (2013)	Test article NC; generation method, analytical method and concentrations NR, static chamber type	++ N = 10 females/group; test animals adequately characterized	+ <i>Limited study design</i> focused on ovarian toxicity, estradiol (E2) levels, and MOA	++ Methods were appropriate for the evaluation of ovarian toxicity and E levels	++ Group mean data and variance presented (graphically) for E2 levels and ovarian weights	Low (Test article NC)
(Woutersen et al., 1987)	++ Test article = paraformaldehyde, generation method, analytical methods and concentrations reported, dynamic whole-body chamber	++ N = 40/sex/group; test animals adequately characterized	++ 13-wk subchronic study	Report indicates that testes and ovaries were weighed at necropsy; no indication if histopathology was performed on male or female reproductive organs	Quantitative reproductive organ weight data were not presented in the study results. No histopathology findings for reproductive organs were reported	Low (Limited methods; no data presented)
Xing et al. (2007)	Test article NC; <i>generation method</i> , analytical method and concentrations, <i>chamber type</i> NR	++ N = 12 males and 24 females/group; test animals adequately characterized	+ <i>Limited study design</i> focused on sperm morphology, reproductive success, and micronucleus assay	++ Methods were appropriate for the evaluation of sperm morphology and reproductive outcome.	++ Adequate reporting of reproductive outcome results (group incidence and mean data with variance). Micronucleus data not presented.	Low (Test article NC; exposure generation, strain NR; high exposure levels)
Zhou et al. (2006)	Test article NC; <i>generation method</i> and analytical concentrations NR, <i>static chamber type</i>	++ N = 10 males/group; test animals adequately characterized	+ <i>Limited study design</i> focused on testes weight and histopathology, sperm measures, and MOA; co-exposure of one FA-treated group with vitamin E to assess mediation effects	++ Methods were appropriate for the evaluation of testes weight and histopathology, and sperm measures	++ Group mean data and variance presented (graphically for testes weights); appropriate statistical analysis of data. Vitamin E co-exposure group not included in dose-response assessment for FA outcomes	Low (Test article NC, exposure generation NR; static chamber used)
Zhou et al. (2011a)	Test article NC; generation method, analytical method and concentrations NR; <i>static chamber</i>	++ N = 10 males/group; test animals adequately characterized	+ <i>Limited study design</i> focused on testes and epididymal weight and histopathology, sperm	++ Methods were appropriate for the evaluation of testes and epididymal weight and	++ Group mean data and variance presented (graphically for T levels)	Low (Test article NC; exposure generation NR;

Supplemental Information—Formaldehyde (Inhalation)

Reference	Exposure quality ^a (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
	<i>type, exposure regimen poorly described</i>		measures, testosterone (T) levels, and MOA	histopathology, sperm measures, and T levels		static chamber used)
Zhou et al. (2011b)	Test article NC; generation method, analytical method and concentrations NR; <i>static chamber type, exposure regimen poorly described</i>	++ N = 12 males/group; test animals adequately characterized	+ <i>Limited study design</i> focused on epididymal weight and histopathology, sperm measures, and MOA	++ Methods were appropriate for the evaluation of epididymal weight, histopathology, and sperm measures	++ Group mean data and variance presented (graphically)	Low (Test article NC; exposure generation NR; static chamber used)

NR = Not Reported; NC = Not Characterized.

B.3.9. Cancer

Details of the study evaluation approach methods are provided in Section 2.3.9 of the Toxicological Review. Some additional considerations specific to documenting the review of the human studies are outlined below. Within the tables documenting the results of the study evaluations for certain immune-mediated conditions, studies are arranged alphabetically. Gray shading is indicative of aspects of the study design or analysis that decreased EPA's confidence rating.

Human Studies

Study Exposure Assessment Methods Categorization

Table B-55 categorizes the exposure assessment methods used in the available human cancer studies.

Table B-55. Categorization of exposure assessment methods by study design.

Group	Cohort (and nested case-control within cohort) studies	Case-control and cancer registry-based studies
A	Industrial settings with extensive industrial hygiene data used to determine levels of exposure (and variability within a worksite); job exposure matrix that accounts for variability by time and job/task. (Beane Freeman et al., 2009; Beane Freeman et al., 2013) Highly exposed professions (embalmers) with comparison to general population, or with measures capturing variability within the cohort. (Hauptmann et al., 2009) (Hayes et al., 1990) (Levine et al., 1984c) (Meyers et al., 2013) (Stroup et al., 1986) (Walrath and Fraumeni, 1983) (Walrath and Fraumeni, 1984)	Detailed lifetime job history, more extensive than industry and occupation codes, including information about specific tasks and setting, combined with job exposure matrix that accounts for variability by time, setting, and job/task. Also includes some kind of validation study or congruence of ratings based on different exposure ascertainment measures to be equivalent to Group A cohort studies with extensive industrial hygiene data. (None identified)
B	Industrial settings with more limited industrial hygiene data. (Andjelkovich et al., 1995) (Coggon et al., 2003; Coggon et al., 2014) (Edling et al., 1987b) (Fryzek et al., 2005) (Marsh et al., 2002; Marsh et al., 2007) (Ott et al., 1989) Exposed professions (e.g., pathologists) with comparison to general population, but that do not have measures capturing variability within the cohort. (Bertazzi et al., 1986) (Hall et al., 1991) (Harrington and Oakes, 1984) (Li et al., 2006) (Matanoski, 1989)	Detailed lifetime job history, more extensive than industry and occupation codes, including information about specific tasks and setting, combined with job exposure matrix that accounts for variability by time, setting, and job/task. (Armstrong et al., 2000) (D'Errico et al., 2009) (Gérin et al., 1989) (Gustavsson et al., 1998) (Hildesheim et al., 2001) (Pesch et al., 2008) (Vaughan et al., 2000)
C	Industrial settings that are only able to use duration as a way to distinguish variability in exposure. (Band et al., 1997) (Dell and Teta, 1995)	Lifetime job history coding based only on industry and occupation; more detailed information about specific tasks and setting not included in assessment of exposure potential (or, information on what was collected was not provided).

Supplemental Information—Formaldehyde (Inhalation)

Group	Cohort (and nested case-control within cohort) studies	Case-control and cancer registry-based studies
	• (Pira et al., 2014) Self-report of exposure. • (Boffetta et al., 1989) • (Saber Hosnijeh et al., 2013) • (Stellman et al., 1998)	• (Blair et al., 2001) • (Laforest et al., 2000) • (Luce et al., 2002) • (Olsen et al., 1984) • (Olsen and Asnaes, 1986b) • (Roush et al., 1987) • (Shangina et al., 2006) • (West et al., 1993) • (Wortley et al., 1992) • (Yu et al., 2004) Self-report of exposure. • (Mayr et al., 2010) Lifetime job history, including tasks/exposure information, but analysis conducted only for job categories rather than for an exposure category • (Teschke et al., 1997)
D	Industrial settings that do not include data to distinguish variability in exposure (e.g., wood workers, with no information on which workers were exposed to formaldehyde; textile workers with no formaldehyde exposure measures), or that include few people classified as exposed. • (Hansen et al., 1994) pharmaceuticals • (Hansen and Olsen, 1995) plant used 1 kg/person/year • (Jakobsson et al., 1997) grinding stainless steel • (Malker et al., 1990) fiberboard plants • (Siew et al., 2012) any occupational exposure • (Solet et al., 1989) pulp and paper mills • (Robinson et al., 1987) plywood mill workers • (Wesseling et al., 1996) banana plant workers Methods of exposure assessment rated as higher quality but downgraded due to methods used by study authors which were likely to induce bias. • (Checkoway et al., 2015)	Job history limited to information on a single job (e.g., based on tax record, death certificate, medical record, census data). • (Heineman et al., 1992) • (Pottern et al., 1992) • (Talibov et al., 2014) High proportion (> 40%) of next-of-kin interviews. • (Vaughan, 1989) • (Vaughan et al., 1986a) • (Vaughan et al., 1986b) • (Yang et al., 2005) Methods of exposure assessment rated as higher quality but downgraded due to invalidation by study authors. • (Berrino et al., 2003)

Outcome-Specific Confidence Classifications

An outcome-specific evaluations classified with **High** confidence in the precise effect estimate is expected to be without appreciable bias and thus represents an accurate estimate of any reported association between formaldehyde exposures and the risks of cancer. These evaluations are expected to have methodological features sufficiently sensitive to provide an adequate basis for interpreting null or weak results as evidence of no or weak risk of cancer. Table B-56 identifies the outcome-specific evaluations were classified with High confidence.

Table B-56. Outcome-specific effect estimates classified with High confidence

Reference	Outcome-specific effect estimates	Confidence classification
(Beane Freeman et al., 2009)	Hodgkin Lymphoma	High
(Beane Freeman et al., 2009)	Laryngeal cancer	High
(Beane Freeman et al., 2013)	Lymphocytic leukemia	High
(Beane Freeman et al., 2009)	Multiple myeloma	High
(Beane Freeman et al., 2009)	Myeloid leukemia	High
(Beane Freeman et al., 2013)	Nasopharyngeal cancer	High
(Hauptmann et al., 2009)	Multiple myeloma	High
(Hauptmann et al., 2009)	Myeloid leukemia	High
(Meyers et al., 2013)	Multiple myeloma	High
(Meyers et al., 2013)	Myeloid leukemia	High

An outcome-specific evaluation classified with **Medium** confidence in the precise effect estimate may have some potential for residual bias, but the direction of the observed effect is unaffected and the magnitude of any expected biases are limited. Thus, the observed effect estimates represent a reasonable estimate of the association between formaldehyde exposures and the risk of cancer, and are expected to be sufficiently sensitive to provide an adequate basis for interpreting null or weak results as evidence of no or weak risk of cancer. Table B-57 identifies the outcome-specific evaluations were classified with Medium confidence.

Table B-57. Outcome-specific effect estimates classified with Medium confidence

Reference	Outcome-specific effect estimates	Confidence classification
(Beane Freeman et al., 2009)	Hodgkin lymphoma	Medium

Supplemental Information—Formaldehyde (Inhalation)

Reference	Outcome-specific effect estimates	Confidence classification
(Beane Freeman et al., 2009)	Lymphocytic leukemia	Medium
(Beane Freeman et al., 2013)	Sinonasal cancer	Medium
(Coggon et al., 2014)	Myeloid leukemia	Medium
(Coggon et al., 2014)	Laryngeal cancer	Medium
(Coggon et al., 2014)	Oro/hypopharyngeal cancer	Medium
(Gérin et al., 1989)	Hodgkin lymphoma	Medium
(Hayes et al., 1990)	Multiple myeloma	Medium
(Hayes et al., 1990)	Myeloid leukemia	Medium
(Hauptmann et al., 2009)	Lymphatic leukemia	Medium
(Hildesheim et al., 2001)	Nasopharyngeal cancer	Medium
(Meyers et al., 2013)	Oro/hypopharyngeal cancer	Medium
(Walrath and Fraumeni, 1983)	Myeloid leukemia	Medium
(Walrath and Fraumeni, 1984)	Myeloid leukemia	Medium
(Laforest et al., 2000)	Oro/hypopharyngeal cancer	Medium
(Luce et al., 2002)	Sinonasal cancer	Medium
(Olsen and Asnaes, 1986b)	Sinonasal cancer	Medium
(Olsen et al., 1984)	Nasopharyngeal cancer	Medium
(Roush et al., 1987)	Nasopharyngeal cancer	Medium
(Roush et al., 1987)	Sinonasal cancer	Medium
(Vaughan et al., 2000)	Nasopharyngeal cancer	Medium
(West et al., 1993)	Nasopharyngeal cancer	Medium

An outcome-specific evaluation classified with **Low** confidence in the precise effect estimate is likely to have some residual bias or may lack sensitivity to provide an adequate basis for interpreting null or weak results as evidence of no or weak risk of cancer. For example, an outcome-specific effect estimate based on fewer than five observed or expected cases of a particular cancer would be classified with Low confidence based on a lack of sensitivity, even if there were no appreciable biases. Another study classified with Low confidence might have relied on exposure assessment methodologies that were unbiased, but nonspecific in nature so as to yield effect estimates that were likely biased towards the null, and thus, underestimated any true effect. Similarly, the lack of consideration of latency is a limitation as it may cause measurement error in improperly including exposure of little biological relevance to cancer occurrence. Concern about the potential for confounding is a limitation when a co-exposure is a known cause of a particular cancer endpoint and may be correlated with formaldehyde exposure in a study. Selection bias may be a limitation when survival rates are long as incidence cases may not be readily detected using mortality statistics. In general, outcome-specific effect estimates that underestimate any true effect may still inform a hazard conclusion. However, outcome-specific effect estimates that overestimate any true effect cannot inform a hazard conclusion and are considered to be uninformative as are outcome-specific effect estimates, which suffer from strong bias or a complex mixture of biases.

Studies Judged to be Uninformative for the Evaluation of Causation

In rare circumstances, studies initially judged to be potentially informative were further evaluated and found to be uninformative. For example, studies of specific LHP subtypes, which mention formaldehyde or study the health of workers in an industry expected to be exposed to formaldehyde, but details of the study reveal only extremely limited exposure ([Dell and Teta, 1995](#); [Armstrong et al., 2000](#)) or virtually none at all ([Li et al., 2006](#)). Two outcome-specific associations were judged to be uninformative due, in part, to potential manifestations of the healthy worker effect with standardized mortality ratio for “all cancers” more than 30% below expected values (SMR<0.7: ([Harrington and Oakes, 1984](#); [Hall et al., 1991](#))). Another reason was that a study had co-exposures that are likely to have been highly correlated with formaldehyde and were known risk factors for LHP cancers and the independent effect of formaldehyde cannot be inferred (e.g. ([Fryzek et al., 2005](#); [D'Errico et al., 2009](#))). Studies with co-exposures to known risk factors for LHP cancers that are not likely to be highly correlated for formaldehyde or were not risk factor for the specific LHP subtype in question are included and the potential for confounding is noted for evaluation in the causal synthesis. Table B-58 identifies the outcome-specific evaluations were classified as not iformative.

Table B-58. Outcome-specific effect estimates classified as not informative

Reference	Outcome-specific effect estimates	Confidence classification	Critical limitation(s)
(Armstrong et al., 2000)	Nasopharyngeal cancer	Not informative	Multiple
(Berrino et al., 2003)	Laryngeal cancer	Not informative	Confounding
(D'Errico et al., 2009)	Sinonasal cancer	Not informative	Confounding
(Dell and Teta, 1995)	Nasopharyngeal cancer	Not informative	Sensitivity (minimal exposure)
(Fryzek et al., 2005)	Hodgkin lymphoma	Not informative	Confounding
(Fryzek et al., 2005)	Multiple myeloma	Not informative	Confounding
(Hall et al., 1991)	Hodgkin lymphoma	Not informative	Selection bias (healthy worker effect)
(Hansen et al., 1994)	Hodgkin lymphoma	Not informative	Information bias (minimal exposure)
(Hansen et al., 1994)	Laryngeal cancer	Not informative	Information bias (minimal exposure)
(Hansen et al., 1994)	Multiple myeloma	Not informative	Information bias (minimal exposure)
(Harrington and Oakes, 1984)	Sinonasal cancer	Not informative	Selection bias (healthy worker effect)
(Li et al., 2006)	Nasopharyngeal cancer	Not informative	Sensitivity (minimal exposure)
(Li et al., 2006)	Sinonasal cancer	Not informative	Sensitivity (minimal exposure)
(Matanoski, 1989)	Hodgkin lymphoma	Not informative	Selection bias and Information bias
(Mayr et al., 2010)	Sinonasal cancer	Not informative	Confounding
(Solet et al., 1989)	Hodgkin lymphoma	Not informative	Multiple
(Wesseling et al., 1996)	Hodgkin lymphoma	Not informative	Multiple
(Wesseling et al., 1996)	Multiple myeloma	Not informative	Multiple

Individual Study Evaluations

Studies that evaluated more than one outcome might be categorized differently for each outcome. The classification of a study could also vary among different analytical groups within a study (e.g., studies of children and adults, with separate analyses for each group), depending on the information presented for the different analyses.

Table B-59. Evaluation of occupational cohort studies of formaldehyde and cancers of the URT (NPC, SN, OHPC) and LHP (HL, MM, LL, ML)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Andjelkovich et al. (1995) United States Cohort study of iron foundry workers working during 1960–1987 with follow-up through 1989.	3,929 male workers exposed to formaldehyde ≥ 6 mos. Loss to follow-up 1.3% (1.5% of 2,032 unexposed workers). Median follow-up ≈15 years. Average follow-up ≈20.77 years. All cancer SMR = 0.99.	Individual-level exposure (Yes/No), questionnaire based on industrial hygienist review of detailed work histories; assignments based on job title and industrial hygiene data and information on tasks and plants. Exposure assessment blinded to outcome. Co-exposed to silica. Possibly co-exposed to polycyclic aromatic hydrocarbons, nickel , and chromium .	Mortality: underlying cause of death based on ICD-8 (Social Security Administration Pension Benefit Information, and National Death Index). HL: ICD 201. Higher survival rates for HL could undercount incident cases, but median follow-up is more than 15 years.	Controlled for sex, age, race, and calendar-year specific mortality rates. Nickel and chromium are associated with URT cancers and would likely be positively correlated with formaldehyde exposure. Potential for confounding is unknown but could have inflated the observed effect. Other co-exposures are not known risk factors for these outcomes.	Exposed vs. unexposed. SMRs (95% CI). Latency not evaluated. No specific estimate of the expected number of NPC or SNC cases was reported, so no SMR was reported for these outcomes.	HL: 1 Larynx: 3 NPC: 0 SNC: 0	SUMMARY: HL, Larynx, NPC, SNC: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group B) and lack of latency analysis with attenuation of association. Confounding possible for URT cancers. Low sensitivity (few cases).
Band et al. (1997) Canada Cohort study of pulp and paper workers,	28,200 male workers employed at least one year by January 1950.	Hire and termination dates and type of chemical process of pulping (sulfate vs. sulfite). Individual exposure measures not	Mortality: underlying cause of death obtained from the National Mortality Database based on ICD version in effect	All comparisons adjusted for age and sex. Confounding not evaluated.	SMRs (95% CI). Duration of exposure evaluated.	HL: 7 Larynx: 12 MM: 12	SUMMARY: HL, Larynx, MM: LOW ↓ (Potential bias toward the null)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
working before 1950 with follow-up through 1982.	Loss to follow-up < 6.5% for workers exposed to the sulfate process (67% of original cohort of 30,157 were exposed to the sulfate process) and loss to follow-up < 20% for workers exposed to the sulfite process. Average follow-up ≈19.42 years. All cancer SMP = 1.03.	derived. As a profession, workers were likely exposed to formaldehyde. Formaldehyde is known to be an exposure for pulp and paper mill workers: job-specific exposures range from 0.2 to 1.1 ppm with peaks as high as 50 ppm (Korhonen et al., 2004). Co-exposed to arsenic, chlorophenols, sulfuric acid mists, and chloroform. Co-exposures to dioxin or perchloroethylene are also possible (Kauppinen et al., 1997).	at time of death and standardize to ICD-9 version HL: ICD 201 MM: ICD 203. Higher survival rates for HL could undercount incident cases, but average follow-up is more than 15 years.	Potential confounders for these outcomes include chlorophenols, acid mists, dioxin, and perchloroethylene and would likely be positively correlated with formaldehyde exposure. Potential for confounding is unknown but could have inflated the observed effect. Other co-exposures are not known risk factors for these outcomes.	Latency evaluated as time since first exposure.		Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) with attenuation of association. Confounding possible.
Beane Freeman et al. (2009); (Beane Freeman et al., 2013) United States Cohort study of workers in 10 plants using or producing formaldehyde, follow-up through 2004.	25,619 workers (12% female) followed from plant start-up or first employment. Deaths were identified from the National	Individual-level exposure estimates based on job titles, tasks, visits to plants by study industrial hygienists who took 2,000 air samples from representative jobs, and plant monitoring	Mortality: underlying cause from death certificates, ICD-8. HL: ICD 201 MM: ICD 203 LL: ICD 204 ML: ICD 205. Larynx: ICD 161 NPC: ICD 147	All comparisons adjusted for calendar year, age, sex, and race. Internal analysis adjusted for pay category.	Internal: Poisson regression; RR (95% CI) by exposure categories (4 levels), for peak, average, cumulative exposures.	HL: 27 MM: 59 LL: 37 ML: 48 Larynx: 48 NPC: 11 SNC: 5	SUMMARY: SNC: MEDIUM (No appreciable bias) Low potential for information bias due to uncertainty in exposure assessment (Exposure Group A)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
<u>Related studies:</u> Initial 10 plant cohort follow-up through 1980 Blair et al. (1986); Blair et al. (1987). Second set of 10 plant follow-ups through 1994 Hauptmann et al. (2003); Hauptmann et al. (2004a). Reanalysis of 1 plant Marsh et al. (2002); Marsh et al. (2007). Reanalysis of Beane Freeman et al. (2009) by Checkoway et al. (2015).	Death Index with remainder assumed to be living. Vital status was obtained for 97.4%. Median follow-up 42 years. Average follow-up ≈38.96 years. All cancer SMR = 0.93.	data from 1960 through 1980. Blinded to outcome. Median cumulative exposure was 0.6 ppm-years (range = 0.0–107.4 ppm-years). Co-exposed to antioxidants, benzene, carbon black, dyes and pigments, melamine, hexamethylenetetramine, phenols, plasticizers, urea, wood dust. (Beane Freeman et al., 2013) sampled cohort members and found no association between smoking and formaldehyde. Blair et al. (1986) noted that smoking habits among this cohort did not differ substantially from those of the general population. Checkoway et al. (2015) redefined peak exposures as the referent category for any exposures <2 ppm of hourly, daily, weekly	SNC: ICD 160. Higher survival rates for HL and LL could undercount incident cases, but median follow-up is more than 42 years. Checkoway et al. (2015) AML: 205.0 CML: 205.1	For HL, MM, LL, ML: Benzene is a potential confounder but was controlled for. For NPC, SN: Wood dust is a potential confounder but was controlled for. Eleven co-exposures examined as potential confounders, but none were found to be confounders.	Latency was evaluated. External: SMRs (95% CI). Checkoway et al. (2015) Cox PH regression; HR (95% CI) by exposure categories (4 levels collapsed to 3 by widening the ref. cat. due to small numbers). Latency was evaluated.	Checkoway et al. (2015) AML: 34 CML: 13	Low sensitivity (few cases) HL, Larynx, LL, ML, MM, NPC: HIGH (No appreciable bias) Checkoway et al. (2015) SUMMARY: ML, AML, CML: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group D due to reclassification of peak exposures as unexposed) with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
		or monthly frequency as well as exposures > 2 ppm if they occurred hourly or monthly.					
Bertazzi et al. (1986) . Italy Cohort study of Italian chemical workers in plant producing formaldehyde resins.	1,332 male workers ever employed in the plant between 1959 and 1980. Deaths were identified from vital statistics offices. Vital status was 98.6% complete. Average follow-up ≈15.26 years. All cancer SMR = 1.54.	Individual-level exposure estimates based on occupational histories from the personnel office with supplement information from 350 employed workers alive at the end of follow-up in 1980. 5,731/20,366 (28%) person years were considered to be exposed to formaldehyde. Other exposures included styrene , xylene, toluene, and methyl isobutyl ketone.	Death certificates used to determine cause of deaths from nasal cancer (ICD-8).	Controlled for age, sex and calendar time. Styrene is associated with LHP cancers but not URT cancers. Other co-exposures are not known risk factors for this outcome.	SMRs (95% CI). Latency evaluated.	SNC: 0 cases	SUMMARY: SNC: LOW ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group B) with attenuation of association. Low sensitivity (few cases).
Boffetta et al. (1989) . United States Nested matched case control of MM within general population cohort. Baseline enrollment in 1982 with bi-annual follow-up in 1984 and 1986.	508,637 men and 676,613 women (57%) in American Cancer Society's Cancer Prevention Study II, with sufficient data on occupation.	Self-report from baseline questionnaire occupational history, based on specific question about exposure to formaldehyde (Ever/Never). Other exposures included asbestos ,	Mortality: underlying or contributing cause from death certificates MM: ICD-9: 203. Analysis limited to "incident" cases (i.e., had not indicated a history of cancer in	Matching controlled for sex, age, ethnic group, residence, smoking, education, diabetes, X-ray treatment, farming, pesticide, and herbicide exposure.	Mantel-Haenszel matched OR (95% CI). Latency not evaluated.	MM: 128 (4 exposed)	SUMMARY: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group C) and lack of latency analysis with

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	Loss to follow-up 1.5%. Death certificates for 84% of deceased subjects. Four controls per case were matched for age, sex, ethnic group, and residence.	chemicals, acids , solvents, coal or stone dusts, coal tar, pitch, asphalt, diesel and gasoline exhausts, dyes, pesticides, herbicides, textile fibers/dusts, wood dust, X-rays, and radioactive material.	baseline questionnaire).	Other co-exposures were not associated with LHP cancers.			attenuation of association. Low sensitivity (few exposed cases).
Coggon et al. (2003) ; Coggon et al. (2014) Great Britain Cohort study of British chemical workers in factories using or producing formaldehyde, working before 1940 with follow-up through 2012. <u>Related studies:</u> Initial follow-up through 1981 Acheson et al. (1984b) . Second follow-up through 1989 Gardner et al. (1993) .	14,008 men in six chemical facilities. Cohort mortality followed from 1941 until December 2012. Vital status was 92% complete. Cause of deaths was known for 99% of 5,185 deaths through 2000. This figure was not provided on 7,378 deaths through 2012.	Individual level categorical exposure assessment based on employment records evaluated occupational hygienist who classified job titles according to their exposure to formaldehyde based on measurement made after 1970 and workers' recall of irritant symptoms prior to 1970. Background exposure corresponded to <0.1 parts per million (ppm), low exposure to 0.1–0.5 ppm, moderate exposure to 0.6–2.0 ppm, and high exposure to >2.0 ppm. Blinded to outcome.	Mortality: underlying cause from death certificates, ICD-9. HL: ICD 201 ML: ICD 205 MM: ICD 203. Larynx: ICD 161 MM: ICD 203 NPC: ICD 147 OHPC: ICD 146-149 minus 147 SNC: ICD 160. Note than HL follow-up was through 2000 Coggon et al. (2003) . Higher survival rates for HL and LL could undercount incident	Adjusted for calendar year, age. Styrene is associated with LHP cancers but not URT cancers. Asbestos is associated with URT cancers, including laryngeal cancer. Authors stated that the extent of co-exposures was expected to be low. Potential for confounding may be mitigated by low co-exposures.	SMRs (95% CI) by low/moderate and high exposure categories. Latency not evaluated.	NPC: 1 SNC: 2 OHPC: 16 Larynx: 22 HL: 15 MM: 28 ML: 36 Note that HL results is from 2003.	SUMMARY: NPC, SNC: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group B) and lack of latency analysis with attenuation of association. Low sensitivity (few cases). HL, Larynx, ML, MM, OHPC: MEDIUM ↓ (Potential bias toward the null) High potential for information bias due

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Third follow-up through 2000: Coggon et al. (2003) .	All cancer SMR = 1.10.	Each worker assigned the highest level of exposure ever experienced (i.e., “ever highly exposed”). Subjects’ assigned exposure grade may exceed average workplace exposure. Potential low-level exposure to styrene, ethylene oxide, epichlorohydrin, solvents, asbestos, chromium salts, and cadmium.	cases, but follow-up is more than 50 years.				to uncertainty in exposure assessment (Exposure Group B) and lack of latency analysis with attenuation of association.
Coggon et al. (2014) Great Britain Nested case-control study. <u>Related studies:</u> Initial follow-up through 1981 Acheson et al. (1984a) . Second follow-up through 1989 Gardner et al. (1993) . Third follow-up through 2000 Coggon et al. (2003) .	Internal comparison using nested case-control study within cohort with 10 controls per case individually matched by facility, mortality status and age within 2 years.	Individual level categorical exposure assessment based on employment records evaluated occupational hygienist who classified job titles according to their exposure to formaldehyde based on measurement made after 1970 and workers’ recall of irritant symptoms prior to 1970. Background exposure corresponded to <0.1 ppm, low exposure to 0.1–0.5 ppm, moderate exposure to 0.6–2.0	Incidence or morality: cancer registries and death certificates, ICD-code in effect at time of diagnosis or death. Cases were either incident diagnoses, underlying cause of death, or contributing cause of death. Larynx: 161 MM: ICD 203 NPC: ICD 147 OHPC: ICD 146-149 minus NPC SN: ICD 160.	Matched analysis controlled for facility and age. Styrene is associated with LHP cancers but not URT cancers. Authors stated that the extent of co-exposures was expected to be low. Potential for confounding may be mitigated by	ORs (95% CI) by low, moderate, high exposure for less than 1 year, and high exposure for 1 year or more. Latency only evaluated by exposure duration and category at 5 years prior to diagnosis or death for each matched set.	Larynx: 53 Pharynx: 28 OHPC: 27 ML: 45 MM: 28	SUMMARY: Larynx, ML, MM, OHPC: MEDIUM ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group B) and weak latency analysis with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
		ppm, and high exposure to >2.0 ppm. Blinded to outcome. Each worker assigned the highest level of exposure ever experienced (i.e., “ever highly exposed”). Subjects’ assigned exposure grade may exceed average workplace exposure. Potential co-exposure to styrene and solvents.		low extent of co-exposures.			
Dell and Teta (1995) United States Cohort study of workers in a plastics manufacturing and research and development facility which made phenol-formaldehyde resins, working 1946–1967 with follow-up through 1988.	5,932 white men employed for at least 7 mos. Vital status was 94% complete. Death certificates obtained for 98%. Average follow-up 32 years. All cancer SMR = 1.02.	Individual exposure measures not evaluated. Only 111 men (2%) had work assignments involving formaldehyde. However, as the plant manufactured and used formaldehyde since 1931, a larger percentage may have actually been exposed. Variation in presumed exposure by department and pay status. Co-exposures: acrylonitrile, asbestos ,	Mortality: underlying cause from death certificates, ICD version in effect at time of death. MM: ICD 203.	Adjusted for sex, race, age, and calendar-year. Asbestos is not associated with LHP cancers. Benzene and styrene were not evaluated as potential confounders and would likely be positively correlated with formaldehyde exposure.	SMRs (95% CI) by major department. Latency evaluated with exposure lag times of 10 and 15 years.	MM: 8 NPC: 0	SUMMARY for MM: LOW ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) with attenuation of association. Confounding possible. SUMMARY for NPC: Not informative Potential for information bias due to uncertainty in exposure assessment (Exposure Group C)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
		benzene , carbon black, epichlorohydrin, PVC (vinyl chloride), styrene , and toluene.		Potential for confounding is unknown but could have inflated the observed effect.			with attenuation of association. Confounding possible. Low sensitivity (few cases).
Edling et al. (1987b) Sweden Cohort study of workers in a production plant making abrasives bound with formaldehyde resins, working 1955 to 1981 with follow-up through 1983.	521 male workers employed at least 5 years. Vital status was 97% complete. All cancer SMR = 0.93.	Whole cohort assumed to be exposed with some individual's exposed to high peak exposures. Manufacture of grinding wheels bound by formaldehyde resins exposed company workers to 0.1–1 mg/m ³ formaldehyde. 59 workers (11%) had intermittent heavy exposures to formaldehyde with peaks up to 20–30 mg/m ³ . Co-exposed to aluminum oxide and silicon carbide.	Incidence (ICD-8), from National Cancer Registry. MM: ICD-203.	Controlled for sex, age, and calendar-year-specific mortality rates. Co-exposures are not known risk factors for this outcomes.	SIRs (95% CI). Latency not evaluated.	MM: 2	SUMMARY: MM: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group B) and lack of latency analysis with attenuation of association. Low sensitivity (few cases).
Fryzek et al. (2005) United States Cohort mortality study of workers in motion picture film processing, working 1960 to 2000,	2,646 workers (11% female) employed at least 3 mos. 178 workers (7%) excluded	Individual-level occupational histories were used to classify workers in job families matched to past industrial hygiene surveys conducted in	Mortality: underlying cause from death certificates. HL: ICD-9 201 MM: ICD-9 203.	Controlled for age, sex, race, and time period. Perchloroethylene may be a risk factor for multiple	SMRs (95% CI). Decade of exposure, duration of exposure and time since first	HL: 0 MM: 2	SUMMARY: NOT INFORMATIVE Critical limitation: Confounding

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
with follow-up through 2000.	for missing work histories or work outside the study period. Vital status obtained for 99.7%; cause of death data for 655 of 666 decedents (98.3%). Average length of follow-up ≈20.58 years. All cancer SMR = 1.1.	house and by state program. Formaldehyde used in “film developing” and possibly in ‘maintenance’. Personal and area sample averaged 0.28–0.29 ppm with range 0.06–0.52. Co-exposures included methanol, methyl chloroform, perchloroethylene , and hydroquinone.	Higher survival rates for HL could undercount incident cases, but average follow-up is more than 20 years.	myeloma as may hydroquinone which is a metabolite of benzene, a known cause of LHP cancers. Potential for confounding is unknown but could have substantially inflated the observed effect due to the high correlation of these exposures with formaldehyde.	exposure were evaluated. Latency was evaluated as time since first exposure.		
Hall et al. (1991) Great Britain Cohort study of British pathologists. <u>Related studies:</u> Initial follow-up through 1973 Harrington and Shannon (1975) Second follow-up through 1980 Harrington and Oakes (1984) .	4,512 pathologists from the Royal College of Pathologists and the Pathological Society of Great Britain from 1974–1987. Deaths among those >85 years were censored. Vital status was	As a profession, pathologists were highly exposed to formaldehyde as a main ingredient in tissue fixative. NIOSH (<i>Industry Selection for Determination of Extent of Exposure</i> , 1979) has reported mean formaldehyde concentrations of 4.35 ppm with range (2.2–7.9).	Mortality: cause of death = Hodgkin lymphoma, ICD 8: code 201. Higher survival rates for HL could undercount incident cases, but maximum follow-up is 13 years with 5% mortality during follow-up.	Controlled for age, sex, and calendar year. Chemical co-exposures are not known risk factors for this outcome. Radiation exposure likely to be poorly correlated with formaldehyde.	SMRs (95% CI) developed from the English and Welsh populations. Latency not evaluated.	HL: 1 Low power due to the rarity of cases.	SUMMARY: NOT INFORMATIVE Critical limitations: Selection bias and information bias.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	obtained from the census, a national health registry, and other sources (100%). Cause of death data for 222 of 231 individuals (96.5%). All cancer SMR = 0.44.	Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and ionizing radiation .					
Hansen et al. (1994) Denmark Cohort study of workers at a Danish pharmaceutical plant.	10,889 employees (51% women) ever employed 1964–1988 at a pharmaceutical plant. Cases were extracted from the Danish Cancer Registry. All cancer SIR (men)=0.95 All cancer SIR (women) = 1.16.	No individual-level exposures estimated: whole cohort assumed to be exposed. Formaldehyde was one of many exposures in this industry but <u>not</u> a main ingredient or product. Co-exposures may have included asbestos , antibiotics, chloroform, dichloromethane, enzymes, ethylene oxide , glucagon heparin, insulin, silica, sex hormones, sodium saccharin, and synthetic agents.	Incidence: cases from Danish Cancer Registry classified according to ICD-7. HL: ICD 201 MM: ICD 203. Higher survival rates for HL could undercount incident cases, although average follow-up is 13 years.	Controlled for age, sex, and calendar year. Asbestos is associated with URT cancers. Ethylene oxide is associated with LHP cancers. Neither were evaluated as potential confounders. Potential for confounding is mitigated by low formaldehyde exposure and likely low correlation with asbestos and ethylene oxide.	SIRs (95% CI). Latency not evaluated.	HL: 4 Larynx: 5 MM: 0 Low power due to the rarity of cases and low confidence in formaldehyde exposure.	SUMMARY: NOT INFORMATIVE Critical limitation: Information bias (minimal exposure)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Hansen and Olsen (1995) . Denmark Cohort study of Danish men, URT cancers diagnosed 1970–1984.	2,041 men with incident cancer whose longest work experience occurred at least 10 years before cancer diagnosis. Cases matched with employment records from pension fund (72%) with remainder being self-employed, pensioners, & unemployed. External comparison with general population. Average follow-up ≈13 years.	Individual occupational histories including industry and job title established through company tax records. Considered exposed if worked in plant with more than 1 kg formaldehyde used per employee per year. Very crude exposure assessment. No information on co-exposures except for wood dust .	Incident cases identified in Danish Cancer Registry (ICD-7). NPC: 146 SNC: 160 Larynx: 161 HL: 201. Higher survival rates for HL could undercount incident cases, although average follow-up is approximately 13 years.	Controlled for age, sex, and calendar time. Sinonasal cancer risk was evaluated controlling for wood dust. While other co-exposures were not evaluated, the overall correlation between co-exposures in multiple occupational industries is likely to be low.	SPIRs (95% CI) (Standardized proportionate incidence ratio) - proportion of cases for a given cancer in formaldehyde-associated companies relative to the proportion of cases for the same cancer among all employees in Denmark. Latency addressed by inclusion criteria.	NPC: 4 SNC: 13 Larynx: 32 HL: 12	SUMMARY: HL, Larynx, NPC, SNC: LOW ↓ (Potential bias toward the null) Potential selection bias. High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) with attenuation of association. Low sensitivity for NPC (few cases).
Harrington and Oakes (1984) . Great Britain Second cohort study of British pathologists. <u>Related studies:</u>	2,720 pathologists from the Royal College of Pathologists and the Pathological Society of	As a profession, pathologists were highly exposed to formaldehyde as a main ingredient in tissue fixative.	Mortality: cause of death sinonasal cancer.	Controlled for age, sex, and calendar year. Radiation exposure likely to be poorly correlated with formaldehyde.	SMRs (95% CI) developed from the English and Welsh populations. Latency not evaluated.	SNC: 0 Low power due to the rarity of cases.	SUMMARY: NOT INFORMATIVE Critical limitations: Selection bias and information bias.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Initial follow-up through 1973 Harrington and Shannon (1975) Third follow-up through 1987 Hall et al. (1991) .	Great Britain from 1974–1980. Deaths among those >85 years were censored. Vital status was obtained from the census, a national health registry, and other sources (100%). 96% of death certificates were obtained with 91 reporting a cause of death. All cancer SMR = 0.61.	NIOSH (<i>Industry Selection for Determination of Extent of Exposure</i> , 1979) has reported mean formaldehyde concentrations of 4.35 ppm with range (2.2–7.9). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and ionizing radiation .		Chemical co-exposures are not known risk factors for this outcome.			
Hauptmann et al. (2009) . United States Nested case-control study within extension of embalmers cohorts described in Walrath and Fraumeni (1983, 1984) ; Hayes et al. (1990) .	Embalms (8% women) from national and state funeral directors associations and licensing boards. Died 1960–1986. Participation rate of case interviews was 220/228 (96%)	Individual level, based on lifetime work practices and exposures to formaldehyde obtained by interview with next of kin or co-workers (96% of cases and controls) with information on occupational exposure resulting from embalming.	Mortality: underlying cause from death certificates, ICD-8. MM: ICD 203 LL: ICD 204 ML: ICD 205. Higher survival rates for HL could undercount incident cases, but average follow-up is more than 39 years (485	Controlled for date of birth, age at death, sex, data source, and smoking. Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are not	Logistic regression, OR (95% CI) by exposure categories (4 levels) for duration, number of embalmings, cumulative exposure, average intensity, time-weighted average, and peak exposure measures.	ML: 34 (17 acute) MM: <i>n</i> cases not reported but must be greater than 5 due to size of se(ln(OR)). LL: 99 NPC: 4	SUMMARY: ML: HIGH (No appreciable bias) LL, MM: MEDIUM ↓ (Potential bias toward the null) Low potential for information bias due to uncertainty in exposure assessment (Exposure Group A).

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	and 265/282 eligible controls (94%). Controls randomly selected from individuals in the funeral industry whose deaths were attributed to other causes. Controls stratified to be similar to data source, sex, and dates of birth and death (5-year intervals).	Interviewers blinded to outcome. Exposure levels assigned based on laboratory reconstruction of exposures for specific work practices. Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and ionizing radiation .	cases and controls/19,104 person-years).	known risk factors for this outcome.	Analyses of duration of exposure for ML is proxy for latency. Latency not evaluated for MM or LL.		Potential for information bias due lack of latency analysis with attenuation of association. NPC: MEDIUM ↓ (Potential bias toward the null) Low potential for information bias due to uncertainty in exposure assessment (Exposure Group A) Low sensitivity (few cases).
Hayes et al. (1990) United States Cohort study of embalmers. <u>Related study:</u> Hauptmann et al. (2009)	4,046 deceased male embalmers and funeral directors, derived from state licensing boards and funeral director who died during 1975–1985 and a death certificate could be obtained.	Individual exposure measures not derived. Occupation confirmed from death certificates. Separate study estimated personal formaldehyde exposures from 0.98 ppm (high ventilation) to 3.99 ppm (low ventilation), with peaks up to 20 ppm. Co-exposures may have included: phenol, methyl alcohol,	Mortality: underlying cause of death from death certificates, ICD-8; ICD 201 = HL ICD 203 = MM ICD 204 = LL ICD 205 = ML. Higher survival rates for HL and LL could undercount incident cases, and median follow-up is unknown.	Controlled for calendar year, age, sex, and race. Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are not known risk factors for this outcome.	PMR (95% CI). Latency not evaluated.	HL: 3 Larynx: 7 LL: 7 ML: 24 MM: 20 NPC: 4 SNC: 0 Possible undercounting of cases due to abbreviated death certificate search.	SUMMARY: Larynx, LL, ML, MM: MEDIUM ↓ (Potential bias toward the null) Low potential for information bias due to uncertainty in exposure assessment (Exposure Group A). Potential for information bias due lack of latency analysis with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	Death certificates obtained for 79% of potential study subjects. The 21% missing death certificates considered to missing at random because all embalmers were considered to be exposed to formaldehyde. All cancer PMR (white) = 1.07 (nonwhite) = 1.08.	glutaraldehyde, mercury, arsenic, zinc, and ionizing radiation .					HL, NPC, SNC: LOW ↓ (Potential bias toward the null) Low potential for information bias due to uncertainty in exposure assessment (Exposure Group A). Potential for information bias due lack of latency analysis with attenuation of association. Low sensitivity (few cases).
Jakobsson et al. (1997) Sweden Cohort study of workers grinding stainless steel.	727 male employees of 2 plants producing stainless steel sinks and sauce pans employed at least 1 year during 1927–1981 with minimum 15-year follow-up.	No individual exposure measures. Presumed exposure was to phenol-formaldehyde resins on ribbons or plates in grinding workers. Co-exposures may have included chromium, nickel, and abrasive dusts including silicon	Incidence: cases from Swedish Tumor Registry SN ICD-7 160.	Adjusted for sex, age, and calendar year. Nickel and chromium are associated with URT cancers and would likely be positively correlated with formaldehyde exposure.	SIRs (95% CIs). Latency addressed by enforcing a 15-year waiting period to begin observation.	Larynx:1 SNC: 0 Low power due to the rarity of cases.	SUMMARY: Larynx, SNC: LOW ↓ (Potential bias toward the null) High potential for information bias with attenuation of association (Exposure Group D). Confounding possible. Low sensitivity (few cases).

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	Of 823 original workers, 23 (3%) could not be identified, 12 died or emigrated before 1952 (1%), and 61 did not exceed the 15 year waiting period. No further losses to follow-up. All cancer SIR = 0.9.	carbide, aluminum oxide, silicon dioxide, and clay. No wood dust exposures.		Potential for confounding is unknown but could have inflated the observed effect. Other co-exposures are not known risk factors for these outcomes.			
Levine et al. (1984a) Canada Cohort study of undertakers.	1,477 male undertakers first licensed during 1928–1977 with mortality follow-up from 1950–1977. Vital status was 96% complete with cause of death available for 94%. Average follow-up 25 years.	As a profession, undertakers/embalmers were highly exposed to formaldehyde as a main ingredient in tissue fixative. Kerfoot and Mooney (1975) reported mean formaldehyde concentrations for embalmers in funeral homes of 0.74 ppm with range (0.09–5.26). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde,	Mortality: underlying cause from death certificates (ICD-8). Nose, middle ear, sinuses: 160 Larynx: 161.	Controlled for calendar year, age, and sex. Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are not known risk factors for this outcome.	SMR, 95% CI. Latency was not evaluated for these endpoints.	SNC: 0 Larynx: 1 Low power due to the rarity of cases.	SUMMARY: Larynx, SNC: LOW ↓ (Potential bias toward the null) Low potential for information bias due to uncertainty in exposure assessment (Exposure Group A). Potential for information bias due lack of latency analysis with attenuation of association. Low sensitivity (few cases).

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	All cancer SMR = 0.87.	mercury, arsenic, zinc, and ionizing radiation.					
Li et al. (2006) China Nested case-cohort study within a cohort study of textile workers.	67 women diagnosed during 1989–1998 with nasopharyngeal cancers were identified in a cohort of 267,400 female textile workers born during 1925–1958. Nine additional cases (12% of total) were excluded due to lack of occupational histories. 3,188 controls randomly selected from the cohort; frequency matched by age.	Individual level, based on job exposure matrix developed for this industry/setting (unclear extent of industrial hygiene specifically for formaldehyde). No historical measurements of exposures. No cases were classified as exposed and only 10/3,188 controls (0.3%) were classified as exposed. EPA considered the potential for formaldehyde exposure to be exceedingly low. Co-exposed to cotton dust.	Incidence or mortality. Diagnosis of nasopharyngeal cancer or sinonasal cancer reported to a cancer and death registry operated by the Shanghai Textile Industry Bureau. NPC: ICD-9 147 SN: ICD-9 160.	Controlled for age and sex. Dusts could be a potential confounder but due to the rarity of formaldehyde exposure the correlation would be minimal.	Cox proportional hazards modeling adapted for case cohort design. Hazard ratios (95% CI). Duration and latency were not evaluated.	NPC: 10 No cases exposed. Very low power due to the rarity of exposure.	SUMMARY: NOT INFORMATIVE Critical limitation: Very low sensitivity rare exposure.
Walker et al. (1990) Sweden Cancer registry-based study, NPC diagnosed 1961–1979.	471 employed men with incident NPC cancer.	No individual exposure measures. Occupations and industries with potential exposure to	Incident cases identified in Swedish Cancer-Environment Registry.	Controlled for age and region. Variation in exposure was not evaluated.	SIRs (95% CI). Latency not evaluated.	NPC: 4 cases in workers exposed in fiberboard plants	SUMMARY: NPC: Low ↓ (Potential bias toward the null) High potential for information bias due

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
		formaldehyde: bookbinders, fiberboard makers, textile workers, furniture makers, chemical workers, physicians, foundry workers, biologists, tanners, and skin processors, worker employed in veneer and plywood plants and in sugar processing plants. Co-exposure information not provided.	Microscopic confirmation obtained for 99.6% of NPC cases. 48% squamous cell carcinomas, 37% unspecified carcinomas, 5% transitional cell carcinomas, and 3% adenocarcinomas.	Co-exposures were also not evaluated. Fiberboard workers are also exposed to wood dust. Wood dust is associated with URT cancers and would likely be positively correlated with formaldehyde exposure. Potential for confounding is unknown but could have inflated the observed effect.			to uncertainty in exposure assessment (Exposure Group D) and lack of latency analysis with attenuation of association. Confounding possible. Low sensitivity (few exposed cases).
Marsh et al. (2002) ; Marsh et al. (2007) United States Nested case-control study within a cohort study of workers in one plant using formaldehyde. <u>Related studies:</u> Initial 10 plant cohort follow-up through 1980 Blair et al.	7,328 workers employed at a formaldehyde using plant in Connecticut followed from 1945 through 1998. Vital status was identified from the National Death Index, private businesses, or	Worker-specific exposure measures from job exposure matrix based on available sporadic plant monitoring data from 1965–1987, job descriptions, and verbal job descriptions by plant personnel and industrial hygienists. Exposure assessment did not include the	Mortality: oropharyngeal code ICD-9: 146. Hypopharyngeal code ICD-9: 148. Nasopharyngeal code ICD-9: 147. Pharyngeal ICD-9: 146–149. Death certificates used to determine underlying cause of death according to	Controlled for age, race, sex, and time period. Comparison was with U.S. death rates and with death rates in 2 counties. Benzene is not associated with URT cancers. Potential	SMR (95%CI) Secondary analysis for NPC. EPA derived SMRs for the combination of oropharyngeal, hypopharyngeal and unspecified pharyngeal cancer by NPC cases from all pharyngeal cancers.	Oro: 5 Hypo: 3 Low power due to the rarity of cases. NPC: cases included in Beane Freeman et al. (2013) .	SUMMARY: Oro- alone & Hypo- alone: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group B) and lack of latency analysis with attenuation of

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
(1986); Blair et al. (1987). Second set of 10 plant follow-ups through 1994 Hauptmann et al. (2003); Hauptmann et al. (2004a). Third set of 10 plant follow-ups through 2004 Beane Freeman et al. (2009); Beane Freeman et al. (2013).	state and local agencies, and was 98.4% complete; cause of death data for 95% of 2,872 deaths. Average follow-up ≈32.89 years. All cancer SMR = 1.08.	same industrial hygiene sampling conducted by Stewart et al. (1986) used in the Beane Freeman et al. (2009); Beane Freeman et al. (2013) analyses which included this plant. Exposure estimates were on average 10 times lower than those of other studies in this plant (Blair et al., 1986; Beane Freeman et al., 2009; Beane Freeman et al., 2013). From Beane Freeman et al. (2009); Beane Freeman et al. (2013): Co-exposed to antioxidants, benzene, carbon black, dyes and pigments, melamine, hexamethylenetetramine, phenols, plasticizers, urea, wood dust.	the ICD codes at time of death. Histological typing not reported.	confounders were evaluated but only smoking was found to be a potential confounder and was controlled for. Co-exposures to pigments and particles were evaluated and were found not to be confounding. Marsh et al. (2002) attempted to evaluate smoking but data were incomplete. No other potential confounders were evaluated. Beane Freeman et al. (2009); Beane Freeman et al. (2013) evaluated 11 potential confounders among a set of 10 plants that included this one and did not find any confounding.	Latency not evaluated.		association. Low sensitivity (few cases). OHPC together: MEDIUM ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group B) and lack of latency analysis with attenuation of association.
Matanoski (1989) United States	3,644 deceased male	As a profession, pathologists were	Mortality: death certificates and	Controlled for sex, race, age, and	SMRs (95% CI).	HL: 2 cases total	SUMMARY: NOT INFORMATIVE

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Prospective mortality cohort study with two external comparison groups.	pathologists, derived from membership rolls of multiple professional societies. Mortality followed through 1978. Death certificates obtained for 94% of potential study subjects, 3% from obituary notices and 3% presumed dead. All cancer SMR = 0.78.	highly exposed to formaldehyde as a main ingredient in tissue fixative. NIOSH (<i>Industry Selection for Determination of Extent of Exposure</i>, 1979) has reported mean formaldehyde concentrations of 4.35 ppm with range (2.2–7.9). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and ionizing radiation.	obituary notices used to determine cause of death from Hodgkin lymphoma (ICD-8: 201). Higher survival rates for HL could undercount incident cases, although median follow-up is probably more than 15 years since follow-up was from the early 20th century through 1978.	calendar-year-expected deaths from the U.S. population and psychiatrists. Variation in exposure was not evaluated. Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are not known risk factors for this outcome.	Latency not evaluated.	Low power due to the rarity of cases.	Critical limitation: Selection and information biases.
Meyers et al. (2013) United States Prospective cohort mortality study. <u>Related studies:</u> Initial cohort follow-up Stayner et al. (1988). Second follow-up Pinkerton et al. (2004)	Workers in 3 U.S. garment plants (n=11,043) in Georgia and Pennsylvania exposed for at least 3 mos (82% female). Vital status was followed through 2008 with 99%	Individual-level exposure estimates for 549 randomly selected workers during 1981 and 1984 with 12–73 within each department. Formaldehyde levels across all departments and facilities were similar.	Mortality: death certificates used to determine the underlying cause of death (ICD-10): NPC: C11 OHPC: C09-C10, C12-C14 SN: C30-31 Larynx: C32. HL:C81	Adjusted for sex, age, race, and calendar-year specific US mortality rates. No other chemical exposures were identified by the industrial hygiene surveys that could influence the findings.	SMRs (95% CI), by exposure categories (3 levels) for duration, time since first exposure measures. SRRs (95% CI) (internal comparison), by 3 categories of	NPC: 0 OHPC: 6 SNC: 0 Larynx: 4 ML: 21 (14 acute; 5 chronic) LL: 6 HL: 4 MM: 23	SUMMARY: LL, ML: HIGH (No appreciable bias) Larynx, NPC, SNC: LOW ↓ (Potential bias toward the null) Potential for information bias due lack of latency analysis with attenuation of

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	completion. Causes of death were obtained for 3,904 (99.7%) of the 3,915 identified deaths. Average follow-up ≈37.52 years. All cancer SMR = 0.96.	Exposures ranged from 0.09–0.20 ppm. Overall geometric mean concentration of formaldehyde was 0.15 ppm, (GSD 1.90 ppm). Area measures showed constant levels without peaks. No other chemical exposures were identified by the industrial hygiene surveys. There was no information on smoking in this analysis, however, according to Stayner et al. (1988), “the overall prevalence of cigarette smokers was 29.4%. In plant 1 the prevalence was 26.6%, in plant 2 it was 33.5%, and in plant 3 it was 29.4%. These figures are similar to those reported in a 1980 survey of adult Americans, in which 29.2% of females and 38.3% of males over the age of 20 were current cigarette smokers [NCHS, 1985].”	LL: C91.0-91.3, C91.5-91.9 ML: C92 MM: C88.7, 88.9, 90. Higher survival rates for HL could undercount incident cases, but average follow-up is more than 37 years Histological typing not reported.		duration of exposure. Latency effects were examined for leukemia only.		association. Low sensitivity (few cases). HL, MM, OHPC: MEDIUM ↓ (Potential bias toward the null) Potential for information bias due lack of latency analysis with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
Ott et al. (1989) United States (West Virginia) Nested case-control study within two chemical manufacturing plants.	29,139 male workers followed from 1940–1978. Loss to follow-up 3.6%. 95.4% of death certificates obtained. Frequency matching of controls (5:1) from the total employee cohort according to a group-matched incidence density sampling design.	Individual-level exposure classification based on company records of work assignments linked to records on department usage of formaldehyde. Exposures during 1940 to 1978. 21 different chemicals were evaluated including benzene with much cross exposure.	Mortality: underlying cause from death certificates, ICD version in effect at time of death. Higher survival rates for LL could undercount incident cases, but average follow-up is likely more than 15 years as follow up was initiated in 1940 and ceased in 1978.	Unconditional logistic regression. Controlled for sex and age. Controlling for age did not change results. Benzene was not evaluated as a potential confounder and may be positively correlated with formaldehyde exposure. Potential for confounding is unknown but could have inflated the observed effect. Potential for confounding may be mitigated by rarity of co-exposures among cases.	OR (95% CI). Analyses conducted with a 5-year exposure lag. Limited adjustment for latency.	MM: 20 ML: 39 LL: 18 ≤2 exposed cases for each endpoint Low power due to the rarity of exposure.	SUMMARY: LL, ML, MM: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group B) and weak latency analysis with attenuation of association. Confounding possible. Low sensitivity due to rarity of exposure.
Pira et al. (2014) Italy Prospective cohort mortality study with	2,750 workers employed at a laminated plastic factory in Italy for at least 180 days	Formaldehyde is a byproduct from the resins used in production process and all workers were	Death certificates used to determine UCOD from multiple myeloma (ICD-9: 203).	Adjusted for sex, age, and 5-year calendar periods using mortality rates from the Piedmont region.	SMRs (95% CI). Latency not evaluated.	MM: 0 cases	SUMMARY: MM: LOW ↓ (Potential bias toward the null)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
external comparison group.	between 1947 and 2011 followed until May 2011. Deaths were identified from population registries. Vital status was 96.9% complete and only 3.1% lost to follow-up.	presumed to have been exposed. Exposure period from 1947 through 2011. Median length of follow-up: 23.6 years. Duration and timing since first exposure were not evaluated.		Coexposures not evaluated.			Potential selection bias. High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) and lack of latency analysis with attenuation of association. Confounding possible. Low sensitivity (few cases).
Robinson et al. (1987) United States Prospective cohort mortality study.	Plywood mill workers (<i>n</i> = 2,283) employed at least 1 year during 1945–1955 followed for mortality until 1977 with vital status for 98% and death certificates for 97% of deceased. Average follow-up ≈25.22 years. All cancer SMR = 0.7.	Individual exposure measures not derived. Presumed exposure to formaldehyde-based glues used to manufacture and patch plywood. Co-exposure to carbon disulfide, pentachlorophenol , wood dust.	Mortality: underlying cause from death certificates (ICD-7) HL: 201 MM: 203. Higher survival rates for HL could undercount incident cases, but average follow-up is more than 25 years.	Adjusted for sex, age, race, and calendar-year-specific U.S. mortality rates. Some exposed workers also exposed to pentachlorophenol for more than 1 year. EPA concluded that penta-chlorophenol is likely to be carcinogenic based on strong evidence from epidemiologic studies of increased risk of MM.	SMRs (90% CI). Latency not evaluated.	MM: 3 cases HL: 2 cases (2 cases, whole cohort of mill workers; 2 cases, subcohort of exposed workers)	SUMMARY: MM: Not informative, Critical limitations: Selection bias, information bias, confounding. HL: LOW ↓ (Potential bias toward the null) Potential selection bias. High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) and lack of latency analysis with attenuation of association. Low sensitivity (few cases).

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
				Potential for confounding is unknown but could have inflated the observed effect for MM but not for HL.			
Saber Hosnijeh et al. (2013) Europe Prospective cohort study.	241,465 men and women recruited from 10 European countries during 1992–2000. Participants were predominantly ages 35–70 at recruitment and were followed up through 2010.	Occupational histories obtained by questionnaire about ever working in any of 52 occupations considered to be at high risk of developing cancer. Occupational exposures estimated as “high,” “low,” and no exposure by linking to a JEM.	Incident primary leukemias identified from cancer registries, health insurance records, pathology registries and contact with subjects of their next of kin.	Controlled for age, sex, smoking, alcohol, physical activity, education, BMI, family history of cancer, country, other occupational exposures, and radiation.	Proportional hazards regression; HRs (95% CI). Latency was not evaluated.	LL: 67/225 exposed ML: 49/179 exposed	SUMMARY: LL, ML: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group C) and lack of latency analysis with attenuation of association.
Siew et al. (2012) Finland National cohort study.	All Finnish men born during 1906–1945 who participated in census and were employed in 1970 ($n=1.2$ million). Cancer cases identified by national	Occupational history from census records were linked to the national JEM to code each cohort member with “any” exposure to formaldehyde or “none.” Only some use of “industry” information.	Diagnosis of cancer reported to the Finnish Cancer Registry.	Controlled for age, sex, socioeconomic status, smoking, and wood dust.	SIRs (95% CI). A 20-year latency period was assumed.	NPC: 149 SNC: 167. Baseline incidence of NPC in this population is the lowest in the world.	SUMMARY: NPC, SNC: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) with attenuation of association. Low

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	registry during 1971–1995.	3% of NPC cases exposed 5% of SNC cases exposed Co-exposure wood dust was collected.					sensitivity (low background rate of cancer).
Solet et al. (1989) United States Proportionate mortality study of pulp and paper workers.	201 white male pulp and paper producing workers who died during 1970–1984 and had at least 10 years of experience in the industry. All cancer PMR = 1.31.	Occupational history from union records identified workers in the pulp and paper producing jobs. Formaldehyde is known to be an exposure for pulp and paper mill workers: job-specific exposures range from 0.2 to 1.1 ppm with peaks as high as 50 ppm (Korhonen et al., 2004). From Band et al. (1997) , co-exposed to arsenic, chlorophenols , sulfuric acid mists , and chloroform. According to a review Kauppinen et al. (1997) co-exposures to dioxin or perchloroethylene are also possible.	Mortality: underlying cause from death certificate submitted to the Union Pension Fund. HL: ICD-8 201. Higher survival rates for HL could undercount incident cases, but average follow-up is probably more than 15 years because workers had to have at least 10 years of experience in the industry.	Controlled for age, sex, race, age at death, and calendar time. Confounding not evaluated. Potential confounders for these outcomes include chlorophenols, acids mists, dioxin, and perchloroethylene, which are likely to have been positively correlated with formaldehyde exposure. Other co-exposures are not known risk factors for these outcomes.	PMRs (95% CI). Latency not evaluated.	HL: 1 case Low power due to the rarity of cases.	SUMMARY: NOT INFORMATIVE Critical limitation: (Information bias and sensitivity)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
				Potential for confounding is unknown but could have inflated the observed effect.			
Stellman et al. (1998) United States General population cohort. Baseline enrollment in 1982; follow-up through 1988.	317,424 men enrolled in the American Cancer Society's Cancer Prevention Study II in 1982. Follow-up was 98% complete. Median follow-up 6 years. Average follow-up ≈5.79 years.	Individual level, based on questionnaire response (Yes/No) on formaldehyde exposure. Excludes wood-related occupations. Specific co-exposures included asbestos and wood dust .	Mortality: death certificates, MM: ICD-9 203.	Controlled for age, sex, and smoking. Co-exposures are not associated with LHP cancers.	Poisson regression (internal comparison) RRs (95% CI). Latency not evaluated.	MM: 14 (4 exposed) Low power due to the rarity of exposure.	SUMMARY: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group C) and lack of latency analysis with attenuation of association. Low sensitivity (few cases).
Stroup et al. (1986) United States Retrospective cohort mortality study.	2,239 deceased white male anatomists identified from professional societies who died during 1925–1979. 91% of death certificates of	As a profession, anatomists were highly exposed to formaldehyde as a main ingredient in tissue fixative. Akbar-Khanzadeh and Mlynec (1997) reported mean formaldehyde	Mortality: underlying cause from death certificates (ICD-8), HL: 201 Larynx: 161 ML: 205 SNC: 160. Higher survival rates for HL could undercount incident	Controlled for calendar year, age, sex, race compared with U.S. population. Radiation exposure likely to be poorly correlated with formaldehyde.	SMR (95% CI). Latency not evaluated.	HL: 0 Larynx: 1 ML: 5 (1 acute, 3 chronic, 1 unspecified) SNC: 0 Low power due to the rarity of cases.	SUMMARY: HL, Larynx, ML, SNC: LOW ↓ (Potential bias toward the null) High potential for selection bias. Low potential for information bias due to uncertainty in

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	known deceased obtained. Average follow-up ≈22.52 years. All cancer SMR = 0.64.	concentrations in anatomy laboratories of 1.9 ppm with range (0.3–4.5). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and ionizing radiation . Anatomists may also be co-exposed to stains, benzene , toluene, xylene, chlorinated hydrocarbons, dioxane, and osmium tetroxide.	cases, but average follow-up is more than 22 years.	Benzene not evaluated as potential confounder but is a risk factor for ML. Potential for confounding is unknown but could have inflated the observed effect.			exposure assessment (Exposure Group A). Potential for information bias due lack of latency analysis with attenuation of association. Confounding possible for ML. Low sensitivity (few cases).
Walrath and Fraumeni (1983) United States Cohort mortality study. <u>Related study:</u> Hauptmann et al. (2009)	1,132 deceased white male embalmers identified from NY state license board. Died 1925–1980. Death certificates obtained for 75%. The 25% missing death certificates considered to missing at random because all	As a profession, embalmers were highly exposed to formaldehyde as a main ingredient in tissue fixative. Kerfoot and Mooney (1975) reported mean formaldehyde concentrations for embalmers in funeral homes of 0.74 ppm with range (0.09–5.26). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde,	Mortality: underlying cause from death certificates (ICD-8) HL: 201 LL: 204 ML: 205. Higher survival rates for HL and LL could undercount incident cases, but average follow-up is likely more than 15 years as follow up was initiated in 1925 and ceased in 1980.	Controlled for calendar year, age, sex, and race. Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are not known risk factors for this outcome.	PMR, 95% CI. Latency was not evaluated for these endpoints.	HL: 7 Larynx: 2 LL: 4 ML: 7 SNC: 0 Low power for LL due to the rarity of cases.	SUMMARY: Larynx, LL, SNC: LOW ↓ (Potential bias toward the null) Low potential for information bias due to uncertainty in exposure assessment (Exposure Group A). Potential for information bias due lack of latency analysis with attenuation of association. Low sensitivity (few cases).

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	embalmers were considered to be exposed to formaldehyde. All cancer PMR = 1.11.	mercury, arsenic, zinc, and ionizing radiation .					HL, ML: MEDIUM ↓ (Potential bias toward the null) Potential for information bias due lack of latency analysis with attenuation of association.
Walrath and Fraumeni (1984) United States Cohort mortality study. <u>Related study:</u> Hauptmann et al. (2009)	1,007 deceased white male embalmers identified from CA state license board. Died 1925–1980. Death certificates obtained for 100%. All cancer PMR = 1.04.	As a profession, embalmers were highly exposed to formaldehyde as a main ingredient in tissue fixative. Kerfoot and Mooney (1975) reported mean formaldehyde concentrations for embalmers in funeral homes of 0.74 ppm with range (0.09–5.26). Co-exposures may have included: phenol, methyl alcohol, glutaraldehyde, mercury, arsenic, zinc, and ionizing radiation .	Mortality: underlying cause from death certificates (ICD-8) HL: 201 LL: 204 ML: 205. Higher survival rates for HL and LL could undercount incident cases, but average follow-up is likely more than 15 years as follow up was initiated in 1925 and ceased in 1980.	Controlled for calendar year, age, sex, and race. Radiation exposure likely to be poorly correlated with formaldehyde. Chemical co-exposures are not known risk factors for this outcome.	PMR, 95% CI. Latency was not evaluated for these endpoints.	ML: 8 Larynx: 2 LL: 4 HL: 0 SNC: 0 Low power due to the rarity of cases.	SUMMARY: HL, Larynx, LL, SNC: LOW ↓ (Potential bias toward the null) Low potential for information bias due to uncertainty in exposure assessment (Exposure Group A). Potential for information bias due lack of latency analysis with attenuation of association. Low sensitivity (few cases). ML: Medium ↓ (Potential bias toward the null) Low potential for information bias due to uncertainty in exposure assessment (Exposure Group A).

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
							Potential for information bias due lack of latency analysis with attenuation of association.
Wesseling et al. (1996) Costa Rica Cohort study of banana plantation workers.	26,565 male workers on the payrolls of banana companies as reported to the Social Security Administration between 1972 and 1979. Cohort follow-up in the cancer registry from 1981 to 1992. Losses to follow-up and poor record keeping resulted in difficulty in assessing participation rates. Very low confidence in data quality. Average follow-up ≈11.83 years.	A list of names of workers sterilized by dibromochloropropane was used to identify banana plantations whose workers may have been exposed to formaldehyde. Co-exposed to maneb, dibromochloropropane, mancozeb, benomyl, chlorothalonil.	Incidence: National Tumor Registry. HL: ICD-9 965-966 MM: ICD-9 973. Higher survival rates for HL and LL could undercount incident cases, but average follow-up is 12 years.	Controlled for age and sex. Banana plantation workers are co-exposed to several potential carcinogens such as dibromochloropropane, maneb, mancozeb, benomyl, and chlorothalonil. While these chemical co-exposures are not known risk factors for these outcomes the fact that co-exposures were so high as to cause sterility in workers strongly suggests a large potential for confounding.	SIR (95% CIs). Latency was not evaluated for these endpoints.	Males: HL: 9 cases MM: 6 cases	SUMMARY: NOT INFORMATIVE Critical limitations: (Selection bias, information bias, confounding)

Reference, setting, and design	Participants and selection	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results	Study sensitivity	Evaluation of major bias categories
	All cancer SIR = 0.76 (men).						

Table B-60. Evaluation of case-control studies of formaldehyde and cancers of the URT (NPC, SN, OHPC) and LHP (HL, MM, LL, ML)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Armstrong et al. (2000) Malaysia Population-based case-control study of NPC.	Prevalent and incident NPC cases (31% female) during 1987–1992 identified through treatment or diagnosis records from 4 radiotherapy centers. Participation of cases was 53% due to death and illness and difficulty in locating them. Participation of living cases who could be located was 89% ($n = 282$) and 90% for	Individual-level exposure status based on occupational history obtained by interview including job description, worked performed, calendar time, machines, tools, substances used, and exposures to dusts, smoke, gases, and chemicals. Exposure assessment blinded to outcome. 8/564 subjects (1.4%) had more than 10 years of potential exposure outside of a 10-year latency period. This suggests additional information bias.	Prevalent and incident cases. Diagnosis of NPC: confirmed by histological review. All cases were squamous cell carcinomas.	Design controlled for age, sex, Chinese ethnicity, and neighborhood. Analysis adjusted for social class, diet, smoking, and wood dust. Other exposures evaluated were wood dust, industrial heat, textile dusts, metals, acids, bases, solvents, detergents, and soaps. Wood dust is a potential confounder but was controlled for.	Conditional logistic regression; ORs (95% CI) for each of 22 separate occupational exposures. Latency was evaluated (exposures < 1, 5, 10, 15, and 20 years prior to diagnosis).	NPC: 282 The power to evaluate formaldehyde as a hazard is diminished as fewer than 10% of cases had any exposure to formaldehyde and 1.4% had exposure beyond a 10-year period.	Selection issue with substantial difference in participation rates. Exposure Group B Lack of latency data. Combination of rarity of exposure among cases with adequate latency resulted in very low power to detect any effects beyond a 10-year period. SUMMARY: NOT INFORMATIVE (Critical limitation: information bias)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	eligible controls ($n = 282$). Selection bias possible. Cases and controls were matched on age, sex, Chinese ethnicity, and neighborhood. Participation rate was somewhat lower in more affluent neighborhoods (80% vs. 90%).						
Berrino et al. (2003) Europe Population-based case-control study of larynx and hypopharynx cancer.	Male residential populations of 6 cancer registries in 4 European countries during 1979–1982. All patients with newly diagnosed cancer were identified with participation	Individual-level exposure status based on lifetime occupational history for all jobs held for more than 1 year obtained from questionnaire including job title, specific tasks, and calendar time. Multiple exposure metrics including peak, average, and cumulative exposure	Incident cases. Diagnosis of cancer of the larynx or hypopharynx confirmed by pathology review. Cancer of the larynx divided into epilarynx and endolarynx. Analyses of hypopharynx grouped together with epilarynx while endolarynx analyzed separately.	Controlled for age and sex by selecting controls from stratified population samples. Analysis controlled for study center, age, tobacco smoking, socioeconomic status, alcohol, and diet.	Unconditional logistic regression; OR (95% CI). Lagged exposures were evaluated to account for cancer latency in selected analyses.	Larynx (endolarynx): 213 total cases 37 cases exposed at least 10 years and more than 20 years since first exposure.	Exposure Group B downgraded to Group D based on poor performance of JEM. Confounding likely due to collinearity of exposures to other risk factors and potentially poor-quality exposure data which minimized ability to control. SUMMARY: NOT INFORMATIVE

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	rates of 70% to 92% by center. Controls participated at an average rate of 74%. Controls were selected from age and sex stratified random samples of the local general population.	developed by job exposure matrix. However, the quality of the exposure assessment is further degraded by the authors' statements. Namely, the authors regarded the "JEM performance as poor for formaldehyde where 14% of jobs classified as category 1 (unexposed) by the matrix were judged as definitely exposed by the experts." Co-linearity among crude exposures (e.g., solvents and formaldehyde had Spearman correlation of 0.4).	No separate analysis of hypopharynx without epiglarynx.	Exposures to other compounds were identified and evaluated as risk factors including asbestos, arsenic, solvents, and dusts (wood and other). Note that solvents were a stronger risk factor for laryngeal cancer than formaldehyde (OR=2.21 vs. 1.7). Co-exposures were controlled for but poorly measured covariates cannot be well controlled for.			Critical limitation: Confounding
Blair et al. (2001) United States Population-based case control of leukemia.	White men, ages ≥ 30 years. Cases (<i>n</i> = 513) identified 1980–1983 (cancer registry and hospital network). Controls (<i>n</i> = 1,087) selected by random digit dialing	Individual-level exposure status based on lifetime farm and nonfarm occupational history for all jobs held for more than 1 year obtained from interview including job title, industry, and calendar time. Other exposures evaluated included	Incident cases. Diagnosis of myeloid leukemia and lymphatic leukemia confirmed by pathology review.	Analysis controlled for age, state, direct or surrogate interview, and smoking. Other co-exposures were not evaluated as potential confounders.	Logistic regression; ORs (95% CI) by exposure categories (3 levels) for intensity, probability, duration, and time since first exposure measures.	ML: 22/59 exposed (14 acute; 8 chronic) LL: 30/190 exposed	SUMMARY: LL, ML: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group C) and lack of latency analysis with attenuation of

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	(under age 65) otherwise from lists provided by the HCFA and state death files. Controls were frequency-matched by 5-year age groups, vital status at interview, and state of residence. Cases participation rate was 86%. Control participation rate was 77–79%.	benzene, other organic solvents, petroleum-based oils & greases, cooking oils, ionizing radiation, paper dusts, gasoline and exhaust vapors, paints, metals, wood dust, asbestos, asphalt, cattle, meat, solder fumes.			Latency not evaluated.		association. Confounding possible.
D'Errico et al. (2009) Italy Hospital-based case-control study of SNC in the Piedmont region of Italy.	154 sinonasal cases during 1996–2000 identified through treatment or diagnosis records from all Piedmont hospital departments. 5 cases excluded (3 prevalent	Lifetime job history (all jobs); company, job title, tasks, size of work environment, and other details. Probability of exposure was determined by blinded expert staff for jobs lasting 6 or more mos.	Incident cases by cell type were taken from the regional Sinonasal Cancer Registry reported to them by hospitals in the region.	Analysis controlled for age, sex, province of residence, smoking and co-exposures. Wood dust is a considered an extremely strong risk factor for SNC and a potential confounder and	Unconditional logistic models; ORs (95% CI). Latency was evaluated with a 10-year latency period.	SNC: 7/113 exposed The power to evaluate formaldehyde as a hazard is diminished as fewer than 10% of cases had any exposure to formaldehyde.	Exposure Group B Wood dust is a likely confounder and no effect estimate adjusted for wood dust was presented. Low power SUMMARY: NOT INFORMATIVE

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	cases, 2 <30 years old). Participation of incident cases using full questionnaire was 76% (113/149). Participation of eligible hospital controls (n=336) was 95%. Controls frequency matched for age, sex, and province of residence.	Other exposures evaluated were arsenic, wood dust, leather dust, nickel, chromium, PAHs, welding fumes, oil mists, flour dust, cocoa powder, silica, coal dust, textile dusts, acid mists, paint mists, organic solvents.		was controlled for but adjusted results not presented; just “loss of statistical significance.”			Critical limitation: Confounding
Gérin et al. (1989) Canada Population-based case-control study. <u>Related study:</u> Siemiatycki et al. (1987)	3,726 male cases, 1979–1985, from 14 major area hospitals, which report to the Quebec Tumor Registry (97% of all cancers reported). 533 population controls participated	Lifetime job history included company activities, raw materials and final product, machines, tasks involving machine maintenance, type of room. A team of chemists and hygienists (likely blinded to outcome) translated each job into a list of potential	Incident cases histologically confirmed diagnosis of Hodgkin lymphoma (ICD: 201).	Controlled for age, ethnic group, socio-economic status, smoking, and dirtiness of jobs held (white vs. blue collar). Additional control for any of 300 of the most common occupational exposures if the inclusion changed the formaldehyde	Logistic regression; OR (95% CI). Latency not evaluated.	HL: 8/53 exposed.	SUMMARY: HL: MEDIUM ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group B) and lack of latency analysis with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	out of 740 selected (72%). Interviews and questionnaires completed for 82% of eligible cases of which 18% of interviews were completed by next of kin. Internal and external comparison. Controls were patients with cancer at other sites with all lung cancers excluded. External comparison with general population.	formaldehyde exposures based on their confidence level, the frequency, and the duration of exposure.		OR by more than 10%.			
Gustavsson et al. (1998) Sweden. Population-based case-control study of squamous cell carcinoma of oro-/hypo-pharynx.	138 men between 40-79 years old residing in Sweden identified by hospitals reports or	Detailed occupational history obtained through unblinded interview yielding information on all jobs held >1 year, starting and stopping	Incident cases of squamous cell carcinomas of the oro-/hypo-pharynx. Diagnosis of cancer based on ICD-9 codes 146 (oropharynx) and 148 (hypopharynx)	Controlled for sex, age, region, drinking, and smoking. Other exposures evaluated included:	Unconditional logistic regression; RRs (95% CI). Latency not evaluated.	OHPC: 13/138 exposed. The power to evaluate formaldehyde as a hazard is diminished as	SUMMARY: OHPC, Larynx: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	regional cancer registries with squamous cell carcinoma of oro-/hypo-pharynx during 1988-1990. Controls were randomly selected from population registries and frequency-matched by sex, age and region. Interviews completed for 90% of cases and 85% of controls.	times, job title, tasks, and company. Histories reviewed by a blinded industrial hygienist who coded jobs based on intensity and probability of exposure to 17 occupational factors.	but not including code 147 (nasopharynx) on weekly reports from departments of otorhinolaryngology, oncology, and surgery and from regional cancer registries.	polycyclic aromatic hydrocarbons, asbestos, quartz, dusts (general, leather, wood, metal, paper, textile), oil & acid mists, phenoxy acids, welding fumes, manmade mineral fibers, nickel, hexavalent chromium. Co-exposures were not evaluated as potential confounders.		fewer than 10% of cases had exposure to formaldehyde.	assessment (Exposure Group D) and lack of latency analysis with attenuation of association. Confounding possible. Low sensitivity (exposure was rare).
Heineman et al. (1992). Denmark. Cancer registry-based case-control study, MM diagnosed 1970–1984.	2,098 men registered in both the national cancer registry and pension fund. All men with a specific occupational history were included.	Individual-level exposure estimated by industrial hygienists based on occupation listed on most recent tax documents.	Incident cases identified in Danish Cancer Registry. 92% of cases were histologically confirmed.	Controlled for age and gender. Other compounds were identified and evaluated as independent risk factors including: gasoline, oil products, engine exhausts, benzene, dyes, phthalates, vinyl chloride,	Logistic regression, ORs (95% CI) by likelihood of exposure in 3 categories. Latency not evaluated.	MM: 835 (185 exposed).	SUMMARY: MM: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) and lack of latency analysis with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	Controls frequency matched on age, sex, and year of diagnosis.			asbestos, and pesticides. Asbestos is not a risk factor for LHP. 'Possible' benzene exposure was associated with MM but not 'probable' Benzene exposure, so confounding is considered to be unlikely.			
Hildesheim et al. (2001) . Taiwan. Population-based case-control study. Related studies: Yang et al. (2005) ; Hildesheim et al. (1997) ; Cheng et al. (1999)	375 men and women with NPC and 375 controls. Ages <75 years, July 1991 and January 1995, from 2 hospitals. Participation of eligible cases was 99% and 87% for controls. Controls individually matched 1:1 on age, sex, and district/township of residence.	Lifetime job history (jobs held for at least 1 year since age 16); job title, typical activities/duties, type of industry, and tools and/or materials used. Industrial hygienist assigned scaling to subjects based upon intensity and probability of exposure on a scale from 0–9.	Incident cases. Diagnosis of nasopharyngeal was confirmed by histological review with >90% diagnosed with nonkeratinizing and undifferentiated carcinomas and 9% with squamous cell carcinoma.	Adjusted for age, sex, education, ethnicity, and HLA. Did not adjust for residence. Other exposures identified included: wood dust, solvents, and smoking. All subjects were tested for EBV. The observed associations were not materially affected when controlling for wood dust, smoking and solvent exposure.	Logistic regression; ORs (95% CI) by exposure intensity, exposure probability, cumulative exposure and an induction period of 10 years used to account for latency. Conditional logistic regression was not used; however, logistic regression did control for age and sex. Area of residence was expected to be related to referral patterns and may	NPC: 375 cases (74 ever exposed)	SUMMARY: NPC: MEDIUM ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group B) with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
					not be related to exposure independent of occupational history.		
Laforest et al. (2000) France Hospital-based case-control study of hypopharyngeal and laryngeal cancer.	Male cases (201 primary hypopharyngeal squamous cell cancer, 296 laryngeal cancer), diagnosed during 1989–1991, from 15 French hospitals. Interviews completed for 79.5% of eligible cases and 86% of eligible controls. Controls frequency matched on sex, age, and the same or similar nearby hospital.	Occupational histories from questionnaires; industry and occupation coding used with job exposure matrix for formaldehyde (and other exposures). Exposure assessment based on job-exposure matrix that included level and probability of exposure to formaldehyde as well as duration and cumulative exposure to formaldehyde.	Incident cases. Diagnosis of hypopharyngeal and laryngeal cancers was histologically confirmed.	Controlled for sex, age, alcohol, and smoking. Induction periods of 5, 10, and 15 years was also used to account for latency in evaluating risk. Other exposures evaluated included: coal dust, leather dust, wood dust, flour dust, silica, and textile dust. Of these, only coal dust significantly increased the risk of hypopharyngeal cancer in this study but coal dust was controlled for in the OHPC analysis.	Unconditional logistic regression; OR (95% CI). Latency was evaluated.	OHPC: 201 Larynx: 296	SUMMARY: OHPC, Larynx: MEDIUM ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) and lack of latency analysis with attenuation of association.
Luce et al. (2002) China, France, Germany, Italy, Sweden, United States	Pooled analysis of 12 case-control studies. Men and	Occupational histories from interview or questionnaires; industry and	Diagnoses originally assessed in 12 studies. 195 cases were	Adenocarcinoma results in men controlled for age, study, and	Unconditional logistic regression; OR (95% CI).	SNC: 627 cases (135 adenocarcinomas exposed. 132	SUMMARY: SNC: MEDIUM ↓ (Potential bias toward the null)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Zheng et al. (1992) ; Vaughan et al. (1986a, 1986b) ; Vaughan (1989) ; Vaughan and Davis (1991) ; Merler et al. (1986) ; Magnani et al. (1993) ; Luce et al. (1992) ; Luce et al. (1993) ; Leclerc et al. (1994) ; Hayes et al. (1986b) ; Hayes et al. (1986a) ; Hardell et al. (1982) ; Comba et al. (1992a) ; Comba et al. (1992b) ; Bolm-Audorff et al. (1990) Mack and Preston-Martin (unpub. data) Brinton et al. (1984) ; Brinton et al. (1985)	women. All from 7 different countries diagnosed with sinonasal cancer during 1968–1990. Each individual study selected controls intended to be comparable to the cases in that study.	occupation coding used with job exposure matrix for formaldehyde (and other exposures).	adenocarcinomas (169 men and 26 women) and 432 were squamous cell carcinomas (330 men and 102 women).	cumulative exposure to wood and leather dust. All other results adjusted for age and study. Co-exposures were evaluated as potential confounders. Other occupational exposures potentially affecting risk estimates were controlled for including dusts (wood, leather, coal, flour, textile), silica, asbestos, and man-made vitreous fibers.	Latency evaluated.	squamous cell carcinomas exposed)	Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) with attenuation of association.
Mayr et al. (2010) Germany Hospital-based case-control study.	Hospital patients diagnosed at the University of Erlangen-Nuremberg, Germany during 1973–2007. 31 of 58 patients with	Structured interview with specific questions about exposure to formaldehyde (and other exposures). Both cases and controls were blinded to case status and study hypotheses, and were not aware of their “case” status.	Prevalent cases. Diagnosis of sinonasal adenocarcinoma in the Department of Otolaryngology, Head and Neck Surgery.	Controlled for age and sex. Other exposures: Wood dust, preservatives, stains, varnishes, solvents, and pickling solutions. Wood dust is a considered an	Crude ORs (95% CI). Methods unstated for OR determinations. Latency not evaluated.	SNC: 2/31 exposed Low power due to the rarity of exposure.	Potential selection issue (prevalent cases) Exposure Group C Latency not evaluated Wood dust is a likely confounder. SUMMARY: NOT INFORMATIVE

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	identified adenocarcinoma (53%) were followed up with a standardized questionnaire. 85 of 110 patients with cancer of the oral cavity (77%) included as controls. Controls were other hospital patients diagnosed with oral cancer during the same time period as cases and in the same hospital. Oral cancer could be related to formaldehyde exposure but this would bias towards the null.			extremely strong risk factor for SNC was not controlled for so there is a strong possibility of confounding.			Critical limitation: Confounding
Olsen and Asnaes (1986b) Denmark	310 men with incident SN cancer. 215 (69%) squamous cell	Employment histories from 1964 based on linkage to population registry data; includes industry and job title.	Incident cases identified in Danish Cancer Registry. Cancer of the nasal cavity (ICD-7 160.0) or	Matched for age, sex, and year of diagnosis. Mantel-Haenszel summary estimates of the	OR (95% CI) calculated using the method of Rothman and Boice (1979).	SNC: 215 squamous cell and lymphoepitheliomas	SUMMARY: SNC: MEDIUM ↓ (Potential bias toward the null)

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Cancer registry-based case-control study, SNC diagnosed 1970-1982. Related study: Olsen et al. (1984)	& lymphoepithelioma. 39 (13%) adenocarcinomas. 2,465 controls, selected among people with colon, rectum, prostate, and breast cancer diagnosed during the same time period as cases. Controls were selected to be similar with regard to age, sex, and year of diagnosis.	Occupational exposure to formaldehyde estimated by industrial hygienists based on industry or occupations.	sinuses (ICD-7 160.2–160.9) was histologically confirmed. Of all male cases for cancer of the nasal cavity and paranasal sinuses. 82% were squamous cell, lymphoepithelioma 18% were other types.	relative risk were used to account for possible confounding because the subjects were stratified according to several variables. Wood dust is a considered an extremely strong risk factor for SNC so exposure to wood dust was evaluated as a potential confounder and as an effect modifier.	Latency was evaluated.	(13 exposed to formaldehyde) and 39 adenocarcinomas (17 exposed to formaldehyde)	Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) with attenuation of association.
Olsen et al. (1984) Denmark Cancer registry-based case-control study, NPC diagnosed 1970–1982. Related study: Olsen and Asnaes (1986b)	266 incident NPC and 488 incident SN cases; matched approximately 3 controls per case. Controls matched on age, sex, and year of diagnosis from the Registry.	Employment histories from 1964 based on linkage to population registry data; includes industry and job title. Occupational exposure to formaldehyde estimated by industrial hygienists based on industry or occupations. Authors reported that 4.2% of control males	Incident cases identified in Danish Cancer Registry. NPC: ICD 146 SN: ICD 160.0 and 160.2–160.9 9% of NPC and SNC cases were sarcomas and 91% were carcinomas. Sarcomas were excluded but gender-specific case counts were not	Controlled for age, sex, and year of diagnosis from the registry. Other exposure evaluated included: wood dust, paint, lacquer, and glue. Wood dust is associated with SNC and was	OR (95% CI) calculated using programs developed by Rothman and Boice (1979). Latency was evaluated.	NPC: 266 cases (number exposed is not stated) SNC: cases included in Olsen and Asnaes (1986a).	SUMMARY: NPC: MEDIUM ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
		and 0.1% of females were exposed to formaldehyde.	provided for carcinomas.	evaluated as a potential confounder of NPC but was not a risk factor.			
Pesch et al. (2008) Germany Insurance-based case-control study.	Male workers insured by a liability insurance association for the German wood-working industries with an occupational disease during 1994–2003. 86/129 cases (67%) participated (including 29 next of kin). 204/272 controls (75%) participated (including 69 next of kin). Controls were selected from the same insurance database of workers with registered accidents. Controls were	Lifetime job history, with focus on tasks and exposures in wood industries. Because next-of-kin information on exposure to wood additives was considered poor, the probability of exposure to formaldehyde was rated by an expert team as none, low, medium, or high.	Prevalent cases. Cases were ever employed in German wood industries and diagnosed with histopathologically confirmed sinonasal adenocarcinoma. Because cases and controls were stratified by age less than 60 years and greater or equal to 60 years, the older cases may have been selected for survival. If so, this may have resulted in a downward bias.	Controlled for age, smoking, region, interviewee, and average wood dust exposure. Co-exposure to wood preservatives, varnishes, and pigment stains likely. Wood dust is a considered an extremely strong risk factor for SNC but was controlled for.	Logistic regression. OR (95% CI). A 5-year latency period was applied.	SNC: 47/86 cases exposed	SUMMARY: SNC: LOW ↓ (Potential bias toward the null) Selection bias possible. High potential for information bias due to uncertainty in exposure assessment (Exposure Group B) and weak latency analysis with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	crudely frequency matched on age with a cut-off at 60 years. Median ages were both 69 years with cases ranging from 41–84 years and controls ranging from 37–85 years).						
Pottern et al. (1992) Denmark Cancer registry-based study, MM diagnosed during 1970–1994.	363 female incident cases; included if found in pension fund registry. 1,517 age and sex matched controls alive at time of case diagnosis. All women with a specific occupational history other than “Homemaker” were included.	Individual-level exposure estimated by industrial hygienists based on occupation listed on most recent annual income tax documents and the industry associated with that occupation.	Incident cases identified in Danish Cancer Registry. ICD code at time of diagnosis.	Controlled for age, sex, and vital status. Other exposures evaluated included 19 categories grouping 47 substances. Co-exposures were not evaluated for confounding but exposure to organic solvents (including benzene) and radiation were not risk factors for MM.	Logistic regression, ORs (95% CI) by likelihood of exposure in 3 categories. Latency not evaluated.	MM: 60/363 exposed	SUMMARY: MM: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) and lack of latency analysis with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Roush et al. (1987) United States Population-based case-control study.	173 male cases of NPC, 198 male cases of sinonasal cancer identified from the Connecticut Tumor Registry who died during 1935–1975; and 605 male controls dying during the same time period and randomly selected from state death certificates. Controls were matched on sex, date of death, and state of residence.	Job history obtained by city directories and death certificates, which yielded information on job, industry, employer, and year of employment. Job data sought for 1, 10, 20, 25, 30, 40, and 50 years prior to death. An industrial hygienist, blinded to case status, classified likely exposure to formaldehyde on basis of job title.	Incident cases (from state tumor registries) who had died. Diagnosis of nasopharyngeal cancer and sinonasal cancer based on case registration by the Connecticut Tumor Registry. Clinical records reviewed for >75% of cases. Histological typing not reported.	Controlled for age at death, year at death, and availability of occupational information. Exposure to wood dust was not found to be a risk factor for all nasal cancers (NPC+SNC). This suggests a lower potential for confounding by wood dust.	Logistic regression; ORs (95% CI). Intensity of the likelihood of exposure and latency evaluated.	NPC: 21/173 exposed SNC: 21/198 exposed	SUMMARY: NPC, SNC: MEDIUM ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) with attenuation of association.
Shangina et al. (2006) Europe Multicenter case-control study.	316 male cases of laryngeal cancer between the ages of 15–79 years residing in 4 European countries that were diagnosed	Occupational histories obtained by interview and yielded information on all jobs held >1 year. A general questionnaire obtained information of job titles, tasks, industries, starting and stopping times,	Diagnosis of laryngeal cancer was histologically or cytologically confirmed and included topographic subcategories from ICD-O code C32 (glottis, supraglottis, subglottis, laryngeal	Controlled for age, country, smoking, and alcohol. Other exposures that were found to be risk factors included dusts of “hard alloys” (16 cases) and	Logistic regression; ORs (95% CI). Latency was evaluated.	Larynx: 18/316 exposed The power to evaluate formaldehyde as a hazard is diminished as fewer than 10% of cases had any	SUMMARY: Larynx: MEDIUM ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) with

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	during 1999–2002 and identified by study centers in Romania, Poland, Russia, and Slovakia. 728 male hospital controls selected within 6 mos of case recruitment from diagnoses excluding disease related to alcohol or tobacco. Controls frequency matched by age +/- 3 years.	full-time/part-time status, working environments, and specific exposures. A specific questionnaire was completed for employment in defined jobs or industries.	cartilage, overlapping lesion of the larynx, and larynx, unspecified).	chlorinated solvents (15 cases). As formaldehyde, hard alloy dust and chlorinated solvents were each found in fewer than 6% of cases, the correlation between them is considered to be small enough to make confounding unlikely.		exposure to formaldehyde.	attenuation of association. Low sensitivity (exposure was rare).

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Talibov et al. (2014) Europe Multicountry case-control study.	Individuals from Finland, Iceland, Norway, and Sweden who were recorded in various censuses from 1960–1990. Acute myeloid leukemia cases identified by national registries up until 2003–2005 depending on the country.	Occupational history from census records were linked to the Nordic Occupational Cancer Study (NOCCA) JEM to code each cohort member as exposed to formaldehyde. Exposures were quantified based on the proportion of people in each occupation considered to be exposed and the mean level of exposure during specific time periods. 8% of AML cases and controls were exposed. Co-exposures to solvents was evaluated.	Diagnosis of incident cancer reported to the National Cancer Registries.	Controlled for age (<50, 50+), sex, and solvents. Solvents included: aliphatic and alicyclic hydrocarbons, aromatic hydrocarbons, benzene, toluene, trichloroethylene, 111-trichloroethane, methylene chloride, perchloroethylene, other organic solvents, and ionizing radiation.	HRs (95% CI). A 10-year latency period was assumed.	AML: 1201/15,332 exposed	SUMMARY: LOW ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group D) with attenuation of association.
Teschke et al. (1997) Canada Population-based case-control study of nasal cancer.	48 incident cases of nasal cancers (31% female) older than 19 years, 1990–1992. Controls were randomly selected from age and sex	Standardized questionnaire including occupational, residential, smoking, and medical histories aimed at identifying exposures considered to be probably carcinogenic by IARC.	Incident cases from British Columbia Cancer Agency registry. Histologically confirmed primary malignant tumors of the nasal cavity. SNC: ICD-O 160.	Controlled for age and sex. More than 40 specific occupational groups were evaluated without control of confounding.	ORs (95% CIs). Latency was evaluated.	SNC: 48 3 cases exposed to pulp and paper mills.	SUMMARY: SNC: LOW ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) with attenuation of

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	strata of voter list of the same time period. 6 of 54 cases (11%) were excluded for lack of interview as were 36 of 195 controls (18%). Controls matched on age and sex.	Occupational data reviewed by an industrial hygienist blinded to case-status. EPA considered that workers in the textile and pulp and paper mill industries may have been exposed to formaldehyde but the exposure questionnaire did not identify them as exposed. Pulp and paper mill workers may also be co-exposures to dioxin or perchloroethylene (Kauppinen et al., 1997).		Confounding not evaluated. Potential confounders for these outcomes include chlorophenols, acid mists, dioxin, and perchloroethylene and would likely be positively correlated with formaldehyde exposure. However, on acids mists are associated with URT cancers. Potential for confounding is unknown but could have inflated the observed effect.			association. Potential confounding. Low sensitivity (rare exposure).
Vaughan et al. (2000) United States Population-based case-control study of nasopharyngeal cancer.	196 cases (32% female) ages 18–74 diagnosed during 1987–1993 identified from 5 population	Individual-level exposure based on industrial hygienist review of detailed occupational histories including industry, job title, duties and dates used to estimate probability, intensity,	Incident cases. Diagnosis of nasopharyngeal (any histological type) based on clinical records. Histological typing reported.	Controlled for age, sex, race, registry, smoking, proxy status, and education. Wood dust evaluated as an independent risk	Logistic regression; ORs (95% CI) by probability of exposure, duration, and cumulative exposure.	NPC: 79 exposed cases.	SUMMARY: NPC: MEDIUM ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
	based cancer registries. Interviews completed for 82% of cases and 76% of the 244 controls. 19% of case interviews completed by next of kin. Controls selected by random digit dialing in the same geographical region frequency matched by age, sex, and cancer registry.	and cumulative exposure.		factor for NPC controlling for formaldehyde and it was not a risk factor in this data set. Therefore, wood dust should not be a confounder in this data set.	Separate analyses by histological type. Latency evaluated.		Group B) with attenuation of association.
Vaughan (1989) United States Population-based, case control study of squamous cell cancers of the pharynx and sinonasal cavity. <u>Related studies:</u>	231 cases (32% female) ages 20–74 years residing in the area covered by Washington State Cancer Surveillance System during 1980–1983.	Individual-level exposure based on job exposure matrix by occupation and industry for each individual job used to estimate probability and intensity of exposure. Formaldehyde exposure from	Incident cases. Diagnosis of squamous cell cancers of the pharynx and sinonasal cavity based on review of hospital medical records, surveillance of radiotherapy and pathology practices, and state death certificates.	Controlled for age, sex, smoking, and alcohol. NPC analyses controlled for race. Wood dust is associated with risk of URT cancer and was not	Logistic regression; ORs (95%CI). Duration of employment and occupation are surrogates for intensity of exposure. Latency was evaluated.	NPC: 3/21 exposed OHPC: 11/183 exposed SNC: cases included in Luce et al. (2002). Low power for NPC.	SUMMARY: NPC: LOW ↓ (Potential bias toward the null) Potential selection bias. High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) with attenuation of association. Low

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Vaughan et al. (1986a, 1986b) ; Included in Luce et al. (2002)	Participation for all cases was 69% (see (Vaughan et al., 1986a)) and 80.0% for controls ($n = 552$). ≈50% of cases interviews completed by next of kin. Controls selected by random digit dialing in same residential area as cases and were frequency matched on age and sex with at 2 controls per cases in each 5-year age and sex category. May result in poorer quality exposure data and a bias towards the null.	available industrial hygiene data, NIOSH and other data, and NCI job exposure linkage system. Occupation as a carpenter or employment in the “lumber and wood product manufacturing” industry presumed to be exposed to formaldehyde.		evaluated as a confounder. However, as this is a case-control study and the correlation between formaldehyde and wood dust is expected to be small and thus wood dust would not be expected to be a confounder.			sensitivity (rare exposure). OHPC: LOW ↓ (Potential bias toward the null) Potential selection bias. High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) with attenuation of association.
Vaughan et al. (1986a) United States	285 cases (35% female) ages 20–74 years	Individual-level exposure based on job exposure matrix	Incident cases. Diagnosis of squamous cell cancers	Controlled for age, sex, smoking, and alcohol.	Logistic regression; ORs (95%CI).	NPC: 11/27 occupationally exposed.	SUMMARY:

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Population-based, case control study of cancers (all types) of the pharynx and sinonasal cavity. <u>Related studies:</u> (Vaughan et al., 1986b); Vaughan (1989) @@author-year; SNC cases included in Luce et al. (2002) but not here.	residing in the area covered by Washington State Cancer Surveillance System during 1980–1983. Participation for all cases was 69% and 80% for controls (<i>n</i> =552). ≈50% of cases interviews completed by next of kin. Controls selected by random digit dialing in same residential area as cases and were frequency matched on age and sex with at 2 controls per cases in each 5-year age and sex category.	by occupation and industry for each individual job used to estimate probability and intensity of exposure. Formaldehyde exposure from available industrial hygiene data, NIOSH, and other data, and NCI job exposure linkage system.	of the pharynx and sinonasal cavity based on medical records, surveillance of radiotherapy and pathology practices, and state death certificates. 2% of cases were nonsquamous cell cancers Vaughan (1989) .	NPC analyses controlled for race. Wood dust is associated with risk of URT cancer and was not evaluated as a confounder. However, as this is a case-control study and the correlation between formaldehyde and wood dust is expected to be small and thus wood dust would not be expected to be a confounder.	Latency was evaluated.	OHPC: 58/205 occupationally exposed. SNC: cases included in Luce et al. (2002) .	OHPC, NPC: LOW ↓ (Potential bias toward the null) Potential selection bias. High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) with attenuation of association.
Vaughan et al. (1986b) United States	285 cases (35% female) ages 20–74 years	Presumed exposure to formaldehyde based on structured	Incident cases. Diagnosis of squamous cell cancers	Controlled for age, sex, smoking, and alcohol.	Logistic regression; ORs (95% CI).	NPC: 8/27 lived in mobile home.	SUMMARY:

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Population-based, case control study of cancers (all types) of the pharynx and sinonasal cavity. <u>Related studies:</u> Vaughan et al. (1986a); Vaughan (1989); SNC cases included in Luce et al. (2002) but not here.	residing in the area covered by Washington State Cancer Surveillance System during 1980–1983. Participation for all cases was 69% (see (Vaughan et al., 1986a)) and 80% for controls ($n=552$). ≈50% of cases interviews completed by next of kin. Controls selected by random digit dialing in same residential area as cases and were frequency matched on age and sex with at 2 controls per cases in each 5-year age and sex category.	telephone interview information on occupational and residential history. Interview-based information on lifetime residential history from cases, next of kin, and controls.	of the pharynx and sinonasal cavity based on medical records, surveillance of radiotherapy and pathology practices, and state death certificates. 2% of cases were nonsquamous cell cancers (Vaughan, 1989).	NPC analyses controlled for race. Wood dust is associated with risk of sinonasal cancer and was not evaluated as a confounder. However, as this is a case-control study and the correlation between formaldehyde and wood dust is expected to be small and thus wood dust would not be expected to be a confounder.	Latency was evaluated.	10/27 exposed to particleboard. OHPC: 28/205 lived in mobile home. 68/205 exposed to particleboard. SNC: cases included in Luce et al. (2002) .	OHPC, NPC: LOW ↓ (Potential bias toward the null) Potential selection bias. High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
West et al. (1993) Philippines Hospital-based case-control study. <u>Related study:</u> Hildesheim et al. (1992)	104 cases (27% female), 11–83 years old, predominantly non-Chinese, from the Philippine General Hospital diagnosed before 1992. 100% of cases participated. All 104 hospital controls participated while only 77% of 101 community controls participated (Hildesheim et al., 1992). Hospital controls were matched on age, sex, and hospital ward type (private/public). Community controls were matched on age, sex, and neighborhood of residence.	Lifetime job history; details not provided. Occupational exposure to formaldehyde classified by blinded industrial hygienist as likely or unlikely to be exposed; appendix provides formaldehyde exposure rating for each job category.	Incident cases. Diagnosis of NPC pathologically confirmed by histological review for all cases. Histological typing not reported.	Controlled for age, sex, hospital ward type (or neighborhood), for education, years since first exposure to dust and exhaust fumes, diet including processed meats, fresh fish, smoking, anti-mosquito coils, and herbal medicines. Note that anti-mosquito coils emit formaldehyde 0.87–25 µg/m³ (Liu et al., 2003). Controlling for mosquito coils may have underestimated the effect of formaldehyde.	Conditional logistic regression; ORs (95% CI). Latency was evaluated.	NPC: 27/104 exposed	SUMMARY: NPC: MEDIUM ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Wortley et al. (1992) United States Population-based, case control study of cancers (all types) of the larynx.	235 cases (21% female) ages 20–74 years residing in the area covered by Washington State Cancer Surveillance System during 1983–1987. Participation for all cases was 81% and 80% for controls (<i>n</i>=547). 7% of cases interviews completed by next of kin. Controls selected by random digit dialing in same residential area as cases and were frequency matched on age and sex with at 2 controls per cases in each 5-year age and sex category.	Individual-level exposure based on job exposure matrix by occupation and industry for each individual job used to estimate duration and intensity of exposure. Formaldehyde exposure from available industrial hygiene data, NIOSH, and other data, and NCI job exposure linkage system.	Incident cases. Diagnosis of cancer of the larynx based on medical records, surveillance of radiotherapy and pathology practices, and state death certificates. 94.5% of cases were squamous cell cancers.	Controlled for age, smoking, and alcohol. Further adjustment for sex did not change results. Other exposures: asbestos, chromium, nickel, cutting oils, and diesel fumes. High risk occupations (e.g., mechanics, carpenters, painters, textile machine operators) likely had co-exposures to unidentified substances. However, as this is a case-control study the correlation between formaldehyde and those potential confounders is expected to be small and thus wood dust would not be expected to be a confounder.	Logistic regression; ORs (95%CI). Latency was evaluated.	Larynx: 58/235 occupationally exposed	SUMMARY: Larynx: MEDIUM ↓ (Potential bias toward the null) Potential for information bias due to uncertainty in exposure assessment (Exposure Group C) with attenuation of association.

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Yang et al. (2005) Taiwan Family-based case-control study. <u>Related studies:</u> Hildesheim et al. (1997) ; Hildesheim et al. (2001) ; Cheng et al. (1999)	502 cases recruited from 265 families with 2 or more NPC cases identified from earlier study (Hildesheim et al., 2001). Additional cases obtained from hospitals that treat NPC. Occupational data available for 65% of cases and 57% of controls. 203 cases represented by next of kin (>40%). Cases were matched with 2 groups: First with 1,944 familial controls; and second with 327 population controls.	Lifetime job history (jobs held for at least 1 year since age 16); job title, typical activities/duties, type of industry, and tools and/or materials used. Exposures coded by industrial hygienist. Exposures in 10 years preceding diagnosis of interview were excluded. Collected information on cigarette smoking, betel nut consumption, wood and formaldehyde exposure, and Guangdong and other salted fish consumption during childhood.	Original case series were incident cases. Unclear if supplemental cases were incident or prevalent. Diagnosis NPC confirmed by histological review on 502 cases from national tumor registry.	Three analyses (check each and be specific). Family control analysis controlled for family, age, sex, education, and ethnicity. This analysis did not control for partial matching on education, ethnicity, or area of residence. Nor did it control for smoking, betel nut consumption, or wood. In this study, smoking was inversely associated with NPC. Because smoking is positively associated with formaldehyde, there may be negative confounding by smoking in this study.	Unconditional logistic regression (95%CI) controlling for age and sex. Lagged exposure partially address latency. Controls used here were originally matched to an earlier set of cases, some of whom were included here.	NPC: 502	SUMMARY: NPC: LOW ↓ (Potential bias toward the null) Potential selection bias. High potential for information bias due to uncertainty in exposure assessment (Exposure Group D) with attenuation of association. Confounding possible.
Yu et al. (2004) Hong Kong	Men and women.	Occupational history obtained from union	Mortality: Underlying cause of death from	MOR with Internal control group	Logistic regression. Mortality odds	NPC: 21	SUMMARY:

Supplemental Information—Formaldehyde (Inhalation)

Reference, setting, and design	Participants, selection, and comparability	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results (estimate and variability)	Study sensitivity	Evaluation of major bias categories
Mortality odds ratio. Related studies: Ho et al. (2006); EHS Consultants Ltd. (1999)	Restaurant workers ($n=1,225$) who died during 1986–1995 and were registered as union members by 4 major Chinese-style restaurant workers' unions in Hong Kong. Cause of death available for more than 80% of restaurant workers.	records. 415 deceased waiters and 140 deceased waitresses and kitchen workers likely exposed to formaldehyde based on independent studies of air quality in service areas of restaurants. Authors discuss sources of exposure. Co-exposures include Epstein-Barr virus (EBV), smoking, salted and preserved foods, and other combustion by-products.	Hong Kong Census and Statistics Department. NPC: ICD-9 147 Histological typing not reported.	adjusted for age at death, sex, year of death, and place of origin. External control group Adjusted for age at death, sex, and year of death. Most adults (90+ %) are seropositive for EBV and thus it cannot be a confounder. Smoking was evaluated as a potential confounder because 49% of staff smoked compared to 27% of population, but it was insufficient to explain the observed effects. Authors stated that with free fresh food available to workers, the availability of preserved or salted food was unlikely to explain the observed effect.	ratios (MORs) calculated for waiters and waitresses by internal and external controls and for waiters, length of union membership (a surrogate for duration of exposure). Latency was not evaluated.		NPC: LOW ↓ (Potential bias toward the null) High potential for information bias due to uncertainty in exposure assessment (Exposure Group C) and lack of latency analysis with attenuation of association. Confounding possible.

Animal Studies

Animal studies in Tables B-61 (respiratory tract cancers) and B-62 (LHP cancers) are grouped by exposure duration, and then organized alphabetically by first author. Specific study details (or lack thereof) that highlight a limitation or uncertainty in answering each of the domain criteria are noted in the table cells. For those identified as having a substantial limitation likely to influence the study results (major limitations), the relevant study details are bolded. Italics are used to highlight other limitations not viewed as substantial in the context of respiratory tract pathology (minor limitations). A ‘Good’ domain rating is denoted by ‘++’; an ‘Adequate’ rating by ‘+’; and a ‘Deficient’ rating by gray shading.

Respiratory Tract Cancer Study Evaluations

Table B-61. Evaluation of controlled inhalation exposure studies examining respiratory tract cancer or dysplasia in animals

Reference and species	Exposure quality (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
<i>Respiratory Tract Cancers—Chronic</i>						
(Appelman et al., 1988) Rat	++	+ <i>Small N (N=10);</i> Note: randomized	1-year duration short to allow for cancer development	+ <i>Blinding of slides for evaluation NR</i>	++	<i>Medium</i> [1 year duration]
(Dalbey, 1982) Hamster	++ Note: 5 hr/d exposure; days and timing of exposure NR	++	++ Note: single concentration (12.3 mg/m ³) lifetime study	<i>Blinding of slides for evaluation NR; only 2 nasal sections; limited reporting of histopathology methods; unclear if dysplasia considered</i>	+ <i>Locations and specific incidence of lesions and other minor details NR</i>	<i>Medium</i> [Limited sampling, evaluation, and reporting]
(Holmstrom et al., 1989b)	++ Note: high concentration exposure (15.3 mg/m ³); exposed	+ <i>Small N (N = 15–16); some cannibalism; non-</i>	+ <i>2/16 animals in formaldehyde group developed emphysema</i>	++ Note: slides blinded	+ <i>Locations of lesions and other minor details NR</i>	<i>Medium</i> [Some health issues noted; limited reporting]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
	nocturnally, in contrast to other studies	URT tumors ≈50% across groups	Note: single concentration (15.3 mg/m ³) 2 year study			
(Kamata et al., 1997) Rat	+ Formalin exposure, with a methanol control (assumed to be based on levels in formalin) Note: methanol considered unlikely to affect endpoint	+ Small N for interim sacrifices (N = 2–5) Note: mortality rate doubled at 18.3 mg/m ³ ; exposure begun at ≈PND35	++ Note: 2 year study	+ Blinding of slides for evaluation NR	++	Medium [Formalin (with methanol control)]
(Kerns et al., 1983) Mouse See also (Battelle, 1982) and (Swenberg et al., 1980b)	++	+ Survival to 18 mos was <33% in all groups (N is >25) Note: randomized	++ Note: data from this study based on a 2 year GLP study (Battelle, 1982)	+ Only three nasal sections evaluated; blinding of slides for evaluation NR	+ Limited reporting of dysplasia findings	High [Note: somewhat limited sampling and high mortality]
(Kerns et al., 1983) Rat See also (Battelle, 1982) and (Swenberg et al., 1980b)	++	+ Viral infection at weeks 52–53 Note: considered unlikely to influence these outcomes; randomized	++ Note: data from this study based on a 2 year GLP study (Battelle, 1982)	+ Blinding of slides for evaluation NR Note: routine analysis of nasal tissues only	+ Limited reporting of dysplasia findings	High [Note: transient viral infection]
(Monticello et al., 1996) Rat	++	++ Note: randomized	++ Note: 2 year study	+ Blinding of slides for evaluation NR Note: routine analysis of nasal tissues only	++	High
(Sellakumar et al., 1985)	+	++	++	+	++	High

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
Rat see also (Albert et al., 1982)	<i>Air controls direct into chamber, not through apparatus</i> Note: PFA in paraffin oil (commonly used in bubbler-type units); high concentration exposure (18.2 mg/m ³)		Note: single concentration (18.2 mg/m ³) lifetime study	<i>Blinding of slides for evaluation not specified</i>		
(Woutersen et al., 1989) Rat, 28-month exposure	++	++ Note: randomized	++ Note: 2 year study	+ <i>Blinding of slides for evaluation NR;</i> Note: routine analysis of nasal tissues only	++	High
<i>Respiratory Tract Cancers—Subchronic (note: includes 1 study with only 8 weeks of exposure in genetically modified mice)</i>						
(Andersen et al., 2010) Rat	+ <i>Analytic concentrations NR</i>	Small N (N = 8) Note: randomized	13 wk duration with no follow up to allow for cancer development	+ <i>Blinding NR; limited reporting of slide selection, analysis methods, and number of slides evaluated</i>	+	Low [Short duration; small sample; short duration of exposure with no follow up]
(Arican et al., 2009) Rat	Analytical method and concentrations NR	+ Small N (N = 10) Note: randomized	12 wk duration with no follow up to allow for cancer	Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR	+ <i>Qualitative descriptions only</i>	<i>Not informative</i> [short duration; exposure and outcome methods lacking]
(Casanova et al., 1994) Rat	++	Small N (N = 3) Note: randomized	12 wk duration with no follow up to allow for cancer	Blinding NR; slide selection, analysis methods, and number of slides or	+ <i>Qualitative descriptions only</i>	<i>Not informative</i> [short duration; small N; outcome methods lacking]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
				regions evaluated NR		
(Coon et al., 1970) Dogs	++	Small <i>N</i> (<i>N</i> = 2); limited reporting (e.g., age, weight, health status, etc.)	Multiple species housed and exposed simultaneously; continuous exposure (>22 hr/d); 90d study does not allow for cancer to develop Notes: single concentration (4.6 mg/m ³) study	Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR	+ <i>Qualitative descriptions only</i>	<i>Not informative</i> [outcome methods lacking; short duration; group housed for exposure]
(Coon et al., 1970) Guinea pig	++	NR age or number of male vs. female guinea pigs ; small <i>N</i> (<i>N</i> = 15); limited reporting (e.g., age, weight, health status, etc.)	Multiple species housed and exposed simultaneously; continuous exposure (>22 hr/d); 90 d study does not allow for cancer to develop Notes: single concentration (4.6 mg/m ³) study	Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR	+ <i>Qualitative descriptions only</i>	<i>Not informative</i> [outcome methods lacking; short duration; group housed for exposure]
(Coon et al., 1970) Monkey	++	Small <i>N</i> (<i>N</i> = 3); limited reporting (e.g., age, weight, health status, etc.)	Multiple species housed and exposed simultaneously; continuous exposure (>22 hr/d); 90 d study does not allow for cancer to develop Notes: single concentration (4.6 mg/m ³) study	Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR	+ <i>Qualitative descriptions only</i>	<i>Not informative</i> [outcome methods lacking; short duration; group housed for exposure]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
(Coon et al., 1970) Rabbit	++	Small <i>N</i> (<i>N</i> = 2); limited reporting (e.g., age, weight, health status, etc.)	Multiple species housed and exposed simultaneously; continuous exposure (>22 hr/d); 90 d study does not allow for cancer to develop Notes: single concentration (4.6 mg/m ³) study	Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR	+ <i>Qualitative descriptions only</i>	<i>Not informative</i> [outcome methods lacking; short duration; group housed for exposure]
(Coon et al., 1970) Rat	++	NR number of male vs. female nor how many of each strain exposed; limited reporting (e.g., age, weight, health status, etc.)	Multiple species housed and exposed simultaneously; continuous exposure (>22 hr/d); 90 d study does not allow for cancer to develop Notes: single concentration (4.6 mg/m ³) study	Blinding NR; slide selection, analysis methods, and number of slides or regions evaluated NR	+ <i>Qualitative descriptions only</i>	<i>Not informative</i> [outcome methods lacking; short duration; group housed for exposure]
(Feron et al., 1988) Rat	++ Note: high concentration exposure (> 12 mg/m ³)	++	+ 13 wk duration, but long-term follow up to allow for cancer to develop	+ Blinding NR; limited reporting of analysis methods	+ <i>Limited information (deaths only) to inform timing of tumor development</i>	<i>Medium</i> [Short duration of exposure; limited reporting]
(Horton et al., 1963) Mouse	+ <i>Analytic concentrations</i> NR Note: excessive exposure level (≈200 mg/m ³)	+ <i>Limited reporting (e.g., age, weight, health status, etc.); high mortality</i>	35 wk duration with no follow up to allow for cancer; exposure paradigm of 1 hr/wk considered less informative	Nasal tissue not examined; blinding NR; limited reporting	+ <i></i>	<i>Not informative</i> [Primary target tissue not examined; study design limited]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
(Maronpot et al., 1986) Mouse	Formalin, methanol concentrations NR, and no controls	+ <i>Small N (N = 10)</i> Note: randomized	13 wk duration with no follow up to allow for cancer	+ <i>Blinding NR; limited reporting of analysis methods</i>	++	<i>Low</i> [Formalin; small sample; short duration of exposure with no follow up]
(Morgan et al., 2017) National Toxicology Program (2017) Mouse	+ <i>Analytic concentrations NR</i>	++ Note: “randomly assigned”; Males only; ≈25 mice/group; genetically modified (p53+/-)	8 wk exposure duration; follow up for 32 wks Note: although unclear if exposure or follow up duration was adequate, the study employed maximally tolerated cumulative dose	+ <i>Blinding NR; examined 3 nasal cavity sections (and 1 larynx)</i> Note: 4 additional pathologists reviewed all tumor slides	++	<i>Low</i> [very short (8 wk) exposure duration and limited follow up (32 wk) for cancer development]
(Rusch et al., 1983) Rat	++ Note: test article was not stabilized (negligible methanol) formaldehyde; concentration <3.6 mg/m ³	++	26 wk duration with no follow up to allow for cancer	+ <i>Blinding NR; limited reporting of analysis methods</i>	++	<i>Low</i> [Short duration of exposure with no follow up]
(Rusch et al., 1983) Monkey	++ Note: test article was not stabilized (negligible methanol) formaldehyde; concentration <3.6 mg/m ³	++	26 wk duration with no follow up to allow for cancer	+ <i>Blinding NR; limited reporting of analysis methods</i>	++	<i>Low</i> [Short duration of exposure with no follow up]
(Rusch et al., 1983) Hamster	++ Note: test article was not stabilized (negligible methanol)	++	26 wk duration with no follow up to allow for cancer	+ <i>Blinding NR; limited reporting of analysis methods</i>	++	<i>Low</i> [Short duration of exposure with no follow up]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality (see Appendix B.3.1)	Test subjects	Study design	Endpoint evaluation	Data considerations & statistics	Confidence classification
	formaldehyde; concentration <3.6 mg/m ³					
(Wilmer et al., 1989) Rat	+ <i>Analytic concentrations</i> NR Note: concentration tested <5 mg/m ³	++ Note: randomized	13 wk duration with no follow up to allow for cancer development	+ <i>Blinding NR</i>	++	<i>Low</i> [Short duration of exposure with no follow up]
(Woutersen et al., 1987) Rat	++	+ <i>Small N (N = 10)</i> Note: randomized	13 wk duration with no follow up to allow for cancer	+ <i>Blinding NR</i>	++	<i>Low</i> [Short duration of exposure with no follow up]
(Woutersen et al., 1989) Rat, 3-month exposure	++	++ Note: randomized	+ <i>12 wk duration, but long-term follow up to allow for cancer to develop</i>	+ <i>Blinding of slides for evaluation NR;</i> Note: routine analysis of nasal tissues only	++	<i>Medium</i>
(Zwart et al., 1988) Rat	++ Note: concentration <3.6 mg/m ³	++	13 wk duration with no follow up to allow for cancer	+ <i>Blinding NR</i>	+ <i>Qualitative descriptions only</i>	<i>Low</i> [Short duration of exposure with no follow up]

NR = not reported; N/A = not applicable. Lymphohematopoietic Cancer Study Evaluations

Table B-62. Evaluation of controlled inhalation exposure studies examining lymphohematopoietic cancers in animals

Reference and species	Exposure quality	Test subjects	Study design	Endpoint evaluation ^c	Data considerations and statistics	Confidence classification
(Kamata et al., 1997) Rat	+	+ <i>Small N for interim</i>	++ Note: 2 year study	+ <i>Blinding of slides for evaluation NR; specific,</i>	+ <i>Limited reporting (incidence data)</i>	<i>Medium</i> [Formalin (with methanol control);

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality	Test subjects	Study design	Endpoint evaluation ^c	Data considerations and statistics	Confidence classification
	<i>Formalin exposure, with a methanol control</i>	<i>sacrifices (N = 2–5); Note: mortality rate doubled at 18.3 mg/m³; exposure begun at ≈PND35</i>		<i>routine histopathology of several tissues relevant to LHP cancer (e.g., femur)</i>	<i>for non-nasal lesions not reported)</i>	limited reporting of LHP cancers]
(Kerns et al., 1983) Mouse See also (Battelle, 1982) and (Swenberg et al., 1980b)	++	+ <i>Survival to 18 mos was <33% in all exposed groups of male mice (N is still >25); randomized</i>	++ Note: relevant data from the 2-year GLP study report (Battelle, 1982)	+ <i>Blinding of slides for evaluation NR; reported gross lesions only for most exposure levels; slides examined for highest exposure level only</i>	+ <i>Limited reporting (incidences only reported for highest exposure level)</i>	<i>Medium</i> [limited sampling and reporting of potential LHP cancers; some increased mortality in exposed males]
(Kerns et al., 1983) Rat See also (Battelle, 1982) and (Swenberg et al., 1980b)	++	+ <i>Viral infection at weeks 52–53 (Note: considered unlikely to influence these outcomes); high mortality in highest exposure groups; randomized</i>	++ Note: relevant data from the 2-year GLP study report (Battelle, 1982)	+ <i>Blinding of slides for evaluation NR; reported gross lesions only for most exposure levels; slides examined for highest exposure level only</i>	+ <i>Limited reporting (incidences only reported for highest exposure level)</i>	<i>Medium</i> [limited sampling and reporting of potential LHP cancers; high mortality in highest exposure groups; transient viral infection]
National Toxicology Program (2017) (Morgan et al., 2017) Mouse	+ <i>Analytic concentrations NR</i>	++ Note: “randomly assigned”; Males only; ≈25 mice/ group; genetically	8 wk exposure duration; follow up for 32 wk Note: although unclear if exposure or follow up duration was adequate, the study employed maximally tolerated	+ <i>Blinding NR; slide evaluation details NR, but assessed multiple relevant tissues</i>	++	<i>Low</i> [very short (8 week) exposure duration and limited follow up (32 wk) for cancer development]

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure quality	Test subjects	Study design	Endpoint evaluation ^c	Data considerations and statistics	Confidence classification
		modified (p53+/-)	cumulative dose; however, no increase in any tumors noted (even nasal SCCs, which were the focus of the study hypothesis)	Note: 4 additional pathologists reviewed all tumor slides		
(Sellakumar et al., 1985) Rat see also (Albert et al., 1982)	+ <i>Air controls direct into chamber, not through apparatus</i> Note: PFA in paraffin oil (commonly used in bubbler-type units); high concentration exposure (18.2 mg/m ³)	++	++ Note: single concentration (18.2 mg/m ³) lifetime study	Does not appear to be an explicit, routine examination of tissues relevant to LHP cancers, or an evaluation of bone marrow, in particular (<i>"histologic sections were prepared from... other organs where gross pathology was present"</i>); <i>Blinding of slides for evaluation not specified</i>	++	<i>Low</i> [no routine examination of tissues relevant to LHP cancers, and lack of evaluation of bone marrow specifically, severely limits detection ability]

B.3.10. Mechanistic Information Related to Genotoxicity and Cancer

Details of the study evaluation approach methods are provided in Section 2.3.10 of the Toxicological Review. As described in Section 2.3.10, many of the studies synthesized for the MOA analyses of respiratory tract and LHP cancers were evaluated in the sections above, most notably Appendix B.3.6. The study evaluations specific to genotoxicity in epidemiology studies are documented in Table B-63 below, with studies organized alphabetically. These study evaluations are discussed further as one component of the broader genotoxicity evaluation in Appendix C.3.

Table B-63. Evaluation of genotoxicity endpoints in epidemiology studies of formaldehyde exposure

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Aglan and Mansour (2018) (Egypt) Hair stylists	Passive air sampling (Umex-100) at fixed position in breathing zone, 15-min samples during hair straightening process; 15-min TWA Group 1 (work duration < 5 years): 1.68 ± 0.27 ppm Group 2 (work duration > 5 years): 1.83 ± 0.16 ppm	Blood collected at end of 8-hr shift on day hair straightening occurred, processed within 6 hrs. Cytokinesis block micronucleus test in lymphocytes Maffei et al. (2002) . Replicate cultures for each sample, incubated 72 hrs, cytochalasin-B added for the last 28 hrs. 1,000 binucleated cells examined per person. 2,000 binucleated cells from coded slides (1,000 from each replicate culture), scored using criteria by Fenech et al. (2003) . MN frequency % altered cells. MN in exfoliated buccal cells. Cheeks scraped with wooden spatula, fixed in 3:1 methanol/acetic acid and dropped onto slides. Air dried slides stained with Feulgen/Fast Green, examined at 400× according to Tolbert et	60 female hairstylists selected between June 2015 and September 2016, aged 20–36 years with comparable work hours, number of clients, usual tasks included hair straightening and no gaps in employment. Excluded subjects with chronic disease and /or regular medications, family history of cancer, recurrent abortions, smoking or pregnancy. Comparison group was 60 healthy female hair stylists who did not straighten hair “matched age, residency, nutritional habits, and socio-economic standard.” Participation rates not reported. No data provided to confirm asserted comparability	Exposed participants were comparable for work tasks, number of clients and work duration. Only nonsmokers were included, and all were female. Exposed and unexposed were “matched” for age, residency, nutritional habits, and SES.	Comparisons between unexposed, group 1 and group 2 using Kruskal Wallis test for nonnormally distributed variables (MNL and MNB) and least significant difference. Comparisons were across duration (greater or less than 5 years) and 15-min TWA concentrations were higher in Group 2 ($p = 0.03$, t test).	Unexposed n = 60 Group 1 n = 31 Group 2 n = 29	Reporting deficiencies result in some concern about potential for selection bias. Comparisons were for duration of exposure (greater or less than 5 years) and 15-min TWA concentrations also were statistically different in these groups.

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		al. (1991) . Analyzed independently by 2 people, 1,500 cells scored per person using criteria by Sarto et al. (1987) % altered cells.	between exposed and referents.				
Attia et al. (2014) (Egypt) Cosmetic manufacture	Urine formic acid according to Hopner and Knappe (1974) ; unclear how to relate urine formic acid levels to air concentrations	Peripheral blood; plasma MDA (commercial kit), plasma p53 (p53 enzyme-linked immunosorbent assay kit. Blinding not stated, but likely minimal bias because interpretation not required	40 employees at company randomly selected compared to referent ($N = 20$) selected from hospital administrative department with comparable gender and SES & no history of occupational exposure to formaldehyde	Age differed between exposed and referent, but age and gender were not associated with formate levels, MDA levels, or p53 levels	Analyses of coded data (blinded assumed) Exposed compared to referent, means (Student's t -test), correlation between urinary formate and MDA or p53 using linear regression	Exposed $n = 40$, referent $n = 20$	No obvious bias
Aydin et al. (2013) (Turkey) Medium density fiberboard plants (prevalence study)	24 area samples in workplaces; personal samples in breathing zone over 8-hr period. 8-hr TWA calculated	Peripheral blood lymphocytes; samples processed within 6 hr, comet assay, tail intensity, tail moment, and tail migration, alkaline conditions, Singh et al. (1988) , cells lysed >1 hr, electrophoresis 20 min, 100 cells/ subject (2 replicates), image analysis software. Blinding not stated	Selection & recruitment of exposed and referent not described. Participation rates not reported. 46 male workers compared to 46 nonexposed males in same area (administrative government offices and maintenance services)	Exposed and referent comparable with respect to age, sex, lifestyle, and smoking habit. No history of occupational exposure to formaldehyde or other chemicals	ANOVA or Kruskal-Wallis H test depending on test for normality; presented mean & SD by exposure group, stratified by smoking status Results of test for normality were not reported, comet assay endpoints were not In-transformed	Exposed $N = 46$ Referent $N = 46$	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Ballarin et al. (1992) (Italy) Plywood factory	Personal samplers, Sampling in warehouse ($N = 3$) shearing-press ($N = 8$) & sawmill ($N = 1$), sampled formaldehyde and wood dust Calculated 8-hr TWA, reference for measurements (NIOSH, 1977).	Nasal respiratory mucosa cells, cell collection using endocervical brush, smeared onto previously coded slides, stain Feulgen's reaction plus Fast Green, MN, analysis blinded by one reader for cytogenetic, 6,000 cells/subject, scoring criteria Sarto et al. (1987)	Selection & recruitment of exposed and referent not described. Participation rates not reported. Referent from different source population: university or hospital clerks; excluded heavy drinkers	All nonsmokers, matched to referent for age and sex	Differences analyzed using Mann-Whitney test	Exposed $n = 15$; Referent $n = 15$	Small sample numbers; no obvious bias
Bauchinger and Schmid (1985) (Germany) Papermaking	Exposure assessment based on air monitoring and job-function. Sampling design and duration was not described.	Peripheral lymphocytes, CA/ cell (scored 500 cells/subject), Giemsa staining; SCE/cell (scored 50/subject) analyzed using coded slides	Selection & recruitment of exposed and referent not described. Participation rates not reported. Exposed and referent worked at same factory	All male, Comparable for age, more smokers among referent; no previous radiation history or exposure to other industrial chemicals	Mann-Whitney rank U test to compare groups, SCE analysis stratified by smoking	Exposed $N = 20$; Referent $N = 20$	Possible bias toward null because no adjustment for smoking in CA analysis
Bono et al. (2010) (Italy) Pathology labs	Personal sampling over an 8-hour shift in each subject; LOD $0.05 \mu\text{g}/\text{m}^3$; questionnaire data on job-specific work (work in production room where slides were fixed or other areas) & use of personal protection	M_1dG adducts in DNA extracted from whole blood, methods described in van Helden et al. (2009) ; evaluated in 20 out of 40 exposed and 20 out of 32 referent workers (selection criteria were not described)	Selection & recruitment of exposed and referent not described. Participation rates not reported. Recruited workers from 3 pathology labs and workers & students from a university lab with	Mean formaldehyde levels varied by age, smoking, and exposure status (referent, work in production room, work in other areas); confounding assessed in analysis	Formaldehyde exposure tertiles based on 8-hr average formaldehyde concentration, compared mean log-transformed M_1dG adducts by exposure tertile or exposure status, using ANCOVA	Exposed $N = 20$ Referent $N = 20$	No obvious bias; small sample size especially for analysis of effect modification by smoking

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
			no exposure to formaldehyde		adjusting for sex, age, smoking; evaluated multiple comparisons using Dunnett tests		
Bouraoui et al. (2013) (Tunisia) Anatomy/pathology lab in hospital	Area sample in macroscopic room, diffuse radical samplers containing 2,4-dinitrophenylhydrazine, 24-hr duration, 3 samplings.	Cytokinesis-blocked MN assay in peripheral lymphocytes in combination with FISH using all-chromosome centromeric probe Sari-Minodier et al. (2002) ; cultured 72 hr, smeared onto slides, stain 5% Giemsa, 2,000 binucleated cells scored/subject, criteria Fenech (2000) blinding not described.	Recruitment and selection not described. Participation rates not reported. Excluded x-ray history during previous 6 mos, use of drugs	Comparison groups were similar for potential confounders	Multivariate regression of genotoxic markers with possible confounders excluding smokers; age and gender were associated but exposure groups were comparable	Exposed $n = 31$ Referent $n = 31$	No obvious bias
Burgaz et al. (2001) (Turkey) Anatomy/pathology departments in hospital & university	Stationary area measurements; number of samples and duration not reported	Nasal respiratory mucosal cells; collected using endocervical brush, cells smeared onto previously coded slides, stain Feulgen's reaction plus Fast Green, MN, 3,000 cells/subject counted, scoring criteria Sarto et al. (1987) and Tolbert et al. (1992)	Recruitment and selection not described. Referents worked in same hospital & university	Higher proportion of females in exposed (referent was only male), slightly older individuals, and smokers (and heavy smokers) in referent. Analyses stratified by smoking. Stated that referents had no occupational exposure to genotoxic agents.	Comparison of means using nonparametric methods, two-tailed tests, stratified by smoking; correlation using Spearman's test	Exposed $n = 23$, Referent $n = 25$	Possible bias to null because of age in referent

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Burgaz et al. (2002) (Turkey) Anatomy/pathology departments in hospital & university Possible overlap with Burgaz et al. (2001)	Stationary area measurements; number of samples and duration not reported	Buccal mucosal cells; cells collected with wooden spatula, smeared onto slides, stain Feulgen's reaction plus Fast Green, MN, 3,000 cells/ subject counted, coded slides, scoring criteria Sarto et al. (1987) and Tolbert et al. (1992)	Recruitment and selection not described. Referents worked in same hospital & university	Higher proportion of females (referent was only male), and smokers in referent. Age comparable. Stated that referents had no occupational exposure to genotoxic agents;	Comparison of means using nonparametric methods (Mann-Whitney test), two-tailed tests, correlation using Spearman's test Multifactorial ANOVA adjusting for smoking, exposure and gender and age	Exposed $n = 28$, Referent $n = 18$	No obvious bias
Costa et al. (2008) (Portugal) Hospital pathology laboratories ($n = 4$) (prevalence)	Samples in breathing zone, NIOSH method #3500. Sampling duration, sample number were not given. 8-hr TWA calculated for each worker	Peripheral lymphocytes; blood samples collected 10–11 am; processed immediately; Scored blind to exposure status; Comet assay, parameter: tail length, alkaline conditions (pH = 13), Singh et al. (1988) lysis 1 hr, 20 min electrophoresis, 100 cells/ subject, image analysis software; Cytokinesis-blocked MN test, Teixeira et al. (2004) ; culture incubation 72 hr; samples applied by smears to slides, stain 4% Giemsa; scored 1,000 binucleated cells/subject, scored	Selection & recruitment of exposed and referent not described. Participation rates not reported. Unexposed worked in administrative offices in hospitals in proximity to pathology labs	Exposed matched to unexposed by age, gender, lifestyle and smoking habits; unexposed worked in same area in administrative offices Demographic information provided	Analyses by one-way ANOVA and Student's t -test	Exposed $n = 30$; Referent $n = 30$	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		blind by one reader, criteria Caria et al. (1995) ; SCE/ cell, 50 2nd division metaphases scored by one observer, Scored blind to exposure status					
Costa et al. (2011) (Portugal) Hospital pathology laboratories (<i>n</i> = 5) (prevalence)	Samples in breathing zone, NIOSH method #3500. Sampling duration, sample number was not given. 8-hr TWA calculated for each worker	Peripheral lymphocytes; blood samples collected 10–11 am; processed immediately; scored blind to exposure status; comet assay, parameter: tail length and % tail DNA; alkaline conditions, Singh et al. (1988) 100 cells/subject, image analysis software; Cytokinesis-blocked MN test Teixeira et al. (2004) ; culture incubation 72 hr; samples applied by smears to slides, stain 4% Giemsa; scored 1,000 binucleated cells/subject, scored blind by one reader, criteria Fenech (2007)	Selection & recruitment of exposed and referent not described. Participation rates not reported. Excluded exposed with <1 year employment. Unexposed worked in administrative offices in hospitals in proximity to pathology labs.	Exposed matched to unexposed by age, gender, and smoking habits. Demographic information provided	Comet assay: normal distribution, analyses by one-way ANOVA and Student's <i>t</i> -test MN: not normal distribution, used nonparametric tests, Mann-Whitney U test and Kruskal-Wallis test	Exposed <i>n</i> = 48; Referent <i>n</i> = 50	No obvious bias.

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Costa et al. (2013) (Portugal) Anatomy/pathology lab workers	# samples and duration not reported. Air sampling in breathing zone. Calculated 8-hr TWA for each subject; NIOSH method # 3500	Peripheral blood samples collected between 10–11 am. Samples processed and assays conducted blinded. Cytokinesis-blocked MN test Teixeira et al. (2004) . 1,000 cells analyzed/subject, MN per 1,000 binucleated cells, scored blindly by one reader, criteria Fenech (2007) . SCE, scored 50 M2 metaphases/ subject by one reader T-Cell Receptor mutation assay in mononuclear leukocytes, # events in mutation cell window (CD3-CD4+ cells) divided by total number of events for CD4+ cells	Included workers with at least 1-year employment in 4 hospital pathology anatomy labs; referent worked in administrative offices in same area & no occupational exposure history to formaldehyde	Similar in gender distribution, age, BMI, and smoking habit Demographic information provided	Difference in means, Student's <i>t</i> -test; tested for normal distribution multivariate analysis adjusted for age, gender, and smoking	Exposed <i>n</i> = 35; referent <i>n</i> = 35	No obvious bias
Costa et al. (2015) (Portugal) Anatomy/pathology laboratories	Samples in breathing zone for periods during formaldehyde-related tasks, NIOSH method #3500. Sampling duration, sample number was not given.	Peripheral blood samples collected between 10–11 am. Samples processed and analyzed blinded. Chromosome aberrations (structural and numerical), duplicates cultured 51	Included workers with at least 1-year employment in 4 hospital pathology anatomy labs; referent worked in administrative offices in same area & no occupational exposure history to	Similar distributions by exposure group for age, gender, and smoking. Evaluated possible confounding by other measures (diet) and found confounding by fruit consumption for frequency of	Exposed compared to unexposed using Student's <i>t</i> test for ln % tDNA or Mann-Whitney U-test for CA measures; linear regression of ln %tDNA; negative binomial regression for untransformed	Exposed = 84; Unexposed = 87	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	8-hr TWA calculated for each worker	hrs cited Roma-Torres et al. (2006) , 4% Giemsa stain; coded slides; scored 100 metaphases per person, 1,250× magnification; CTAs & CSAs according to Savage (1976) ; gaps not included. Comet assay: alkaline conditions according to Singh et al. (1988) ; Scored blind 100 cells/donor from two gels; % DNA in comet tail.	formaldehyde; exclusions cancer/tumor history, radiation therapy or chemotherapy treatments, last year surgery with anesthesia and blood transfusions.	multiaberrant cells and %tDNA.	total-CAs, CSAs, CTAs, gaps, aneuploidies, & aberrant cells; Poisson regression for untransformed multiaberrant cells. Models adjusted for age, gender and smoking plus actual confounders for specific parameters. Analyzed effect modification by genotype (homozygous variant plus heterozygous) compared to homozygous wildtype, genotype frequency compared by Pearson's chi-square test		
Costa et al. (2019) (Portugal) Anatomy/ pathology laboratories	Samples in breathing zone for periods during formaldehyde-related tasks and at other sites "considered relevant", NIOSH method #3500. Sampling duration and number were not given.	Peripheral blood samples collected and processed and assays conducted blinded. Exfoliated cells were collected for each cheek separately. Cytokinesis-blocked MN test, Costa et al. (2008) ; culture incubation 72 hr; samples applied by smears to slides, stain 4% Giemsa; scored	This study analyzed additional endpoints using blood and buccal cell samples collected in Costa et al. (2015) . Selection & recruitment of exposed and referent not described. Participation rates not reported. Included workers	Similar distributions by exposure group for age, gender, and smoking. Exposed smokers smoked less than unexposed smokers (11 vs. 15 pack-years). Evaluated possible confounding by other measures (diet) and found confounding by fruit consumption	Sample size varied by endpoint because of "sample limitation and/or technical losses," although missingness likely not associated with exposure. Data were log transformed to approximate normal distribution for TCR-Mf and Mann-Whitney U test	MNL Exposed = 84; Unexposed = 87 SCE/cell Exposed = 84; Unexposed = 87 MNB Exposed = 63; Unexposed = 69 BNbud	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	8-hr TWA calculated for each worker	1,000 binucleated cells/subject, scored blind by one reader, criteria defined by Fenech (2007) Buccal MN cytome assay. Scored blind by same reader, 2,000 differentiated cells scored for frequency of MN, nuclear buds and nucleoplasmic bridges according to Tolbert et al. (1992); Thomas et al. (2009). SCE/ cell, 50 2nd division metaphases scored by one observer, Scored blind to exposure status. T-Cell Receptor mutation assay in mononuclear leukocytes, flow cytometry, minimum of 2.5×10^5 lymphocyte-gated events were acquired, # events in mutation cell window (CD3-CD4+ cells) divided by total number of events for CD4+ cells	with at least 1-year employment in 9 hospital pathology anatomy labs; referent worked in administrative offices in same area & no occupational exposure history to formaldehyde.	for frequency of multiaberrant cells and %tDNA. The association of exposure with possible confounders was examined using linear regression. Dietary habits were reported to be parameter-specific actual confounders for white blood cell counts.	applied to MN in lymphocytes and buccal cells and nuclear buds in buccal cells. Associations (mean ratio (MR), 95% CI) with SCE, MNB, BNbud and log TCR-Mf were assessed using Poisson regression. Untransformed MNL also were modeled using negative binomial regression. Models adjusted for age, gender, smoking habits and dietary habits. Effect modification by genotype analyzed using Mann-Whitney U test for specific polymorphisms in CYP2E1, GSTM1, GSTT1, GSTP1, SRCC1, PARP1, MUTYH, RAD51 BRIP1 and FANCA.	Exposed = 63; Unexposed = 69 TCR-Mf Exposed = 61; Unexposed = 64	

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Fleig et al. (1982) (Germany) Formaldehyde manufacturing	Personal sampling, 8-hr shift, number of measurements or people with monitors not reported. Measurements were not reported. Provided categories of maximum exposure as % of MAK value for 25%, 60%, and 100% of MAK for two periods (before and after 1971)	Chromosome aberrations, peripheral blood lymphocytes cultured 70–72 hrs, 10% Giemsa stain; coded slides. Presented aberrant cells/ individual both including gaps and excluding gaps	Recruitment and selection of participants not described. Referent group from administrative or office staff at same site with no formaldehyde exposure	Referent matched to exposed by age and gender; stated smoking not associated with CA (data not reported)	Fisher-Yates exact test	Exposed $n = 15$, referent $n = 15$	Cell incubation period 72 hrs
Gomaa et al. (2012) (Egypt) Pathology, histology and anatomy laboratories at a university	No formaldehyde measurements	Chromosome aberrations (structural and numerical) cited Verma (1998), peripheral blood lymphocytes cultured 72 hrs, 5% Giemsa stain; blinding not described; scored total CA and types, analyzed 50–100 metaphases per subject. Comet assay, alkaline conditions according to Singh et al. (1988) ; tail length & tail moment; blinding not described; analyzed 50 cells per subject.	Recruitment and selection of participants not described. Referent group described to be unexposed	Age comparable between exposed and referent; data analysis by gender; no evaluation of smoking	Difference in mean values between exposed and referent, Student's t -test	Exposed $n = 30$, referent $n = 15$	Cell incubation period 72 hrs; blinding not described; no evaluation of smoking

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Hayes et al. (1997) (USA) Panel study, 9 wks embalming course Related to Suruda et al. (1993)	Personal sampling; cumulative exposure estimated using sampling data and time-activity data; continuous area samples at head height over embalming tables for short-term peak concentrations; monitored for other compounds: glutaraldehyde, methanol, isopropyl alcohol, and phenol	Blood samples collected in morning before 1 st class and after 9 wks; analysis blinded to exposure status; O ⁶ -alkylguanine DNA alkyl-transferase activity in peripheral blood lymphocytes (according to Klein and Oesch, 1990), expressed as pmol AGT/mg protein (LOD 0.006 pmol AGT/mg protein), blind to period of sample (before or after)	Recruited volunteers prior to beginning of course; reported loss to follow-up.	15 students had some prior embalming experience during lifetime; exposure to other chemicals below LOD or very low; confounding not likely	Change in individual; Individual data pre- and postcourse AGT activity in peripheral blood lymphocytes depicted in graphs by embalming experience during previous 90 days (yes/ no), ANOVA adjusting for age, sex, and smoking.	N = 29	No obvious bias, small sample size
He et al. (1998) (China) Prevalence Anatomy students	Breathing-zone samples during dissection; number, duration of sampling not described	Blood collection not described. Assays used whole blood. Cytokinesis-blocked MN assay, cultured 72 hr, cells processing (Fenech and Morley, 1985), blinding not described (scored 1,000 cells per individual), CA analyzed 100 metaphases, modified fluorescence-plus-Giemsa stain; SCE analyzed 50 metaphases, Giemsa stain, Blinding not described	Recruitment and selection details not described. Demographic data comparing exposed and referent groups were not provided.	All nonsmokers, age and sex similar (data not reported)	Analytic method not described	Exposed n = 13 Referent n = 10 (# in table reported as 13)	Deficiencies and inconsistency in reporting, small sample numbers.

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Jakab et al. (2010) (Hungary) Hospital and university pathology department	Area samples, records of measurements within 1–3 years of study 8-hr TWA determined	Venous blood collection, timing not stated, peripheral blood lymphocytes HPRT gene mutations, unscheduled DNA synthesis, CA and SCE whole blood samples, cultures incubated 50 (CA) and 72 (SCE) hours; CA stain 5% Giemsa, SCE fluorescence plus Giemsa; analyses blinded, for CA scored 100 metaphases/ subject. Scored total CA and types, SCE and high frequency SCE, total premature centromere division (PCD) and mitoses with >3 chromosomes with PCD	Recruitment and selection of participants not described. Participation rates not reported. Referent group from health-service staff in same hospitals	Provided data on demographic characteristics; Age comparable, Formaldehyde only group had higher proportion of smokers, more cigarettes/d and higher proportion drinkers. Solvents were ethyl alcohol, acetone, and xylene	Exposure groups compared, student's <i>t</i> -test SCE stratified by smoking, CA frequency analyses not stratified	HCHO alone <i>N</i> = 21; HCHO and solvents <i>N</i> = 16; Referent <i>N</i> = 37	Possible confounding by smoking on CA association not assessed. Direction: potential over-estimation
Jiang et al. (2010) (China) Woodworkers (prevalence study)	Personal samples in breathing zone; 3–5 workers from each job title, 5 referent workers; 8 hr samples; calculated 8-hr TWA	Blood lymphocytes; blinded analysis; comet assay (DNA strand breaks), lymphocytes isolated within 2 hr after blood draw, alkaline conditions, (Singh et al., 1988) ; slides desiccated, shipped to Beijing, >100 cells/ subject, image analysis software.	Selection & recruitment of exposed and referent not described. Participation rates not reported. 263 male workers all Han Chinese; 151 exposed from 2 plywood industries; 112 referents from a	Excluded subjects with recent exposure to known mutagenic agents (x-ray) chronic conditions (autoimmune disease), recent antibiotic use. Structured questionnaire collected info on smoking, alcohol,	Ln-transformed Olive TM and CBMN frequency ANOVA differences by exposure group; <i>t</i> -test for differences in means. ANCOVA differences by years of exposure among exposed adjusted for age, formaldehyde	Referent <i>N</i> = 112 Exposed <i>N</i> = 151	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		MN: cytokinesis-block micronucleus assay (chromosome damage), scoring criteria (Fenech et al., 2003) 1,000 binucleated lymphocytes/ subject	machine manufacturing plant in same town	medical conditions, occupational history & house redecoration in last year. Evaluated mean age and frequency of smoking and alcohol by exposure level.	concentration, smoking and alcohol.		
Kitaeva et al. (1996) (Russia) Translation Formaldehyde production and anatomy lab workers	Exposure definition by job task, no formaldehyde measurements	MN assay in buccal mucosal cells, blinding not described, cell collection using swab, smeared onto slides, stain Feulgen and light green, analyzed 2,000 cell/ subject. CA in peripheral blood (blood from finger), reported % metaphases with aberrations after 72-hrs culture; # metaphases at 72 hrs cultivation was low (148), observed in only 8 exposed workers	Recruitment and selection not described. Referent group not defined clearly.	Referents 10 years younger than exposed; Stated that age and smoking were not related to MN or CA frequency, gender not related among unexposed, Data not shown.	Analysis using Student method with Freeman-Tukey transformation and results were not clearly presented	Female Exposed $n = 8$ Female Referent $n = 7$; Students $n = 12$	Small numbers, reporting deficiencies for details of study design and results, difficult to evaluate
Kurtio et al. (1993) (Finland) Wood plywood/ veneer manufacture	No formaldehyde measurements; exposure defined by task; 5 out of 15 exposed, considered to be exposed to formaldehyde; referent selected from same town employed at municipal energy	Venous blood samples cultured all on same day; cultured for 48 hr according to (Jantunen et al., 1986); slides coded; analyzed 100 metaphases per subject	Selection of exposed and referents not described; referents were employed in other industries (potential for dissimilarities)	All male, matched on age, data analysis excluded 1 smoker	Structural aberrations, mean # per cell by exposure, Mann-Whitney U-test (2-tailed)	Exposed $n = 15$; Referent $n = 15$	5 out of 15 considered exposed to formaldehyde; no formaldehyde-specific data analysis Not informative

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	plant, a loading company, or a health care center						
Ladeira et al. (2011) (Portugal) Histopathology labs in 6 hospitals	Personal air sampling, 6–8 hrs, estimated 8-hr TWA (NIOSH method 2541) Ceiling values for each task	Cell collection between 10 am and noon. Samples coded and analyzed blinded. Peripheral blood lymphocytes, cytokinesis-block micronucleus cytome assay, fresh samples, cultured for 72 hr, applied to slides with cytocentrifuge, May-Grunwald-Giemsa, 1,000 binucleated cells scored/ subject by 2 readers; buccal mucosa cells, collection using endobrush, smeared onto slides, stain Feulgen, 2,000 cells scored/ subject, 2 readers	Recruitment and selection not described. Participation rates not reported. Excluded history of cancer, radio or chemotherapy, use of therapeutic drugs, exposure to diagnostic x-rays in the past 6 mos, intake of vitamins or other supplements like folic acid (no one was excluded)	Exposed were older, with lower proportion of drinkers and smokers	Comparisons by exposure group; binary logistic regression and Mann-Whitney test Stratified by categories of age, gender and smoking	Exposed $n = 56$, referent $n = 85$	No obvious bias
Lan et al. (2015) (China) Formaldehyde-melamine resin production or use (related study related study)	Personal monitors for 3 d over entire shift within a 3-wk period. Formaldehyde concentration: 8-hr TWA Exposed	Postshift and overnight peripheral blood samples. Metaphase spreads from colony forming unit granulocyte macrophage (CFU-GM) cultured for 14 d;	Analyzed aneuploidy among subset with scorable metaphases, high formaldehyde among exposed and existence of comparable	Referents frequency-matched by age (5 year) and gender Personal sampling of volatile organic compounds; concentrations at	Analyzed using negative binomial regression controlling for age and gender. Also evaluated potential confounding from current smoking and	Exposed $n = 29$; Referent $n = 23$	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Zhang et al. (2010) ; Bassig et al. (2016)	Median: 1.38 ppm (1.7 mg/m ³) 10 th & 90 th percentile: 0.78, 2.61 ppm 0.96, 3.2 mg/m ³) Referent 0.026 ppm (0.032 mg/m ³) 10 th & 90 th percentile: 0.015, 0.026 ppm (0.019, 0.032 mg/m ³) LOD: 0.012 ppm	chromosome-wide aneuploidy analysis using OctoChrome FISH; scored minimum 150 cells/subject; analysis blinded to exposure.	referents. Participation rates exposed 92%, referent 95%. Referent from 3 workplaces in same geographic region as exposed, engaged in manufacturing with similar demographic and SES; excluded history of cancer, chemotherapy, and radiotherapy, previous occupations with exposure to benzene, butadiene, styrene, and/or ionizing radiation.	background, urinary benzene at background and comparable between groups	alcohol use, recent infections, current medication use, and body mass index (Supplemental tables in(Lan et al., 2015)).		
Lazutka et al. (1999) (Lithuania) Carpet and plastic manufacturing Prevalence study	Industrial hygiene area measurements reported by plant; carpet plant, formaldehyde 0.3–1.2 mg/m ³ , styrene 0.13–1.4 mg/m ³ , phenol 0.3 mg/m ³ ; plasticware plant, formaldehyde 0.5–0.9 mg/m ³ , styrene 4.4–6.2 mg/m ³ , phenol 0.5–0.75 mg/m ³	Peripheral blood samples; chromosome aberrations, cells cultured 72 hr, differential staining fluorescence–plus–Giemsa, CA scored on coded slides, >100 first mitotic division cells per subject.	Recruitment and selection not described. Participation rates not reported.; Source population for nonexposed referents not described	Nonexposed were “approximately” matched to exposed by age; males and females, smokers and nonsmokers included; demographic information provided; unable to distinguish between formaldehyde and styrene	ANOVA including variable for exposure and age, no adjustment for smoking or gender; CA data transformed using average square root transformation	Carpet plant, exposed 38 male, 41 female; unexposed 64 male, 26 female Plastic plant, exposed 34 male, 63 female; unexposed 64 males, 26 females	Cell incubation period 72 hrs; unable to distinguish between formaldehyde and styrene effects Direction: potentially overestimated

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Lin et al. (2013) (China) Woodworkers (prevalence study) 2009 (cross-shift) 2011	Prevalence: Area samples (2 badges in each of 5 workplaces with differing tasks), 8-hr samples on 2 d. Change over work-shift: badges in breathing zone of 2–4 representative workers conducting different job types (8-hour samples). Referent group exposed, mean 0.13 mg/m ³ (0.019–0.252)	Blood lymphocytes; blinded analysis; comet assay (DNA strand breaks), alkaline conditions (pH = 13) (Olive and Banath, 2006), lysis 2-hr for <i>N</i> = 178 & over-night for <i>N</i> = 62, 50 lymphocytes/sample, image analysis software; cytokinesis-block micronucleus assay, Fenech (1993) analyzed 1,000 binucleated cells/subject, scoring criteria Fenech (1993) , Fenech et al. (2003) ; Zhitkovich and Costa's KCI-SDS assay (DNA-protein crosslinks)	Selection & recruitment of exposed and referent not described. Participation rates not reported. Exposed and referent from same factory.	Excluded subjects with exposure to known mutagenic agents in previous 3 months (radiotherapy & chemotherapy). Structured questionnaire collected info on smoking, alcohol, medical conditions, occupational history, and house redecoration in last year.	Natural log-transformed olive TM. Prevalence: ANOVA differences by exposure group (control, low and high), adjusting for age, sex, smoking, alcohol, # work years) Regression for trend across exposure level adjusting same as above; Poisson regression for MN frequencies, linear regression for Ln(OTM)) Cross-shift: Paired Wilcoxon test (MN freq) or paired <i>t</i> -test (OTM or DPX); regression models for trend with exposure levels	Referent <i>N</i> = 82 Low <i>N</i> = 58 High <i>N</i> = 38	Referent group with significant formaldehyde exposure, potential bias toward null.
Marcon et al. (2014) (Italy) Population living in proximity to chipboard plants	Modeled outdoor formaldehyde concentrations at residential address based on data from 62 monitoring sites in district; four 1-wk sampling periods (2 each in warm and cold seasons); calculated annual	Epithelial mucosal cells using cytology brush; comet assay, alkaline conditions, 50 cells per subject; MN 2,000 cells per subject, according to Tolbert et al. (1991)	Random sample of participants in previous survey (93% of population in Viadana District) with children under 12 years, Italian primary language, and address information; invited stratified random	No adjustment for indoor formaldehyde concentrations; co-exposure with NO ₂	Linear regression for tail length, tail intensity, tail moment and binucleated cells; negative binomial regression for micronuclei and nuclear buds; models adjusted for children's sex, age,	<i>N</i> = 413; Analysis included only complete datasets for comet assay, <i>n</i> = 310 and MN <i>n</i> = 374	Potential exposure misclassification; no obvious bias No indoor formaldehyde exposure measure Not informative

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	average concentration of formaldehyde and NO ₂ ; estimated at each address using ordinary Kriging; formaldehyde 2.5 ± 0.3 mg/m ³ , NO ₂ 16.0 ± 3.5 mg/m ³ ,		sample in 3 strata of distance from wood factories (656 remaining in district since 2006 of 750), participation 63%, participation was not higher in residents closest to wood factories; higher proportion of nonparticipants were of foreign nationality and had smoking parents		nationality, parents' education, parents' smoking, exposure to tobacco smoking at home, time with windows open, traffic near home, orthodontic appliance, condition of teeth, person who collected cell sample		
Musak et al. (2013) (Slovakia) Prevalence study Pathologists	Air monitoring once per year (no details provided)	Chromosomal aberration, peripheral blood lymphocytes, blinded analysis, cultured 48 hr, 100 mitoses scored/subject, 2 scorers	Recruitment and selection of participants not described. Participation rates not reported. Exposed and referent all employed in hospitals	Exposed and referent comparable for age, gender; % smokers slightly higher in exposed; analyses adjusted for age, gender, job type, and smoking	Adjusted odds ratios, Binary logistic regression controlling for age, gender, job type, and smoking	Exposed N = 105; Referent N = 250	No obvious bias
Orsiere et al. (2006) (France) Hospital pathology labs (prevalence)	Personal sampling near breathing zone; Short-term: 15 minutes, Long-term 8 hrs during typical work day.	Peripheral lymphocytes, blood samples taken preshift and postshift; processed within 6 hr, assays conducted blinded. Chemiluminescence microplate assay; cytokinesis-blocked micronucleus assay	Selection & recruitment of exposed and referent not described, however subgroups selected randomly. Exposed and referent worked in same institution.	Groups similar for gender, age, % smokers. No exposure to other genotoxic substances. Excluded history of radiotherapy or chemotherapy and use of therapeutic drugs that were	Differences by group analyzed using nonparametric Mann-Whitney U-test; median DNA repair across shift analyzed using Wilcoxon W-rank sum test. Analyzed binucleated	Exposed n = 59; referent n = 37; Subgroups Exposed n = 18; referent n = 18	No obvious bias.

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		Sari-Minodier et al. (2002) ; cultured 72 hr, smears on slides, stain 5% Giemsa, scoring criteria (Fenech, 2000) ; 1,000 binucleated cells/ subject; FISH with a pan-centromeric DNA probe, same operator scored exposed and referent blinded		known mutagens or reproductive toxicants	micronucleated cell rate (BMCR), and MN measures using multivariate regression adjusting for smoking, drinking, age, and gender.		
Pala et al. (2008) (Italy) Research institute lab (prevalence)	Personal samples, one 8-hr shift; 75% exposed to < 0.026 mg/m ³ .	Peripheral blood samples collected at same time at end of day; processed within 20 hr; analysis blind to exposure. CA, harvested after 48 hr, 100 metaphases/ subject SCE, cultures harvested at 72 hr, analysis of 30 second-division cells/subject; MN: modified cytokinesis-blocked method, Fenech and Morley (1986) ; 72 hr incubation, stain 3% Giemsa, 2,000 cells/subject	Selection & recruitment of exposed and referent not described. Participation rates not reported.	Statistical models adjusted for gender, age, and smoking	Multivariate regression models adjusting for gender, age, and smoking; Poisson model for CA and MN, SCE log-normal random effects model, comparisons were low and high exposure groups, below and above 26 µg/m ³	N = 36	No obvious bias; only 9 exposed above 0.026 mg/m ³ .
Peteffi et al. (2015) (Brazil) Furniture manufacturing	Monitoring in 7 sections in facility; referent monitoring in 5 areas of	Peripheral blood processed within 4 hr. comet assay, alkaline conditions according to	46 workers in furniture manufacturing facility and	Exposed and referent had comparable distributions for age,	Nonparametric tests used because data were not normally distributed. Exposed	Exposed n = 46, referent n = 45	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	university; breathing zone 8-hr samples collected on same day as biological samples. Urine samples collected at end of work day on 5 th day of work; correlation of formaldehyde concentration in air with urinary formic acid concentration, $r = 0.626$, $p < 0.001$	Tice et al. (2000) ; silver nitrate staining according to Nadin et al. (2001) ; 100 cells/person read by two independent observers (50 cells each). Blinding not stated, classified by visual scoring according to Anderson et al. (1994) ; 5 categories based on tail migration (0–IV) and frequency of damaged cells (sum of I–IV), damage index (Pitarque et al., 1999) Oral mucosa samples (scraped with endocervical brush), micronucleus test, DNA-specific Feulgen staining and counterstaining with Fast Green according to Tolbert et al. (1992) ; analyzed 2,000 cells/person by 2 independent observers (1,000 ea)	unexposed group recruited from employees and students of local university with no history of occupational exposure to potentially genotoxic agents or substances metabolized to formic acid	smoking, and alcohol; differed by gender Exposed 56.5% male, referent 33.3% male; no association of any biomarkers with gender (data not shown)	and referent compared using Mann-Whitney test;		
Santovito et al. (2014) (Italy) Hospital nurses	All exposed used protective equipment; no formaldehyde measurements, intensity and	Peripheral blood samples, coded, processed within 2 hr after collection. Cultures incubated for 48 hr for CA and 72 hr for SCE; CA slides	20 female nurses from 2 analogous departments in 2 hospitals; 20 referents from administrative departments of	Accounted for sex, age, smoking, and alcohol in design; referents from same hospitals	Mean frequencies compared, Wilcoxon test; regression analysis, association of age and exposure duration on CA and SCE	Exposed $n = 20$; Referent $n = 20$	Potential for large degree of exposure misclassification and variation in intensity of exposure; bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	frequency likely highly variable	stained with 5% Giemsa, scored 200 metaphases per subject, gaps not scored as CA; SCE 50 metaphases scored per subject	same hospital; all nonsmokers and did not consume alcohol	Nurses exposed to other substances			toward null; small sample size
Santovito et al. (2011) (Italy) Pathology wards	Personal sampling near breathing zone, 8-hr duration	Venous blood sample collected at end of shift, samples coded and processed within 4 hr, same day concentration sampling conducted, cultured 48 hrs; CA 5% Giemsa stain; scored 100 metaphases/ subject	Recruitment and selection of participants not described; participation rates not reported.	All nonsmokers, nondrinkers, no drug use 1 year prior; no information on other exposures (acetone, ethyl alcohol, xylene)	Mean % of cells with aberrations and frequencies of aberrations per cell compared using Mann-Whitney U test, 2-tailed. Generalized linear models (Poisson distribution) adjusting for age, gender, polymorphisms, Cubic spline regression of mean % of cells with aberrations and frequencies of aberrations per cell with number years exposed and age	Exposed $n = 20$; Referent $n = 16$	No obvious bias Small sample size
Schlink et al. (1999) (Germany) Anatomy students	Personal sampling near breathing zone once per week, sampling period not reported. formaldehyde exposed, Mean $\pm$ SD,	Blood samples collected before 1 st class and after days 50 and 111; O ⁶ -alkylguanine DNA alkyl-transferase activity in peripheral blood lymphocytes	Recruitment and participation of students were not described. 41 students from 1 university course, 16 students from a	Considered effects of age, sex, smoking, and alcohol	MGMT activity change compared (U-test, paired data) within categories of sex, smoking, allergy, and alcohol; as well as between groups	Exposed $N = 41$ Referent $N = 10$	No obvious bias, small sample size

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	0.2 ± 0.05 mg/m ³ , 0.14–0.3 mg/m ³	(modification of Klein and Oesch (1990) , expressed as fmoI MGMT/ 10 ⁶ cells (LOD 1 fmoI MGMT/ 10 ⁶ cells), blind to period of sample (before or after)	different university course, and 10 unexposed students		(Wilcoxon, Mann and Whitney U-test)		
Shaham et al. (1997) (Israel) anatomy/ pathology departments (prevalence) also reported in Shaham et al. (1996)	Personal and “field” samples, duration 15 min, multiple times during work day (# not reported).	Peripheral lymphocytes; DPX, K-SDS method; double blinded. SCE at 72 hrs, mean of 30 cells/ individual, blinding not described	Selection & recruitment of exposed and referent not described. Participation rates not reported. Referent group worked at same institution.	Exposed and referent matched by age (matching protocol not described). No exposure to other mutagens or substances known to cause DPX in either exposed or referent.	Analyses by ANOVA adjusting for smoking; difference in means, <i>t</i> -test; linear regression for DPX levels or means SCE per chromosome by years of exposure to formaldehyde	Exposed DPX: <i>N</i> = 12 SCE: <i>N</i> = 13 Referent DPX: <i>N</i> = 8 SCE: <i>N</i> = 20	Low sample numbers; no obvious bias.
Shaham et al. (2002) (Israel) Hospital pathology labs	Personal and area samples, sampling at different points in work day, sampling duration averaged 15 min	SCE in peripheral lymphocytes, blood samples collected at same time in morning; blinding not described, stain fluorescence plus 5% Giemsa, scored 30–32 cells/subject	Recruitment and selection of participants not described. Referent group from administrative sections of same hospitals	Authors presented demographic data. Exposed were higher proportion female, European/ American, education >12 year, and lower proportion smokers. No exposures to other chemicals linked to SCE. Confounding addressed in analysis	Mean # SCEs per chromosome and proportion of high frequency cells compared between exposed and referent. Difference between means assessed using ANOVA (unbalanced design) adjusting for age, gender, smoking, origin and education years	Exposed <i>n</i> = 90; Referent <i>n</i> = 52	No obvious bias
Shaham et al. (2003) (Israel)	Personal and “field” samples, duration 15 min, multiple times	Peripheral lymphocytes; DPX, same protocol as	Selection & recruitment of exposed and	Adjustment for age, sex, smoking, origin, and years of	Analyses: comparisons of mean DPX adjusted for sex,	Exposed <i>N</i> = 186; Referent <i>n</i> = 213	No obvious bias.

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
14 hospital pathology departments (prevalence)	during work day (# not reported).	Shaham et al. (1997) ; SCE; pantropic p53	referent not described. Exposed and referent worked in same institution.	education in analysis. No exposure to other mutagens or substances known to cause DPX in either exposed or referent.	smoking, age, origin, and years education. Comparison of mean DPX by low and high formaldehyde levels and by duration of exposure, Mann-Whitney test		
Souza and Devi (2014) (India) Prevalence study Anatomy Dept (embalming)	No formaldehyde measurements reported.	Total MN/1,000 cells peripheral lymphocytes. Assays conducted blinded. Cytokinesis - blocked micronucleus assay Costa et al. (2008) ; stain 4% Giemsa, scoring criteria Fenech (2000) , 1,000 binucleated cells/subject. Frequency MN compared by exposure group using Student's <i>t</i> -test, and by duration of employment using Pearson's correlation.	Recruitment and selection of participants not described. Participation rates not reported.	Provided characteristics of exposure groups (see Table 1). All male, age comparable, higher prevalence smokers in exposed. Adjustment in analysis. Excluded frequent exposure to x-rays or other radiation, worked in paint or pesticide industries or history of chemotherapy.	Frequency MN compared by exposure group using Student's <i>t</i> -test, and by duration of employment using Pearson's correlation. Exposure and smoking evaluated together using two-way ANOVA.	Exposed <i>N</i> = 30 Referent <i>N</i> = 30	No obvious bias
Speit et al. (2007a) (Germany) Controlled human exposure study	Generation using para-formaldehyde; 10 consecutive d, 5 groups of 3–6 persons in chamber, 4 hr exposures, some exposures masked with ethyl acetate, 3 15-min exercise sessions during exposure;	MN in buccal mucosal cells–1 wk before start, at time=0, after end of exposure, and 1, 2, and 3 wks after end of exposure; cells collected with metal spatula, smeared onto slides, blinded analysis at end of study by 1 person, stain DAPI/	Excluded severe allergy, skin or airways disease, acute infection, current smoking or within last 3 years, contact lenses or glasses, > 50 g alcohol per day, present use of psychotropic agents,	Within person comparison	Post exposure compared to preexposure using Wilcoxon ranked sum test	<i>N</i> = 21	No obvious bias.

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	randomized order of concentration, double blinded	propidium iodide, 2,000 cells/ subject	exposure to ionizing radiation, or cytostatic drugs during the last 6 mos				
Suruda et al. (1993) (USA) Panel study, 85 d Embalming course	Personal sampling for 121 of 144 embalmings; cumulative exposure estimated using sampling data and time-activity data; Continuous area samples at head height over embalming tables for short-term peak concentrations; monitored for other compounds: glutaraldehyde, methanol, isopropyl alcohol, and phenol	Nasal mucosa cells, oral mucosa cells, blood samples collected in morning before 1 st class and after 9 wks; processed on same day, analysis of slides blinded to exposure status; pre- and postslides from each subject stained at same time and read together by one reader, conducted a blinded 10% recount of slides; MN assay buccal and nasal cells Stich et al. (1982) , collected with cytopathology brushes, slides prepared with cytocentrifuge, stain Feulgen/ Fast Green, 1,500 cell/ subject; MN lymphocytes Fenech and Morley (1985) , stain Feulgen 2,000 cells/ subject; SCE 50 s division metaphases scored/ subject	Recruited volunteers prior to beginning of course; reported loss to follow-up. Excluded one student with many embalmings in previous 90 d, & 1 student who chewed tobacco during study	21 students had some prior embalming experience during lifetime; exposure to other chemicals below LOD or very low, confounding not likely	Change in individual; difference in mean pre- and postexposure, matched Student's t-test (SCE) or Wilcoxon sign-rank test (micronuclei); Change with cumulative exposure spearman's rank correlation coefficient & linear regression (if residuals were normally distributed)	N = 29	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Suskov and Sazonova (1982) (USSR) Phenol-formaldehyde resin production	Area samples, # and duration not reported	Cytogenetic analysis in peripheral lymphocytes; Chromosomal aberrations, blinding not described, Buckton and Evans cytogenetic method, 1973	Recruitment and selection not described.	Average age in exposed 39.1 year, referent 34 year. Matched for gender, smoking, alcohol, and medication (data not shown)	Compared chromosome aberration frequency by exposure group, chi-square	Exposed $n = 31$; Referent $n = 74$	Brief report, minimal detail of methods
Thomson et al. (1984) (Great Britain) Pathology lab	Sampling in breathing zone; 26 samples taken for the duration of the task involving formaldehyde exposure, over 1–3 mos, sample duration not reported, calculated TWA Measured peaks in breathing zone on 1 d for different tasks	CA frequency, stain fluorescence plus Giemsa technique Perry and Wolff (1974) , cells harvested 48 hr, slides coded and scored 100 1 st division metaphases/ subject; SCE frequency, cells harvested 72 hr, 50 cells/subject; blinding not reported	All exposed worked in same laboratory; characteristics of referent not provided.	Obtained smoking histories	Data analysis not described	Exposed $n = 6$; referent $n = 5$	Reporting of study methods and group characteristics not adequate; low sample numbers
Titenko-Holland et al. (1996) Same subject as Suruda et al. (1993) (USA) Panel study, 90 d Embalming course	See Suruda et al. (1993) Calculated 2 exposure periods: (1) Lagged 7–10 d before last sampling to account for lag in development of MN (2) 90-d cumulative	Buccal cells, Scored previously unstained and unanalyzed slides. New method: FISH with a centromeric probe—differentiates between clastogenic vs aneuploidogenic mechanism (total MN, MN- and MN+); <1,500 cells scored for 14 of 35 subjects; scored pre- and postexposure slides at same time, blinded.	Subjects with missing MN data were compared to those with complete data by Student's t -test; comparable for age, smoking, and mean exposure	Change in individual. Exposure to other chemicals below LOD or very low, confounding not likely	Change in total MN, MN- and MN+ frequency (per 1,000 cells) and change in mean MN. Excluded subjects with <500 epithelial cells available for analysis. Difference scores evaluated using Wilcoxon sign-rank test. Association with both formaldehyde	Complete MN data from buccal mucosa, $n = 19$ Complete MN data from nasal mucosa, $n = 13$	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		Frequency calculated by dividing # cells with MN by total # cells counted, multiplying by 1,000. 78% of preexposure slides and 76% of postexposure slides were scorable; 10% of slides were rescored			exposure metrics via Spearman non-parametric correlation coefficient, two-sided <i>p</i> -values		
Vasudeva and Anand (1996) (India) Medical student lab	<1 ppm, no data reported to support assertion	Peripheral blood lymphocytes, frequency of aberrant metaphases; cell culture 72 hr, Giemsa staining, blinding not reported	Recruitment and selection of participants not described. No demographic information provided.	Stated that participants had received no or insignificant radiation treatments (no data reported); exposed and referents matched by age, no other potential confounders evaluated	Data analysis not described	Exposed <i>n</i> = 30; referent <i>n</i> = 30	Reporting of methods, design and results not adequate to evaluate; cell incubation 72 hr
Viegas et al. (2010) (Portugal) Formaldehyde & resin production, pathology/ anatomy lab workers Also discussed in Viegas et al. (2013)	Personal air sampling, (<i>N</i> = 2 in factory, <i>N</i> = 29 in labs) 6–8 hrs, estimated 8-hr TWA (NIOSH method 2541). Ceiling values for each task	Buccal mucosa Peripheral blood lymphocytes, sample collection between 10 am & noon. Blinded coding and analysis, buccal cell MN cell collection using endobrush, smeared onto slides, Feulgen stain, 2,000 cells scored/ subject by 4 observers, scoring criteria Tolbert et al. (1992) , peripheral	Recruitment and selection not described. Participation rates not reported.	Presented comparisons for gender, age, and smoking. Difference by gender (higher prevalence males in exposed); genotoxic endpoints were not associated with smoking or gender, and only slightly with age	Correlation evaluated using Pearson or Spearman correlation test depending on distribution	Exposed, Production <i>n</i> = 30, Lab workers <i>n</i> = 50, Referent <i>n</i> = 85	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		lymphocytes, samples processed within 6 hr, cultured for 72 hr, applied to slides with cytocentrifuge, stain May-Grunwald-Giemsa, 1,000 binucleated cells scored/ subject					
Wang et al. (2019) (Shanghai, China) Chemical production	Routine formaldehyde monitoring by factory with sampling site selection using China national standard for hazardous substances air sampling in the workplace. Cumulative dose determined for each worker ($C \times T$). C = geometric mean of concentration for a year at a sampling site, T = years. Serum formaldehyde-albumin adducts (FA-HSA) quantified in fasting venous peripheral blood. Geometric mean range (mg/m^3): Exposed: 0.06–0.25	CBMN according to Fenech (2000) , Fenech (1993) . Blinded analysis. Venous peripheral blood cultured for 44 hr, Cytochalasin-B added to cultures, cells harvested 28 hours later, air dried slides stained with Giemsa, MN detected at 400 $\times$ with confirmation at 1,000 $\times$. 1,000 binucleated cells scored/ subject	Recruitment and selection of participants not described; participation rates not reported. 100 male workers exposed to formaldehyde > 1 year through 4 work processes (i.e., production examination, glue spraying, coating and workplace inspection). Demographic information, smoking and alcohol, medical and occupational history (job types and # years) collected by questionnaire. Unexposed group (n = 100 males) from the logistics workshop in same	Mean age and frequency of smoking and alcohol use were slightly higher in exposed. Work duration was higher in exposed. Age, smoking status and alcohol use were adjusted in statistical models.	MN frequency compared using Poisson regression and frequency ratio (FR) as effect estimate. Exposure was analyzed with quartiles for cumulative dose and FA-HSA concentration. Cumulative dose (mg/m^3): 0.01–0.06 0.06–0.125 0.125–0.9 0.9–3.75	Exposed n = 100 Unexposed n = 100	No obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
	Unexposed: 0.01		factory age matched (likely frequency matched since rates were different).				
Yager et al. (1986) (USA) Anatomy course, 10 wks	Area samples randomly distributed ($N = 13$, 1–4/ wk); breathing zone samples on 30 individuals at 15 tables ($N = 35$, 2–8/ week), mean sampling duration 18 min	Whole blood cultures; stain fluorescence plus Giemsa technique, Mean SCEs per cell in peripheral lymphocytes; before and after samples coded and randomized together for analysis, scored 80 cells/subject	Recruitment and selection not described.	All nonsmokers, 7 female	Paired t -test of before and after samples	$N = 8$	No obvious bias
Vargová et al. (1992) (Czechoslovakia) Woodworking	8-hr sampling duration in breathing zone	CA frequency, peripheral lymphocytes, Giemsa staining, cells harvested 48 hr, 100 cells/ subject. Blinding not described. CA frequency in both exposed and referent was higher than range considered normal	Recruitment and selection of participants not described; participation rates not reported.	Referents were matched to exposed (did not report what matching parameters were), no info on subject characteristics was reported Authors stated questionnaire data suggested that factors such as smoking and alcohol were different between exposed and referent; analyses were not adjusted.	Exposed and referent compared using student's t -test and arcsin-sq rt transformation test	Exposed $n = 20$ (or 25?); Referent $n = 19$	Reporting of study methods and group characteristics not adequate; # exposed in text did not match # exposed in table II in the paper. Lack of adjustment for confounding, bias toward null

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
Ye et al. (2005) (China, 1992) Formaldehyde exposure in factory or indoor air from building materials	Sampling according to NIOSH method; Referent $n = 6$; Waiters $n = 18$; Workers $n = 36$	MN in nasal mucosa, cell collection using swab, cells smeared onto slide, stain Wright's, scoring criteria Sarto et al. (1987) , per 3,000 cells, blinding not stated, results reexamined by another trained staff. SCE in peripheral lymphocytes, time of sample not stated; stain Giemsa solution, analysis blinded, 30 M ₂ lymphocytes analyzed/subject.	Recruitment and selection not described. Included: nonsmokers, no medicines for 3 wks prior and during study, no x-ray history for 6 mos prior, no drug use; comparison groups were from different sources: industrial exposed, wait staff (indoor air exposed), and unexposed student volunteers	Waiters and workers older than referent, % male 52% in referent, 25% in workers, 61% in wait staff; all Han Chinese; no adjustment for age or gender in analyses.	Analysis using one-way ANOVA and tested for multiple comparisons. Data presented in figures and values estimated from graph by EPA.	Workers $n = 18$; waiters $n = 16$; referent $n = 23$	Possible bias away from null; expect higher frequency of MN in older individuals. Small sample numbers.
Ying et al. (1997) ; Ying et al. (1999) (China) Panel study, 8-wk class Anatomy students	NIOSH (1977) method; 3-hr TWA and peaks; sample duration, number and frequency not described	Nasal mucosa cells, oral mucosa cells, blood samples collected before 1 st class and after last class; analysis of slides by 1 blinded observer with reexamination by another, nasal and buccal cells collected with swab, smeared onto slides, MN Nasal and Buccal cells, Wright's stain, scored 4,000 cells/ subject; MN blood lymphocytes, stain 4% Giemsa, scored mean of 2,870–3,167 cells/ subject; MN	Included nonsmokers, students living in dorms, disease-free & no medications prior 3 wks, no x-ray history prior 6 mos	Mean age 18.8 ± 1.0 year, all Han nationality, all lived in dorms, all nonsmokers	Change in individual over time; paired t -tests	$N = 25$	No obvious bias, small sample size

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
		scoring criteria Sarto et al. (1987) , SCE and LTR (Zhao et al., 1994): 30 M ₂ lymphocytes per subject analyzed blind to exposure					
Zendehdel et al. (2017) I(ran) Melamine dinnerware manufacturing Related publication: Zendehdel et al. (2018)	Personal air sampling, NIOSH method 3500, whole shift for each worker. Median time weighted average in three workshops, 0.086 mg/m ³ ; range, 0.02–0.22 mg/m ³ ; authors state that 2/3 of sample were exposed to < 0.1 mg/m ³	Comet assay, alkaline conditions, according to Tice et al. (2000) Blood samples collected same day as air sampling; blinding not described; minimum of 50 randomly selected cells per sample; tail moment and Olive moment	Workers in 3 melamine dinnerware manufacturing workshops (n = 49) and referents matched by age and sex (n = 34) who worked in food industries, # smokers higher in referent (26% versus 16%), >90% male. Recruitment and participation were not described.	Data in Table 1 of paper supported comparability of age, sex, and # smokers in exposed and referent groups.	Normal distribution assessed using Kolmogorov-Smirnov test. Difference in mean tested using Student <i>t</i> -test or Mann-Whitney test	Exposed N = 49; Referent N = 34	No obvious bias blinding not described;
Zhang et al. (2010) (China) Formaldehyde-melamine resin production or use Related publications: Gentry et al. (2013) ; Bassig et al. (2016) ;	Personal sampling for full shift (>240 min) on 3 working d over 3 wks. Exposed: at least 2 samples per individual; Referent: Sampling in subgroup on 1 d. Evaluated for other known or suspected leukemogens (benzene, phenol, chlorinated	Postshift and overnight peripheral blood samples; analysis blinded to exposure. Metaphase spreads from cultured colony forming unit granulocyte macrophage (CFU-GM); identified loss of chromosome 7 and gain of chromosome 8 using FISH	Participation rates exposed 92%, referent 95%. Referent from 3 workplaces in same geographic region as exposed, engaged in manufacturing with similar demographic and SES; excluded history of cancer, chemotherapy, and radiotherapy;	Referents frequency-matched by age (5 year) and gender	Analyzed using negative binomial regression (exposed compared to unexposed) controlling for age, gender, and smoking Mundt et al. (2017) presented individual data in graphs for chromosome 7 and chromosome 8,	High N = 10 Low N = 12	Small sample numbers, no obvious bias

Supplemental Information—Formaldehyde (Inhalation)

Reference and setting	Exposure measures and range	Outcome classification	Consideration of participant selection and comparability	Consideration of likely confounding	Analysis and completeness of results	Study size	Comment
(Mundt et al., 2017) Reanalyses	solvents), found none. Analysis blinded.		previous occupations with exposure to benzene, butadiene, styrene and/or ionizing radiation.		noting smoking status and whether 150 or more cells were evaluated. Gentry et al. reported that < 150 cells per individual were analyzed for several subjects. Not expected to be different between exposed and unexposed, impact likely to increase variability and attenuate association		

APPENDIX C. HAZARD IDENTIFICATION SUPPLEMENT

C.1. TOXICOKINETICS OF INHALED AND ENDOGENOUS FORMALDEHYDE

This chapter presents specific information on the toxicokinetics [absorption, distribution, metabolism, and excretion (ADME)] of inhaled and endogenously produced formaldehyde from human and experimental animal studies. A summary of this information is presented in Section 3.1 of the Toxicological Review. Although toxicokinetics is typically discussed in a sequential manner [i.e., with absorption defined as delivery to the blood; distribution describing delivery to the target tissue(s); metabolism outlining conversion to a more-or-less active chemical species, which often occurs in liver or target tissue elsewhere; and excretion documenting tissue clearance and removal processes], the primary site of action of inhaled formaldehyde is at the portal of entry (POE), specifically within the upper respiratory tract (URT). Therefore, this section will first discuss the uptake (also referred to as “absorption” in the formaldehyde literature) of inhaled formaldehyde into the URT tissue, and its transport, metabolism, and removal within the POE. Following this is a description of what is known regarding the absorption of formaldehyde from the POE into the blood and the potential for distribution of exogenous formaldehyde to systemic sites, along with a discussion of formaldehyde metabolism and excretion processes that may occur outside of the POE.

Formaldehyde is produced endogenously during normal cellular metabolism and as a byproduct of lipid peroxidation, or as a product in the catabolism of other chemicals introduced through dietary, environmental, or pharmaceutical sources. Therefore, discussions of inhaled formaldehyde require a consideration of the potential impact of endogenous formaldehyde on its toxicokinetics, as well as on its toxicity. The available evidence on the metabolism and kinetics of endogenous formaldehyde is discussed within each of the following subsections specifically as it pertains to the toxicokinetics of exogenous formaldehyde.

In the last subsections, the available toxicokinetic models of formaldehyde are presented.

C.1.1. Toxicokinetics of Inhaled Formaldehyde at the Portal of Entry (POE)

Formaldehyde is a highly reactive, highly water soluble, respiratory irritant, towards which the human body has developed several detoxification and removal processes at the site(s) of first contact (e.g., nasal passages for inhalation). Thus, this discussion of the toxicokinetics of inhaled formaldehyde at the POE is organized according to the most likely sites of first contact between inhaled formaldehyde and biological materials, in the context of the known anatomy and potential elimination processes of the respiratory tract tissues. Several of the key considerations for evaluating the toxicokinetics of inhaled formaldehyde at the POE in the rat nose are represented

schematically in Figure C-1. The respiratory tract is divided broadly as (1) upper respiratory tract (URT), which includes the nasal cavity, pharynx, and larynx and (2) the lower respiratory tract (LRT) comprising the trachea, bronchi, and lungs. Species differences in the structure of the airways, as well as the composition of the surface epithelium at various nasal locations, are important considerations to keep in mind when interpreting results in rodents and extrapolating observations to humans. Nasal passages, starting from anterior to posterior, are lined by four different types of epithelia: (1) squamous or keratinized, stratified (nasal vestibule); (2) transitional or nonciliated cuboidal/columnar; (3) respiratory or ciliated pseudostratified cuboidal/columnar (main chamber and nasopharynx); and (4) olfactory (dorsal and dorsoposterior nasal cavity) ([Harkema et al., 2006](#)). It is important to note that rodents and humans differ in the distribution of nasal epithelial surfaces. For example, the olfactory epithelium in rats and mice makes up approximately 50–52% and 45–47%, respectively, of the nasal cavity surface area, whereas in humans, it makes up only 3% ([Sorokin, 1988](#); [Gross et al., 1982](#)).

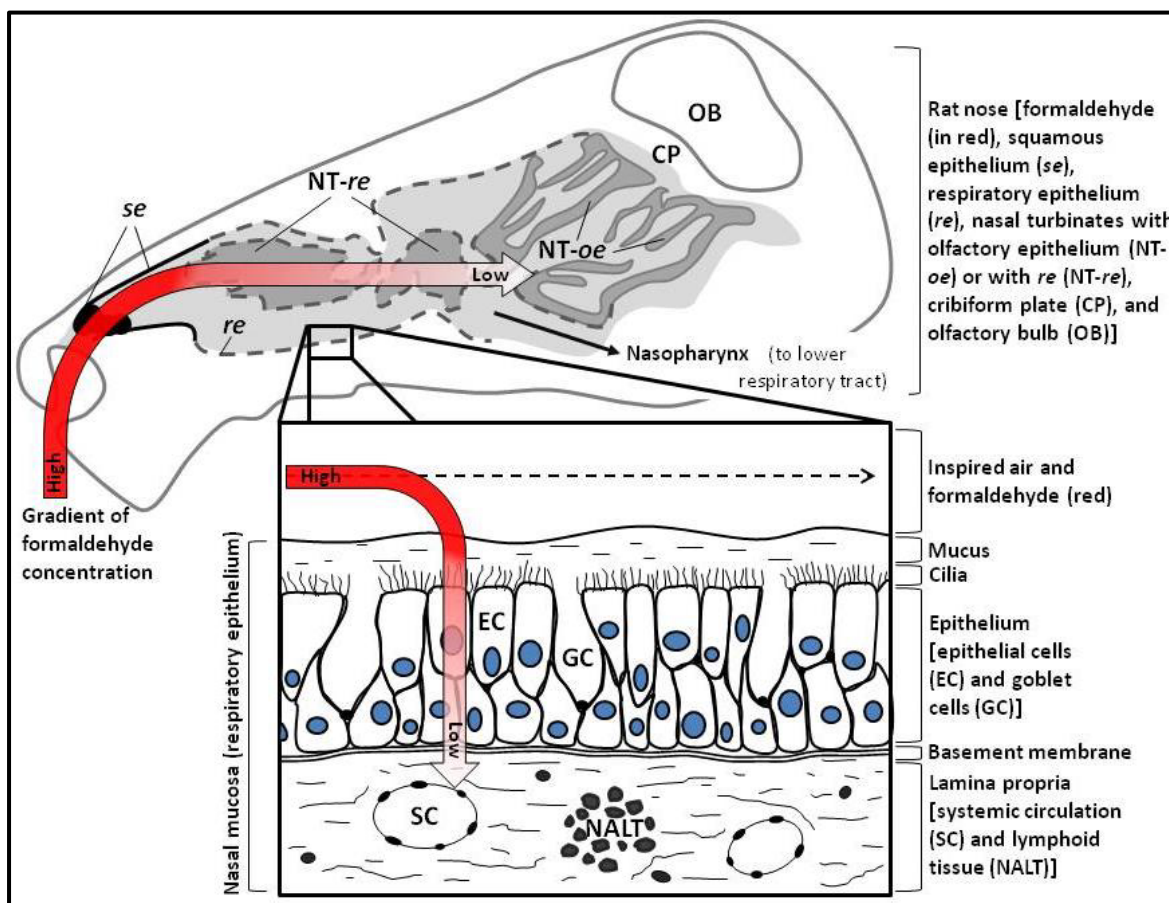


Figure C-1. Schematic of the rat upper respiratory tract depicting the gradient of formaldehyde concentration formed following inhalation exposure, both from anterior to posterior locations, as well as across the tissue depth. Modeling based on observations in rodents predicts a similar pattern of distribution

in humans. Drawn based in part on images by ([NRC, 2011](#)) and ([Harkema et al., 2006](#)). Note: other components (e.g., naris; transitional epithelium) have been omitted to increase clarity.

C.1.2. Spatial Distribution of Tissue Uptake of Formaldehyde at the Portal of Entry

The distribution of inhaled formaldehyde within the URT and LRT can provide information useful to interpreting any potential toxicity. The nasal passages in humans are generally similar to other mammalian species. One key difference, however, is that humans and nonhuman primates have nasal passages adapted for both oral and nasal (oronasal) breathing, as opposed to obligate nasal breathing in rodents. A second key difference is the shape and complexity of the nasal turbinates, with relatively simple shapes in humans, and complex, folded patterns in rodents. In general, these differences provide better protection of the rodent LRT against inhaled toxicants than is provided to the human LRT ([Harkema et al., 2006](#)).

Indirect measurement studies

Much of what is known regarding the uptake of formaldehyde is based on indirect measurements of formaldehyde-induced changes and/or molecular interactions, or removal of formaldehyde from the air. This is because, in biological systems, formaldehyde exists as total or analyzable formaldehyde, which includes free and reversibly bound (acid-labile) forms ([Heck et al., 1982](#)). Conventional methods cannot directly measure low levels of free formaldehyde with certainty in tissues and body fluids. Additionally, carbonyl impurities such as acetone, formaldehyde and acetaldehyde are present even in quartz distilled water and may interfere in the measurements ([Esterbauer et al., 1982](#)). Uptake of formaldehyde (defined as retention within the respiratory tract tissue), based on estimates determined from the amount of formaldehyde removed from the air, indicate that a majority of formaldehyde is removed from inhaled air by the URT.

Indirect estimates of formaldehyde uptake, based on interactions with cellular materials, have been made in experimental animals, including monkeys ([Monticello et al., 1989](#); [Casanova et al., 1991](#)), dogs ([Egle, 1972](#)), and rats ([Kimbell et al., 2001b](#); [Kerns et al., 1983](#); [Heck et al., 1983](#); [Chang et al., 1983](#)) as shown in Table C-1.

Table C-1. Dosimetry and response of formaldehyde in experimental animals by indirect measurements

Reference and species	Exposure and analysis	Observations	
Casanova et al. (1991) ; Monkeys, rhesus;	0.86, 2.46, 7.38 mg/m ³ for 6-hr [¹⁴ C]CH ₂ O from [¹⁴ C]PFA. Estimated the amount of DNA-protein crosslinks (DPX) formed in various tissues	DPX Levels	Area of the respiratory tract
		Highest	Middle turbinate mucosa
		Lower	Anterior lateral wall/septum and nasopharynx

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure and analysis	Observations				
male, n = 9; mean $\pm$ SD = 8.74 $\pm$ 0.84; 4.6 year old		Very low	Larynx/trachea/carina			
		None	Maxillary sinuses and lungs			
Monticello et al. (1989) Monkeys, rhesus; male, n = 9; 4-6 years; 6-7 kg	7.4 mg/m ³ , 6 hrs/d; 5 d/wk; 1 or 6 wk CH ₂ O from PFA. Animals injected with [³ H]-Thd, sacrificed, histoauto-radiography of cell proliferation measured	Proliferation	Area of the respiratory tract			
		Significant	Nasal passages			
		Minimal	Lower respiratory tract			
		None	Maxillary sinuses			
Egle (1972) Dogs/Mongrel; Male and female; n = 4; 13–19 kg	150 to 350 mg/m ³ CH ₂ O vapors from <u>formalin</u> ; nose-only inhalation from a respirometer; animals preanesthetized; aldehydes analyzed by a colorimetric method	Uptake at all ventilation rates and concentrations				
		Total respiratory tract (TRT)			≈100%	
		URT- inhalation			100%	
		URT- inhalation + exhalation			≈100%	
Heck et al. (1983) ; Rats, Fischer; Male, n = 3; 18--250 g	Radioactivity immediately after 6hr exposure to [¹⁴ C]CH ₂ O from [¹⁴ C]PFA, each averaging 3 exposures and 4 rats at 6.2, 12.3, 18.5, or 29.5 mg/m ³		Equivalents of [¹⁴C] in various tissues (μmol/g)^a or mg/m³			
			6.15	12.3	18.5	29.5
		Nasal Mucosa	0.59 $\pm$ 0.18	1.15 $\pm$ 0.29	1.78 $\pm$ 0.4	2.28 $\pm$ 0.61
		Trachea	0.26 $\pm$ 0.13	0.39 $\pm$ 0.13	0.36 $\pm$ 0.09	0.40 $\pm$ 0.13
		Plasma	0.05 $\pm$ 0.01	0.08 $\pm$ 0.01	0.10 $\pm$ 0.04	0.11 $\pm$ 0.05

CH₂O, formaldehyde; PFA, paraformaldehyde; DPX, DNA-protein crosslinks.

^aValues, representing mean $\pm$ SD, were extracted from graphical data using GrabIT software.

As shown in Table C-1, ([Casanova et al., 1991](#)) used DNA-protein crosslinks (DPX) levels as a measure of regional dosimetry of formaldehyde in monkeys exposed to formaldehyde by inhalation assuming that the rate of crosslink formation depends on the concentration of formaldehyde delivered at the portal of entry tissues. They subjected rhesus monkeys to a single 6-hr exposure of formaldehyde over a range (0.9–7.4 mg/m³) and concluded based on the observed pattern of DPX formation that formaldehyde uptake primarily occurs in nasal passages involving middle turbinates, to a smaller extent in the nasopharynx and trachea, but not in maxillary sinuses or lungs ([Casanova et al., 1991](#)). [Monticello et al. \(1989\)](#) predicted the uptake of formaldehyde based on other indirect measures such as cell proliferation in monkeys exposed to 7.4 mg/m³ formaldehyde, 6 hours/day, 5 days/wk for 1 or 6 weeks. They concluded that formaldehyde uptake primarily occurs in nasal passages and middle turbinates, to a smaller extent in the nasopharynx and trachea, with evidence of increased proliferation in proximal regions of the bronchi, but no indication of effects in the maxillary sinuses. In dogs exposed to formalin vapors, almost 100% of

inhaled formaldehyde is retained in the URT, indicating that little, if any, inhaled formaldehyde would reach the LRT, and this is independent of respiration rate, tidal volume, and inhaled formaldehyde concentration ([Egle, 1972](#)).

Similarly, radiolabeling studies, exemplified by ([Heck et al., 1983](#)) in rats show that the majority of the labeled formaldehyde is retained within the nasal passages and, to a far lesser extent, within the other parts of the URT and proximal LRT, with no evidence of significant distribution into plasma. However, because formaldehyde is incorporated into the one-carbon (1C) pool (see discussion later in this section), possibly facilitating its distribution in a toxicologically-inactive form, neither the distribution of radiolabel nor the estimated retention are interpreted to provide a clear picture of the spatial distribution of inhaled formaldehyde within the respiratory tract tissues. Notably, long-term exposure of rats to formaldehyde for 30 months induced lesions in the nasal cavity and proximal trachea ([Kerns et al., 1983](#)). [Kimbell et al. \(2001b\)](#) predicted the uptake of formaldehyde in the nasal passages of F344 rats, rhesus monkeys and humans to be respectively, 90%, 67% and 76% using the computational fluid dynamics (CFD) modeling. Similar to these predictions for rats, ([Morgan et al., 1986c](#)) demonstrated that rat nasal passages scrubbed nearly all of the inhaled formaldehyde (on average $\approx 97\%$). In rats, the evidence suggests that higher concentrations of formaldehyde are taken up in the respiratory mucosa as compared to the olfactory mucosa ([Swenberg et al., 1983a](#); [Casanova-Schmitz et al., 1984b](#)).

Extrapolation using fluid dynamic modeling

There are no studies available in the literature that directly addressed uptake of formaldehyde into the respiratory tract of humans. However, a few modeling studies based on findings in rodents report estimated uptake of inhaled formaldehyde in humans ([Overton et al., 2001](#); [Kimbell et al., 2001b](#); [Kimbell and Subramaniam, 2001](#)). [Kimbell et al. \(2001b\)](#), using a three-dimensional, CFD model of the nose, predicted human nasal uptake of approximately 76% of the inhaled formaldehyde at unidirectional steady-state nasal inspiratory flow corresponding to sleeping activity, decreasing to 58% under heavy exercise activity. [Overton et al. \(2001\)](#) modeled overall uptake in the entire respiratory tract and predicted that 95% of inhaled formaldehyde is retained in the respiratory tract in general in any activity state. description of modeling efforts in human, monkeys and rats is provided in Appendix C.1.12. Overall, dosimetric modeling studies in humans have shown close agreement with observations of exposed rodents: namely, that 90–95% of inhaled formaldehyde is retained in the URT ([Subramaniam et al., 1998](#); [Overton et al., 2001](#); [Kimbell et al., 2001b](#)).

Relationship of formaldehyde uptake to endogenous levels and prior exposure

Heck et al ([1982](#)) developed a gas chromatography-mass spectrometry (GC-MS) method to measure total or analyzable formaldehyde, which includes both free as well as reversibly bound formaldehyde [hydrated formaldehyde bound to glutathione (GSH) and tetrahydrofolate (THF)]. However, this method does not measure irreversibly bound formaldehyde. Based on this method,

endogenous formaldehyde levels were 1.5–4.3 folds higher at the POE (i.e., nasal mucosa; $\approx 12.6 \mu\text{g/g}$ or 0.42 mM) than in other tissues (i.e., testes<liver<brain) ([Heck et al., 1982](#)). It remains to be determined how this may affect the local toxicokinetics of inhaled formaldehyde.

[Heck et al. \(1983\)](#) also examined the effect of prior exposure to formaldehyde on tissue levels of formaldehyde in rats. As shown in Table C-2, no statistically significant changes in total formaldehyde levels in the nasal mucosa were observed following 10-day exposure of F344 rats to 7.4 mg/m^3 formaldehyde ([Heck et al., 1982](#)), suggesting that formaldehyde exposure does not distinguishably augment total levels of formaldehyde in POE tissues. However, rats and mice appear to differ in the uptake of formaldehyde following repeated inhalation exposure to formaldehyde. Prior, short-term exposure to high levels of formaldehyde in rats did not alter uptake of formaldehyde into the respiratory mucosa during a subsequent exposure. This was based on comparisons between a single exposure to 18.5 mg/m^3 in naïve rats compared to repeated exposures in rats exposed to the same dose of formaldehyde for the previous 9 days ([Heck et al., 1983](#)). In a different study, ([Chang et al., 1983](#)) also observed similar uptake in preexposed as well as naïve rats; however, mice responded differently, with naïve mice exhibiting more radioactivity uptake than preexposed mice (see Table C-2). The authors concluded that since mice tend to lower their minute volume with repeated exposures to formaldehyde, they tend to have less absorption, hence less radioactivity compared to naïve mice. So comparing the results in rats, which do not alter their minute volume as mice do, it was suggested that repeated exposure does not affect the uptake of formaldehyde in nasal cavity of rats ([Chang et al., 1983](#)).

Table C-2. Comparison of formaldehyde uptake at the portal of entry with single or repeated inhalation exposure

Reference and design	Exposure and analysis	Observations	
Heck et al. (1982) Rats, Fischer Male, $n = 8$ 200–250 g	7.4 mg/m^3 [^{13}C] CH_2O (from PFA) for 6 hrs/d; 10-d exposure; chamber inhalation; CH_2O measured as PFPH derivative by GC/MS	Nasal mucosa levels total ^a CH_2O ($\mu\text{g/g}^b$)	
		<i>Unexposed</i> 12.6 ± 2.7	<i>Exposed</i> 11.7 ± 3.6
Heck et al. (1983) Rats, Fischer Male, $n = 3$; 180–250 g	2 groups: (a) <i>preexposure</i> ; (b) <i>naïve</i> ; On Days 1–9: <u>group a</u>) received 18.5 mg/m^3 CH_2O (from PFA); whole body exposure, 6 hrs/d; <u>group b</u>): no preexposure. On Day 10: groups a and b received [^{14}C] CH_2O (from PFA) for 6 hrs, nose-only exposure. Tissue homogenates counted with LSC for $^{14}\text{CO}_2$ trapped in ethanolamine in 2-methoxy-ethanol counted for radioactivity.	Equivalents of ^{14}C in respiratory mucosa ($\mu\text{g/g}^c$)	
		naïve rats	67.5 ± 9.2
		preexposed	64.4 ± 7.6
Chang et al. (1983) Rats, Fischer; Male, $N=3$; 180–200 g	i) <i>preexposure</i> : 7.4 or 18.4 mg/m^3 unlabeled CH_2O from PFA, 6 hrs/d, 4-d whole-body exposure; on 5th d $^{14}\text{CH}_2\text{O}$ from PFA, 6 hrs	(No significant difference)	
		Radioactivity in nasal cavity: preexposed rats = naïve rats Radioactivity in nasal cavity:	

Supplemental Information—Formaldehyde (Inhalation)

Reference and design	Exposure and analysis	Observations
Mice, B6C3F1 Male, N=3; 22-26 g	ii) naïve animals: ¹⁴ CH ₂ O, 6 hrs from PFA	naïve mice > pretreated mice

CH₂O, formaldehyde; PFA, paraformaldehyde; PFPH, pentafluorophenylhydrazine; GC/MS, gas chromatography/mass spectrometry; LSC, liquid scintillation counting; CO₂, carbon dioxide.

^aTotal formaldehyde includes free plus reversibly bound formaldehyde.

^bData from ([Heck et al., 1982](#)) given in $\mu\text{mols/g}$ is converted to $\mu\text{g/g}$ by the equation: $\mu\text{mols} \times 30 = \mu\text{g/g}$ (30 is the molecular weight of formaldehyde).

^cData from ([Heck et al., 1983](#)) given in nmols/g is converted to $\mu\text{g/g}$ by the equation: $(\text{nmol/g} / 1,000) \times 30 = \mu\text{g/g}$ (30 is the molecular weight of formaldehyde).

Summary of spatial distribution of POE uptake

To summarize, a majority of inhaled formaldehyde is rapidly absorbed and retained in the URT based on CFD modeling studies in humans ([Subramaniam et al., 1998](#); [Overton et al., 2001](#); [Kimbell et al., 2001b](#); [Kimbell and Subramaniam, 2001](#)), indirect or direct measurements in monkeys ([Monticello et al., 1989](#); [Casanova et al., 1988](#)), and direct measurements in dogs ([Egle, 1972](#)) and rats ([Kimbell et al., 2001b](#); [Kerns et al., 1983](#); [Heck et al., 1983](#); [Chang et al., 1983](#)), despite the anatomical and physiological differences between species, such as obligate nose breathing in rodents (rats and mice) and oronasal breathing in primates (monkeys and humans) ([Schreider, 1986](#); [Harkema et al., 2006](#)). As demonstrated in monkeys and rats, and as modeled in humans, a concentration gradient of inhaled formaldehyde follows an anterior to posterior distribution, with high concentrations of formaldehyde distributed to squamous, transitional, and respiratory epithelia, and less uptake by olfactory epithelium, and very little or no formaldehyde reaching more distal sites such as the larynx or lung. Further, at inhaled concentrations as high as 7.4 mg/m³, exogenous exposure does not appreciably change the levels of formaldehyde over the endogenous levels in the nasal mucosa ([Heck et al., 1982](#)). Also, repeated exposures to formaldehyde do not alter the tissue formaldehyde levels in rats, but naïve mice do show higher tissue uptake than preexposed mice, which is attributed to species differences in minute volume and response to irritant gases ([Chang et al., 1983](#)).

C.1.3. Tissue Penetration of Formaldehyde Within the Upper Respiratory Tract

Within the URT, penetration of formaldehyde follows initial interaction with the mucociliary apparatus followed by diffusion into the epithelial cell layer where it can be metabolized. Important details to consider in evaluating formaldehyde nasal dosimetry and toxicity are the differences in the types of epitheliums lining the nasal surfaces. As described earlier, there are striking differences in the amount of olfactory epithelium and respiratory epithelium present between the noses of rats, which have a highly complex sense of smell, compared to humans, who use the nose primarily used for breathing. In all species, air (and formaldehyde) must first pass over squamous, transitional, and respiratory epithelium before coming in contact with olfactory epithelium. This section will focus on the interaction and fate of inhaled formaldehyde in the URT.

Formaldehyde interaction with the mucociliary layer

The mucociliary apparatus of the URT is the first line of defense against airborne agents in that it may entrap, neutralize, and remove particulates and airborne chemicals from inspired air ([Morgan et al., 1983](#)). The mucociliary apparatus is comprised of three layers: a thick mucus layer (epiphase) at the top, a watery fluid layer (hypophase) in the middle, and a ciliated epithelial layer at the bottom ([Schlosser, 1999](#)). Inhaled formaldehyde must pass through the mucus layer covering the URT before it can react with the cellular components in this region.

The respiratory mucus is composed of 97% water, 2–3% glycoproteins, 0.3–0.5% fats, and about 0.1–0.5% soluble proteins ([Bogdanffy et al., 1987](#)). Formaldehyde gas (unhydrated) is highly soluble in water, in which it hydrolyzes to a reversible hydrated form called methanediol or methylene glycol with a half-life of 70 milliseconds and with an equilibrium constant $[\text{CH}_2\text{O}]/[\text{CH}_2(\text{OH})_2]$ of 4.5×10^{-4} at 22°C ([Sutton and Downes, 1972](#)). In aqueous solution, most of the formaldehyde (99.9%) exists as methanediol in an equilibrium with free (0.1%) formaldehyde ([Fox et al., 1985](#)). Thus, formaldehyde is first hydrated in nasal mucus to form methanediol, which subsequently interacts with the nasal mucociliary apparatus ([Priha et al., 1996](#); [Bogdanffy et al., 1986](#)). Physical-organic chemistry studies of the reaction of formaldehyde with amines (and presumably other biological nucleophiles) have conclusively demonstrated that the unhydrated or free form of formaldehyde, but not the hydrated form or methanediol is the reactive species ([Abrams and Kallen, 1976](#)). Methanediol is either transported to the underlying tissue (presumably by diffusion) or it is removed within nasal mucus by convective flow and subsequent ingestion. [Schlosser \(1999\)](#) estimated that 22–42% of the absorbed formaldehyde in rodents is removed by mucus flow.

Airborne pollutants and reactive gases have been shown to decrease mucus flow rates in several animal models (as reviewed in ([Wolff, 1986](#))). Degradation in the continuity or function of this mucociliary apparatus can impair clearance of inhaled pollutants at the portal of entry. For example, ([Morgan et al., 1983](#)) have shown that a single exposure of 18.45 mg/m³ formaldehyde in Fischer rats causes mucostasis (cessation or severe slowing of mucus flow) in several regions of the nasoturbinates. Exposure (6 hours/day for 1–9 days) results in ciliastasis (loss of ciliary activity) occurring with greater frequency and across more regions of the nasoturbinates in subsequent days of exposure. Thus, continued exposure would be expected to result in an increased uptake, as well as an altered deposition of inhaled formaldehyde within the URT tissue. Further, ([Morgan et al., 1986c](#)) also reported that rats exposed 6 hours daily for 3 weeks showed an increase in mucostasis extending from anterior to posterior regions at the 18.45 mg/m³ dose; however, at lower doses (0.6–7.4 mg/m³) the effect was either undetectable or less severe. In addition, ([Morgan et al., 1986c](#)) showed an increase in mucus flow at lower concentrations after 4 days of exposure, but not after 6 days to 0.6 mg/m³ formaldehyde. Thus, there are some uncertainties regarding the occurrence of mucostasis at lower concentrations of formaldehyde exposure.

In addition, as methanediol and free formaldehyde are transported through the mucociliary apparatus, the free formaldehyde is known to bind to soluble proteins such as albumin in the nasal mucus ([Bogdanffy et al., 1987](#)). Similarly, the nasal lining fluid contains antioxidants, including the thiol GSH with which formaldehyde is known to interact, likely eliciting a transient GSH depletion during and following formaldehyde exposure. However, it is unclear to what extent inhaled formaldehyde interacts with soluble and insoluble factors within the mucociliary layer and whether reactive byproducts may be formed by these interactions. Importantly, endogenous formaldehyde produced during normal cellular metabolism is unlikely to be present at appreciable levels in the mucus, and thus, would not be expected to participate in similar reactions. Interactions with soluble proteins are expected to further reduce the amount of formaldehyde available to react with cellular materials. As such, alterations in the levels of soluble proteins within the mucus could substantially affect tissue uptake.

Formaldehyde diffusion into the epithelial cell layer

The less reactive methanediol is better able to penetrate tissues, while the free formaldehyde reacts with macromolecules. However, when the free formaldehyde ($\approx 0.1\%$) is used up, a fraction of methanediol (from the 99.9%) will convert to free formaldehyde so that the equilibrium of methanediol with free formaldehyde (i.e., 99.9:0.1 ratio) is maintained in the aqueous media ([Fox et al., 1985](#)). However, several uncertainties exist regarding the transition of inhaled formaldehyde from the mucociliary layer to the underlying epithelium. Although direct experimental evidence is lacking, the biochemical properties of formaldehyde make it likely that inhaled formaldehyde (in the hydrated or anhydrated form) undergoes passive transport, via simple diffusion, across biological membranes. Thus, higher extracellular formaldehyde levels would be expected to result in increased diffusion into the cell owing to the concentration gradient formed. However, this concentration gradient may be affected by endogenous formaldehyde levels because in humans, as in other animals, formaldehyde is an essential metabolic intermediate in all cells ([Thompson et al., 2009](#)).

Enzymatic metabolism of formaldehyde within cells of the URT

Formaldehyde, either from exogenous sources (inhaled air) or endogenous sources (enzymatic and nonenzymatic mechanisms as well as that released endogenously from metabolism of xenobiotics), can be metabolized by several different enzyme pathways. Based on studies of endogenous formaldehyde and in vitro enzyme inhibition experiments ([Teng et al., 2001](#)), and as summarized in Figure C-2, formaldehyde has been shown to be predominantly metabolized to formate by GSH-dependent class III alcohol dehydrogenase (ADH3; also described as formaldehyde dehydrogenase or FDH) and by a minor pathway involving mitochondrial aldehyde dehydrogenase 2 (ALDH2) which is GSH-independent. Catalase may also be involved, to a minor extent, in oxidizing formaldehyde, especially under conditions when hydrogen peroxide is formed ([Uotila and Koivusalo, 1974](#)).

Supplemental Information—Formaldehyde (Inhalation)

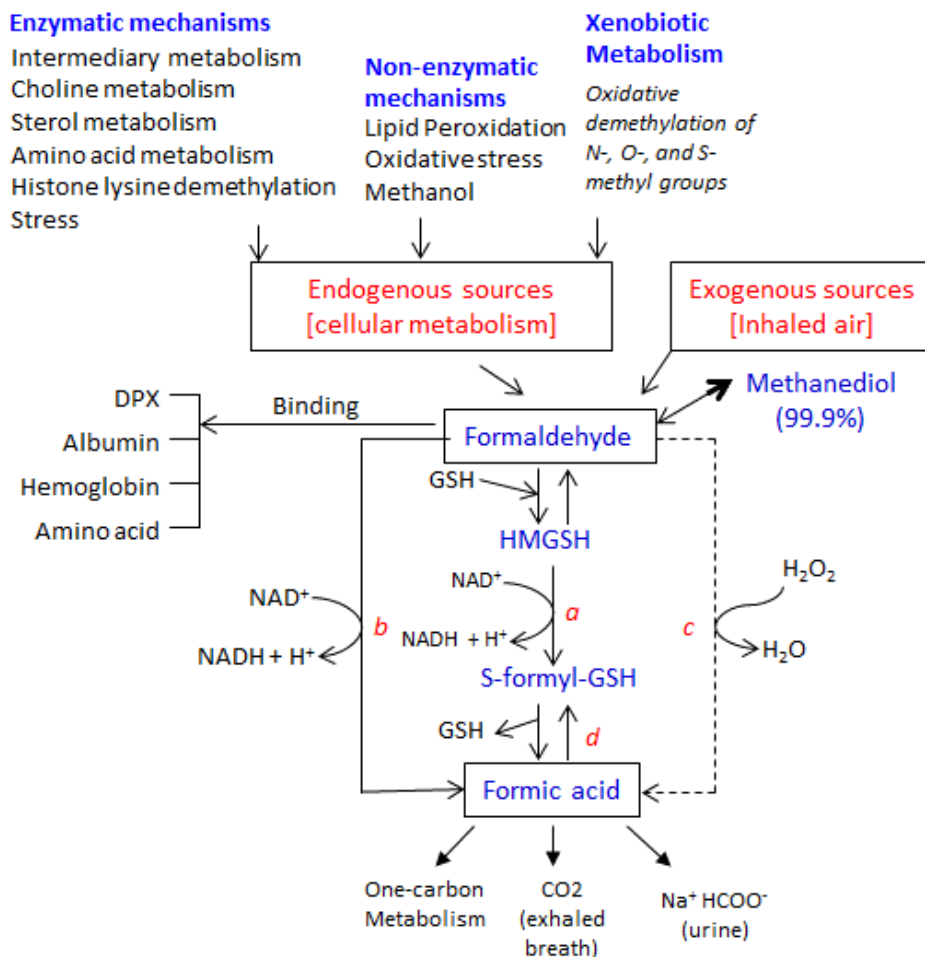


Figure C-2. Metabolism of formaldehyde.

Abbreviations: CO₂, carbon dioxide; DPX, DNA-protein crosslinks; GSH, glutathione; H₂O, water; H₂O₂, hydrogen peroxide; HMGSH, hydroxymethylglutathione; NAD⁺, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced); Na⁺HCOO⁻, sodium formate. Enzymes: *a*, alcohol dehydrogenase-3 (ADH3); *b*, aldehyde dehydrogenase 2 (ALDH2); *c*, catalase; *d*, S-formyl-GSH hydrolase. Adapted from [NTP \(2010\)](#).

Both ADH3 and ALDH2 enzymes have been found across different species and in a broad range of tissues, including the nasal mucosa (Reviewed ([Thompson et al., 2009](#))). In rodents, both ADH3 and ALDH2 exhibit region-specific differences in the nose, in that the specific activity of ADH3 is two times higher in the olfactory mucosa than in respiratory mucosa, while the specific activity of ALDH2 is 5–8 times higher in respiratory than in olfactory tissue ([Casanova-Schmitz et al., 1984a](#); [Bogdanffy et al., 1986](#)). In rats, higher levels of ADH3 activity have been reported in the cytoplasm of the respiratory and olfactory epithelial cells and in the nuclei of olfactory sensory cells, as compared to other regions of the nasal mucosa ([Keller et al., 1990](#)). These enzymes are enriched in the nasal tissues presumably to protect the underlying tissues against respired toxicants. This highlights a significant barrier to the penetration of inhaled formaldehyde beyond

the respiratory epithelium and a means by which these same cells can rapidly metabolize formaldehyde produced endogenously within the cell ([Uotila and Koivusalo, 1974](#)).

The ADH3-mediated pathway of formaldehyde oxidation involves a two-step enzymatic reaction but is preceded by the rapid and reversible nonenzymatic binding of formaldehyde to GSH, which results in the formation of S-hydroxymethylglutathione (HMGS) or the glutathione hemiacetal adduct. In the first of a two-step enzymatic reaction, ADH3 converts HMGS to S-formylglutathione (S-formyl-GSH) in the presence of the co-factor, nicotinamide adenine dinucleotide (NAD⁺). In the second step, another enzyme S-formyl-GSH-hydrolase converts S-formyl-GSH to formate with the concomitant release of free GSH. Under physiological conditions, cellular NAD⁺ levels are two orders of magnitude higher than NADH (reduced form of NAD⁺) and intracellular GSH levels are high enough (in millimolar concentrations) to favor rapid oxidation of HMGS to formate ([Svensson et al., 1999](#); [Meister and Anderson, 1983](#)). Because of this rapid metabolism, formaldehyde is likely to have a short half-life in biological systems. As previously mentioned, and given the importance of this major detoxification pathway, individual variations in GSH levels within the nasal mucosa are of particular importance in formaldehyde metabolism.

ADH3 shows comparable kinetics across rats and humans. As shown in Table C-3, the affinity (K_m) of purified human liver ADH3 for HMGS is 6.5 μM ([Uotila and Koivusalo, 1974](#)) and 4.5 mM for rat liver ([Casanova-Schmitz and Heck, 1983](#)). ([Hedberg et al., 2000](#)) demonstrated that the kinetics of ADH3 in human buccal tissue lysates are in close agreement with those reported for purified human liver ADH3 ([Uotila and Koivusalo, 1974](#)). This is comparable to the rat respiratory and olfactory mucosal K_m values in the presence of GSH as well as the K_m of ADH3 from the rat liver soluble fraction (2.6 μM) ([Casanova-Schmitz et al., 1984a](#)). In contrast, the affinity of ALDH2, presumably in the absence of GSH, is several-fold lower than ADH3 ([Siew et al., 1976](#)). Thus, at lower concentrations of formaldehyde ADH3 is the dominant formaldehyde detoxification pathway. The K_m of ADH3 is in close agreement across species and tissue types, including the nasal mucosa, all of which exhibit similar responses to GSH depletion (i.e., in the absence of GSH, ALDH family members oxidize formaldehyde, which is associated with mitochondrial ALDH2). Both ADH3- and ALDH2-mediated pathways oxidize formaldehyde to formic acid (formate). ADH3 is also known to catalyze the NADP-dependent reduction of the endogenous nitrosylating agent S-nitrosoglutathione (GSNO) and is also referred to as S-nitrosoglutathione reductase (GSNOR) ([Jensen et al., 1998](#)).

Table C-3. ADH3 kinetics in human and rat tissue samples and cultured cells

Source	K _m (μM)	V _{max} (nmol/mg protein x min)	References
Purified human liver ADH3	6.5	2.77 ± 0.12	Uotila and Koivusalo (1974)
Rat respiratory mucosal homogenate (+GSH)	2.6 ± 2.6	0.90 ± 0.24	Casanova-Schmitz et al. (1984a)
Rat respiratory mucosal homogenate (– GSH)	481 ± 88	4.07 ± 0.35	

Supplemental Information—Formaldehyde (Inhalation)

Source	K _m (μM)	V _{max} (nmol/mg protein x min)	References
Rat olfactory mucosal homogenate (+GSH)	2.6 ± 0.5	1.77 ± 0.12	
Rat olfactory mucosal homogenate (– GSH)	647 ± 43	4.39 ± 0.14	
Rat liver (+ GSH) ^a	4.5 ± 1.9 ^a	2.0 ± 0.3	
Human buccal tissue (+ GSH)	11 ± 2	2.9 ± 0.6	Hedberg et al. (2000)
Human buccal tissue (– GSH)	360 ± 90	1.2 ± 0.7	

ADH3 = alcohol dehydrogenase 3; K_m = substrate concentration at which half of the maximum velocity is achieved; V_{max} = maximum reaction velocity at which all enzymes become saturated; GSH = glutathione.

^aSoluble fraction of rat liver homogenate.

Formate can undergo three possible outcomes: (1) enter the one-carbon pool for use in the synthesis of DNA and proteins (aka “metabolic incorporation”), (2) become further oxidized to CO₂ and eliminated in exhaled air, or (3) be excreted in urine (see Figure C-2).

One-carbon metabolism

As summarized in Figure C-3, tetrahydrofolate (THF)-mediated eukaryotic one-carbon (1C) metabolism involves an inter-connected network which is highly compartmentalized between the cytosol, mitochondria, and nucleus (Reviewed in ([Tibbetts and Appling, 2010](#))). A majority of the 1C metabolism takes place in the mitochondria followed by the cytosol and nucleus. In cytoplasmic 1C metabolism, de novo synthesis of purines and thymidylate, and remethylation of homocysteine to methionine takes place. 1C metabolism in the mitochondrial compartment involves formylation of methionyl-tRNA, and oxidation of one-carbon donors, such as serine, glycine, sarcosine, and dimethylglycine (DMG). In addition, mitochondria contribute 1C units for cytoplasmic 1C metabolism in the form of formate. The mitochondrial and cytoplasmic pathways are connected by serine, glycine and formate which are the 1C donors. The nuclear compartment of 1C metabolism predominantly provides de novo synthesis of dTMP from dUMP.

Some of the steps in both cytosolic and mitochondrial 1C metabolism are common. Formate, formed from the metabolism of formaldehyde, enters the 1C pool and is either oxidized to CO₂ and eliminated in exhaled breath or is used in protein and DNA synthesis. As shown in Figure C-3, formate is combined with THF whereby its 1C group is transferred to THF forming 10-formyl-THF (10-CHO-THF), mediated by the enzyme 10-HCO-THF-synthetase. The 10-CHO-THF is then oxidized by CHO-THF dehydrogenase to CO₂ and H₂O and eliminated in exhaled breath, with the release of THF which can be reused for binding with formic acid. Alternatively, 10-CHO-THF can also be converted through two-steps of reversible reactions to 5,10-methenyl-THF (CH⁺-THF) to 5,10-methylene-THF (CH₂-THF). Serine, derived from glycolytic intermediates, is the main source of 1C units. Serine combined with THF is converted reversibly by the enzyme serine hydroxymethyl transferase (SHMT) to glycine and CH₂-THF. Further, the enzyme methylene tetrahydrofolate

reductase (MTHFR) converts $\text{CH}_2\text{-THF}$ to 5-methyl-THF ($\text{CH}_3\text{-THF}$). The 1C metabolism products - $\text{CH}_2\text{-THF}$ and $\text{CH}_3\text{-THF}$ utilize their one-carbon units, respectively, in DNA (dTMP) and protein (methionine) biosynthetic pathways (metabolic incorporation).

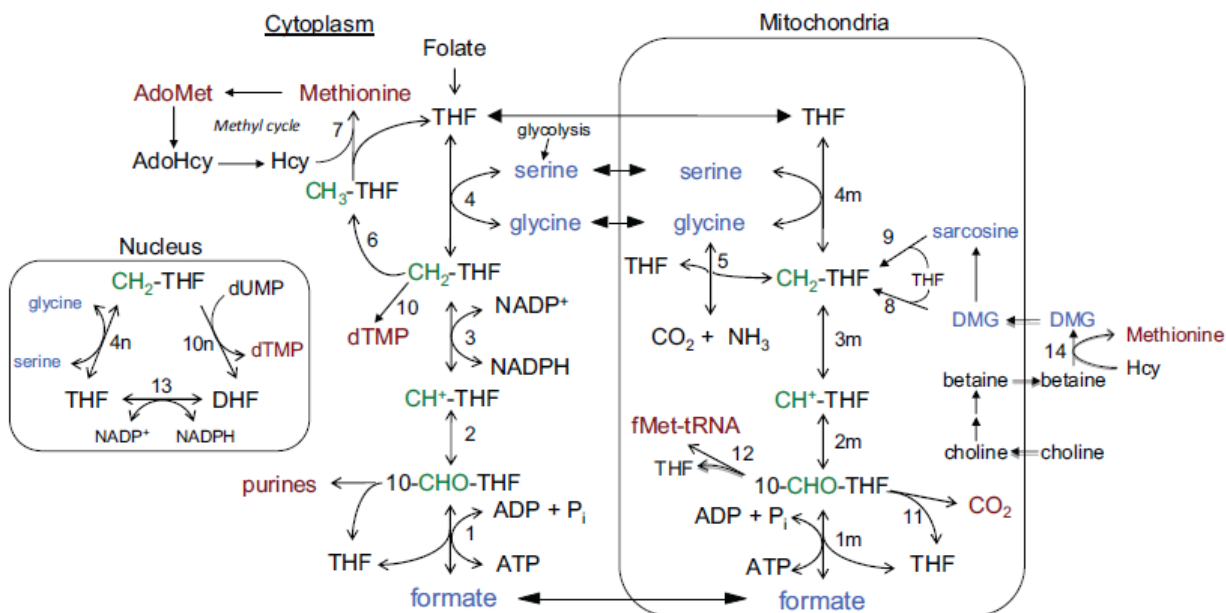


Figure C-3. Compartmentalization of mammalian one-carbon metabolism. The end products, donors, and activated units carried by tetrahydrofolate (THF) of the 1C metabolism are shown in red, blue, and green, respectively. Note that reactions 1–4 are common in both the cytoplasmic and mitochondrial (m) compartments, while reactions 4n and 10n are present in the nucleus (n). Enzymes catalyzing the reactions: 1: 10-formyl-THF synthetase; 2: 5,10-methenyl-THF ($\text{CH}^+\text{-THF}$) cyclohydrolase; 3: 5,10-methylene-THF ($\text{CH}_2\text{-THF}$) dehydrogenase; 4, 4n, and 4m: serine hydroxymethyltransferase (SHMT); 5: glycine cleavage system; 6: 5,10-methylene-THF reductase; 7: methionine synthase; 8: dimethylglycine dehydrogenase (DMGDH); 9: sarcosine dehydrogenase (SDH); 10 and 10n: thymidylate synthase; 11: 10-formyl-THF dehydrogenase (only the mitochondrial activity of this enzyme is shown, but it has been reported in both compartments in mammals); 12: methionyl-tRNA formyltransferase; 13: dihydrofolate (DHF) reductase; 14: betaine-homocysteine methyltransferase. Abbreviations: AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; Hcy, homocysteine.

Source: [Tibbetts and Appling \(2010\)](#).

The rate of formate metabolism depends on the availability of dietary folic acid, which is the main source of THF. It is also important to note that levels of folate intermediates and folate-dependent enzymes show some differences in rats and primates (see Table C-4).

Table C-4. Levels of folate intermediates, activity of folate-dependent enzymes, and the rate of oxidation of formate in the liver of various species

Folate intermediate/folate-dependent enzyme	Rat	Monkey	Human
10-formyl-THF (nmoles/g of liver)	4.6 ± 1.3	10.5 ± 0.8	3.3 ± 0.5
Tetrahydrofolate (nmoles/g of liver)	11.4 ± 0.8	7.4 ± 0.8	6.5 ± 0.3
5-CH ₃ -THF (nmoles/g of liver)	9.3 ± 0.6	7.6 ± 1.1	6.0 ± 0.7
10-formyl-THF synthetase (nmoles of product/min/mg protein)	65.9 ± 0.0	142 ± 16	75.0 ± 8.7
10-formyl-THF dehydrogenase (nmoles of product/min/mg protein)	88.3 ± 1.7	33.0 ± 4.0	23.0 ± 2.2
5,10-CH ₂ -THF reductase (nmoles of product/min/mg protein)	1.21 ± 0.07	0.22 ± 0.02	0.42 ± 0.07
Serine hydroxymethyl transferase (nmoles of product/min/mg protein)	10.8 ± 0.6	17.1 ± 9.7	18.5 ± 0.7
Dihydrofolate reductase (nmoles of product/min/mg protein)	19.8 ± 1.3	4.1 ± 0.7	0.74 ± 0.17
Methionine synthase (nmoles of product/min/mg protein)	0.09 ± 0.007	0.09 ± 0.012	0.10 ± 0.008
Rate of formate oxidation (mg/kg/hr)	78	40	0

Source: [Skrzydłewska \(2003\)](#)

THF, tetrahydrofolate; CH₂-THF, methylene tetrahydrofolate.

As shown in Table C-4, the normal hepatic THF levels of monkeys and humans are 1.5 and 1.75-fold lower than the levels in rats. Also, the levels of 10-formyl-THF-dehydrogenase levels are 2.67- and 3.83-fold lower in the livers of monkeys and humans, respectively, compared to the levels in rat liver, which might cause an accumulation of formate in primates since there is decreased oxidation of formate to CO₂. Thus, primates oxidize formate less efficiently than rats ([Skrzydłewska, 2003](#)).

Interaction of formaldehyde with cellular macromolecules in the URT

As mentioned earlier, it has been shown that “free” formaldehyde (i.e., the 0.1% of total formaldehyde that does not exist in the form of methanediol) reacts with macromolecules ([Abrams and Kallen, 1976](#)). However, it is unclear whether methanediol in certain hydrophobic matrices (e.g., biological membranes, etc.) could be converted to a more reactive form and available to interact with cellular materials. Inhaled formaldehyde interacts at the portal of entry with the nasal passage macromolecules, and these interactions can be either noncovalent (reversible) or covalent (irreversible).

Noncovalent interactions:

Formaldehyde is reversibly bound to GSH and THF in cells forming the glutathione hemithioacetal adduct or hydroxymethylglutathione (HMGS) adduct and 5, 10-CH₂-THF adducts. Levels of the cellular antioxidant glutathione are abundant in the cell ≈5 mM with which formaldehyde readily forms the hemiacetal adduct. The dissociation constant for the hemiacetal and CH₂-THF adducts are approximately 1.5 mM ([Uotila and Koivusalo, 1974](#)) and ≈30 μM,

respectively ([Kallen and Jencks, 1966a, b](#)). Based on in vitro experiments formaldehyde has been shown to reversibly bind to human and rat nasal mucus, in particular the fraction containing albumin ([Bogdanffy et al., 1987](#)).

Covalent binding

Formaldehyde covalently binds to protein and DNA, forming protein adducts, DNA adducts, DNA-protein crosslinks (DPX), and DNA-DNA crosslinks (DDX). A complication that has been explored in some of these studies is that inhaled formaldehyde can also be metabolized and incorporated into DNA and proteins via the 1C pool.

Protein adducts

Formaldehyde has been shown to bind to histones and chromatin forming N⁶-formyllysine ([Edrissi et al., 2013a](#)) and a major source of this adduct has been shown to result from endogenous formaldehyde. Further, in rats exposed to various inhalation concentrations of ¹³C-labeled formaldehyde (0.9–11.2 mg/m³), a concentration-dependent increase in ¹³C-labeled N⁶-formyllysine, which was distinguished from endogenous N⁶-formyllysine, was detectable in the total proteins as well as in protein fractions from different cellular compartments (cytoplasmic, membrane, and nuclear) of the respiratory epithelium ([Edrissi et al., 2013a](#)).

DNA-protein Crosslinks

Formaldehyde-induced DNA-protein crosslinking occurs predominantly between the epsilon-amino groups of lysine, especially at the N-terminus of histones, and exocyclic amino groups of DNA ([Lu et al., 2008](#)). Several analytical methods including radiolabeled formaldehyde have been used to evaluate DPX formation in experimental animals. Earlier experiments have shown that inhalation of F344 rats to 2.46–36.93 mg/m³ of ¹⁴C-formaldehyde (6 hours/day, 2 days) caused a significant increase in the radioactivity of interfacial (IF) DNA³, representing DPX, observed in tissue homogenates from respiratory but not olfactory epithelium at ≥ 7.38 mg/m³ ([Casanova-Schmitz and Heck, 1983](#)). Formaldehyde-induced DPX levels have been shown to have concentration-dependence in both monkeys (0.86 to 7.37 mg/m³) ([Casanova et al., 1991](#)) and rats (0.37–12.1 mg/m³) ([Casanova et al., 1989](#); [Casanova et al., 1994](#)). In both rodents and monkeys there was a nonlinear concentration-response for DPX formation, which has been attributed to saturation of detoxification enzymes at high concentrations ([Casanova et al., 1989](#); [Casanova et al., 1991](#)). In monkeys, the DPX distribution pattern in the nasal passages following formaldehyde inhalation was in the order of middle turbinates > anterior lateral wall/septum > maxillary sinuses and lungs ([Casanova et al., 1991](#)), which corresponded to the location and proliferative response. In

³During a typical DNA extraction of tissue homogenates, the DNA separated into aqueous phase is termed aqueous (AQ) DNA, while the DNA trapped in the protein precipitate from the interphase (between aqueous and organic phases) was washed, treated with protein kinase and reextracted to get the interfacial DNA (IF DNA).

rats the DPX distribution pattern was in the order of lateral meatus > medial and posterior meatus ([Casanova et al., 1994](#)), which corresponded to the high and low tumor incidence sites in the respiratory tract ([Monticello et al., 1989](#)). This is possibly due to the differences in the anatomy of nasal passages and breathing patterns of these two species.

Recently, ([Lai et al., 2016](#)) developed a method that distinguishes deoxyguanosine-methyl-cysteine (dG-Me-Cys), a DPX formed with exogenous formaldehyde, from that formed with endogenous formaldehyde (see Table C-5). In monkeys exposed to 7.4 mg/m³ of ¹³C-labeled formaldehyde for 2 days, both exogenous and endogenous DPXs were detectable, with the levels of exogenous DPXs being 2.8-fold less than the endogenous DPX adducts. In contrast, only endogenous DPXs were detectable in air-exposed monkeys. In rats, a higher dose of 18.5 mg/m³ formaldehyde exposure for 1, 2, or 4 days was tested. DPX levels in nasal tissues were detected and were comparable for endogenous and exogenous formaldehyde among rats exposed 1 or 2 days, but at 4 days, DPX levels from exogenous formaldehyde had increased 5-fold above those from endogenous formaldehyde. Similarly, DPX levels from exogenous formaldehyde increased between 7 days and 28 days in rats exposed to 2.5 mg/m³.

Using in vitro studies, ([Yu et al., 2015b](#)) have shown that DPX such as, dG-CH₂-cysteine or dG-CH₂-GSH can undergo hydrolytic degradation to give rise to hm-dG monoadducts under physiological pH and temperature conditions. These results provide a mechanism which explains why formaldehyde-induced DPX are removed within 12.5–24 hours in cultured human epithelial cell lines ([Quievryn and Zhitkovich, 2000](#)) and lymphoblasts ([Craft et al., 1987](#)). However, the in vivo studies by Lai ([2016](#)) did not replicate this phenomenon. These more precise studies showed that in rats exposed to 2.5 mg/m³ labeled formaldehyde for 28 days, at 1-week postexposure, 87% of the exogenous DPX were retained in the nasal tissues, suggesting a slow repair of these bulky adducts. The potential implications of this for dose-response modeling are discussed in Appendix D.2.2.

Table C-5. Summary of endogenous and exogenous DNA-protein crosslinks in nasal tissues of rats following inhalation exposure of ¹³CD₂-labeled formaldehyde

Reference and design	Exposure and analysis	Exposure duration	CH ₂ O conc.	Observations	
Lai et al. (2016) ; Monkeys, cynomolgus; N = 4–6.	0 (air control) or 7.4 mg/m ³ [¹³ CD ₂]-CH ₂ O from PFA by inhalation; 6 hrs/d; for 2 d; whole-body exposure; nasal tissue collected; DNA extracted with DNAzol reagent, dG-Me-Cys purified on HPLC and analyzed by nano-LC/ESI/MS-MS.		(mg/m ³)	Endogenous adducts	Exogenous adducts
				dG-Me-Cys/10 ⁸ dG	
		2 d	0	3.59 ± 1.01	ND
		2 d	7.4	3.76 ± 1.50	1.36 ± 0.20

Supplemental Information—Formaldehyde (Inhalation)

Reference and design	Exposure and analysis	Exposure duration	CH ₂ O conc.	Observations	
Lai et al. (2016) ; Rats, F344; N = 4–6.	0 (air control) or 18.5 mg/m ³ [¹³ CD ₂]-CH ₂ O from PFA by inhalation; 6 hrs/d; for 1, 2, or 4 d; whole-body exposure; nasal tissue collected; DNA extracted with DNAzol reagent, dG-Me-Cys purified on HPLC and analyzed by nano-LC/ESI/MS-MS.	Exposure Duration	(mg/m ³)	Endogenous adducts	Exogenous adducts
				dG-Me-Cys/10 ⁸ dG	
		4 d	0	6.50 ± 0.30	ND
		1 d	18.5	4.42 ± 1.10	5.52 ± 0.80
		2 d	18.5	4.28 ± 2.34	4.69 ± 1.76
		4 d	18.5	3.67 ± 0.80	18.18 ± 7.23
Lai et al. (2016) ; Rats, F344; N = 4–6.	Rats, inhalation exposure to 2.5 mg/m ³ CH ₂ O for 7 or 28 d and allowed to recover for 1 or 7 d PE. Nasal tissue collected and DNA extracted at the given time points and analyzed for dG-Me-Cys adducts as above.	Exposure Duration	(mg/m ³)	Endogenous adducts	Exogenous adducts
				dG-Me-Cys/10 ⁸ dG	
		7 d	2.5	4.78 ± 0.64	0.96 ± 0.17
		28 d	2.5	4.51 ± 1.48	2.46 ± 0.44
		28 d + 1 d PE	2.5	3.78 ± 0.69	2.12 ± 1.00
		28 d + 7 d PE	2.5	3.51 ± 0.16	2.14 ± 1.02

Abbreviations: PFA, paraformaldehyde; LC, liquid chromatography; MS, mass spectrometry; HPLC, high performance liquid chromatography; CH₂O, formaldehyde; DPX, DNA-protein crosslinks; dG-Me-Cys, deoxyguanosine-methyl-cysteine; PBMC, peripheral blood mononuclear cell; ESI, electron spray ionization; PE, post-exposure; D2, deuterium.

Distinguishing covalent binding of formaldehyde from metabolic incorporation

Three studies from the same research group addressed the issues of differentiating covalently bound (i.e., DPX formation) versus metabolically incorporated formaldehyde in rats exposed to formaldehyde by inhalation ([Casanova-Schmitz and Heck, 1983](#); [Casanova-Schmitz et al., 1984b](#); [Casanova and Heck, 1987](#)).

[Casanova-Schmitz et al. \(1984b\)](#) used dual isotope labeling as a way to partially distinguish between covalent binding (DPX formation) and metabolic incorporation of formaldehyde. In this approach, male F344 rats were exposed to a mixture of ³H- and ¹⁴C-labeled formaldehyde for 6 hours at exposure concentrations ranging from 0.37–18.42 mg/m³, a day after exposure to nonradioactive formaldehyde with the same dose range. The IF DNA was extracted from respiratory and olfactory mucosa, and the ³H/¹⁴C ratios of different phases of DNA extraction (i.e., AQ DNA and IF DNA) were measured. It is important to note that formaldehyde loses the hydrogen atom during oxidation reactions (i.e., metabolic incorporation), but not during covalent binding to DNA. Therefore, the ³H/¹⁴C ratio in a sample that contains adducts and crosslinks should be higher than in a sample that primarily contains DNA with metabolically incorporated formaldehyde.

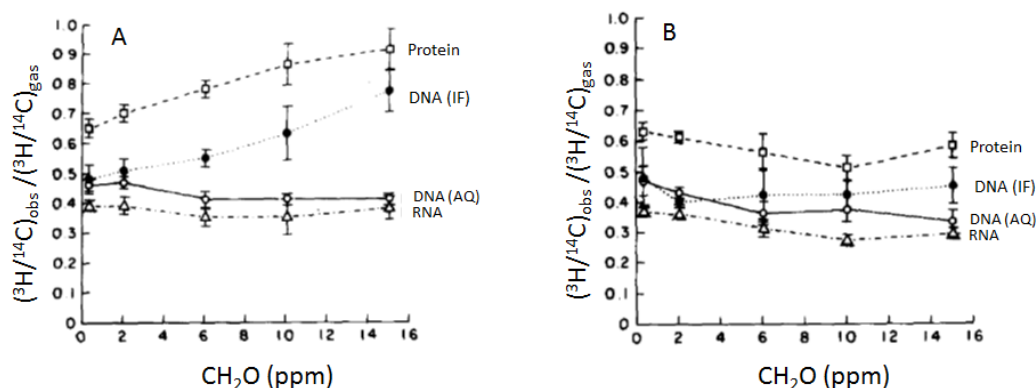


Figure C-4. Metabolic incorporation and covalent binding of formaldehyde in rat respiratory tract. $^3\text{H}/^{14}\text{C}$ ratios in macromolecular extracts from rat respiratory mucosa (A) and olfactory mucosa (B) following 6-hour exposure to ^{14}C - and ^3H -labeled formaldehyde (0.3, 2, 6, 10, and 15 ppm, corresponding to 0.37, 2.46, 7.38, 12.3, 18.42 mg/m^3 , respectively).

Source: Adapted from [Casanova-Schmitz et al. \(1984b\)](#)

As seen in panel A of Figure C-4, ([Casanova-Schmitz et al., 1984b](#)) report that IF DNA from nasal respiratory mucosa has a significantly higher $^3\text{H}/^{14}\text{C}$ ratio (Y-axis) than the aqueous phase (AQ) DNA, with a nonlinear dose response of IF DNA at exposure concentrations equal to or greater than 2.46 mg/m^3 . These data suggest that IF DNA has significantly more ^3H , a phenomenon likely explained by additional ^3H -formaldehyde molecules present as DPXs prior to DNA extraction. These crosslinks were due to exogenous formaldehyde that could be attributed to DPX. The $^3\text{H}/^{14}\text{C}$ ratio was linearly increased for the organic fraction, suggesting covalent binding of formaldehyde to respiratory mucosa proteins. In contrast, olfactory mucosa did not show increased $^3\text{H}/^{14}\text{C}$ ratio in the IF DNA or AQ DNA or proteins phase as a function of formaldehyde concentration (panel B, see Figure C-4). In total, these data suggest that the radiolabeling observed following formaldehyde exposure in rats results from both covalent binding and metabolic incorporation in the nasal mucosa, but not the olfactory mucosa ([Casanova-Schmitz et al., 1984](#)). The respiratory mucosa from unexposed rats appears to contain 15% of DNA as IF DNA ([Casanova-Schmitz and Heck, 1983](#)), possibly as endogenous DPX.

DNA monoadducts

Another form of formaldehyde-induced covalent DNA modifications is hydroxymethyl-DNA (hm-DNA) adducts or DNA monoadducts. Five studies conducted in one laboratory used $^{13}\text{CD}_2$ -formaldehyde in experimental rats and monkeys coupled with an LC/MS approach to distinguish hm-DNA adducts formed by endogenous and exogenous formaldehyde ([Yu et al., 2015b](#); [Moeller et al., 2011](#); [Lu et al., 2010a](#); [Lu et al., 2011](#)), as summarized in Table C-6. In this method, hm-DNA adducts formed by exogenous $^{13}\text{CD}_2$ -formaldehyde are distinguished from unlabeled endogenous hm-DNA adducts based on the differences in their typical m/z ratio ([Lu et al., 2012b](#)). As shown in

Table C-6, both exogenous and endogenous N²-hydroxymethyl-deoxyguanosine (N²-hm-dG) adducts were detected in nasal tissues of cynomolgus monkeys exposed to 2.34 or 7.5 mg/m³ ¹³CD₂-formaldehyde for 2 days, and across several rat studies testing exposures ranging from 0.9–18.7 mg/m³ formaldehyde for several hours up to 28 days ([Yu et al., 2015a](#); [Yu et al., 2015b](#); [Lu et al., 2010a](#); [Lu et al., 2011](#)). Notably, however, these studies demonstrate that the levels of endogenous N²-hm-dG adducts were several folds higher than corresponding exogenous adducts in nasal tissue.

While these studies provide the first insights into the relationship between endogenous and exogenous DNA monoadducts, further study may help to clarify some remaining uncertainties. For example, the potential involvement of different types of DNA monoadducts, as well as their specific toxicodynamic roles (e.g., for cancer development), remain poorly understood. Of the studies which used inhalation exposure to ¹³C-labeled formaldehyde, only ([Lu et al., 2010a](#)) quantified other adduct types; interestingly, while the authors detected ¹³CD₂-labeled N²-hm-dG adducts and dG-CH₂-dG crosslinks, they did not detect N⁶-hydroxymethyl-deoxyadenosine (N⁶-hm-dA) adducts in the nasal epithelium of rats exposed for 1 or 5 days (12.3 mg/m³) to exogenous formaldehyde. However, the same group reported the formation of both N²-hm-dG (most of the tissues) and N⁶-hm-dA monoadducts (only in bone marrow) in rats that were dosed by gavage with ¹³C-labeled methanol, which is a precursor of formaldehyde ([Lu et al., 2012b](#)). Similarly, a different research group reported that rats dosed subcutaneously with nitrosamines ([Wang et al., 2007b](#)), which are precursors to formaldehyde, and smokers ([Wang et al., 2009a](#)) both exhibit N⁶-hm-dA monoadducts in peripheral tissues. Thus, additional sensitive evaluations of dA monoadducts, particularly following longer term formaldehyde exposure and preferably in humans, may be informative. Also of interest, it is important to keep in mind that the experiments conducted to date involve comparisons of endogenous adduct levels, which would represent steady-state formaldehyde levels after having built up over time from the continuous presence of endogenous formaldehyde, to exogenous adduct levels resulting from short-term and/ or episodic (e.g., 6 hours/day) exposures. As an illustration, with exogenous exposure for 6-hour/day, multiple weeks or longer could be needed to reach steady-state levels, and, even so, those levels could be roughly expected to be four-fold lower than if a continuous (24 hours/day) exogenous exposure occurred at the same concentration. The recent study by ([Yu et al., 2015b](#)) begins to address this, noting that “quasi-steady-state” levels appear to be nearing after 6hr-day exposure to 2.46 mg/m³ formaldehyde for 28 days; however, exogenous adducts were still substantially increased with 28 days, as compared to 21 days of exposure, and exogenous adduct levels reached ≈37% of endogenous adduct levels (1.05 versus 2.82 adducts/10⁷ dG, in contrast to the ≈14% observed after 7 days of exposure) under this scenario. Considering these data at 2.46 mg/m³, the comparability of endogenous versus exogenous adducts relevant to lifetime exposure scenarios would be informed by additional studies incorporating a range of experiments and formaldehyde concentrations that span short, episodic exposures to more constant, long-term exposures.

Table C-6. Summary of endogenous and exogenous DNA monoadducts in nasal tissue of monkeys and rats following inhalation exposure of $^{13}\text{CD}_2$ -labeled formaldehyde

Reference and design	Exposure and analysis ^a	Portal of entry tissues	CH ₂ O exposure conc. (mg/m ³)	Observations	
				Endogenous adducts	Exogenous adducts
				N ² -hm-dG/10 ⁷ dG	
Moeller et al. (2011) ; Monkeys, cynomolgus; <i>n</i> =3	2.34 or 7.5 mg/m ³ [¹³ CD ₂]-CH ₂ O; 6 hrs/d; for 2 d (whole-body exposure); sacrificed immediately after exposure; tissues collected.	Nasal maxilloturbinates	2.34	2.50 ± 0.40	0.26 ± 0.04
			7.5	2.05 ± 0.54	0.41 ± 0.05
Yu et al. (2015b) ; Monkeys, cynomolgus; <i>n</i> = 4	0 (air control), 2.4 or 7.5 mg/m ³ [¹³ CD ₂]-CH ₂ O generated from [¹³ CD ₂]PFA; nose-only exposure; 6 hrs/d for 2 consecutive d; Sacrificed immediately after exposure; maxilloturbinates (Animal #1) and all other nasal tissues (Animal #2) were collected.	Nasal maxilloturbinates	2.4	2.50 ± 0.44	0.26 ± 0.04
			7.5	2.05 ± 0.54	0.41 ± 0.05
		Nasal dorsal mucosa	0	3.81 ± 1.19	ND
			7.5	3.62 ± 1.28	0.40 ± 0.07
		Nasal nasopharynx	0	3.48 ± 0.53	ND
			7.5	3.62 ± 1.34	0.33 ± 0.10
		Nasal septum	0	3.75 ± 0.32	ND
			7.5	3.56 ± 0.69	0.39 ± 0.15
		Nasal anterior maxillary	0	4.21 ± 0.53	ND
			7.5	3.80 ± 0.91	0.34 ± 0.12
		Nasal posterior maxillary	0	3.95 ± 0.74	ND
			7.5	3.46 ± 1.05	0.36 ± 0.16
		Trachea carina	0	2.69 ± 0.95	ND
			7.5	2.33 ± 1.12	ND
		Trachea proxima	0	2.35 ± 1.05	ND
			7.5	2.35 ± 1.05	nd
Lu et al. (2010a) ; Rats, Fisher; Male, <i>n</i> = 5–8	12.28 mg/m ³ [¹³ CD ₂]-CH ₂ O generated from [¹³ CD ₂]PFA; 6 hrs/d, 1 or 5 d; nose-only exposure; Sacrificed immediately after exposure; tissues collected.	Nasal tissue ^{b,c}	1-d	2.63 ± 0.73	1.28 ± 0.49
			5-d	2.84 ± 1.13	2.43 ± 0.78
			N ⁶ -hm-dA/10 ⁷ dA		
			1-d	3.95 ± 0.26	ND
			5-d	3.61 ± 0.95	ND
				dG-CH ₂ -dG/10 ⁷ dG	

Supplemental Information—Formaldehyde (Inhalation)

			1-d	0.17 ± 0.05	0.14 ± 0.0
			5-d	0.18 ± 0.06	0.26 ± 0.07
Lu et al. (2011) ; Rats, Fischer; n = 5–6	[13CD2]-CH2O from [13CD2]PFA; 6 hrs, nose-only exposure; Sacrificed immediately after exposure; tissue collected.	Nasal tissue		N ² -hm-dG adducts/10 ⁷ dG	
			0.9 ± 0.25	3.62 ± 1.33	0.039 ± 0.019
			2.5 ± 0.12	6.09 ± 3.03	0.19 ± 0.08
			7.1 ± 0.62	5.51 ± 1.06	1.04 ± 0.24
			11.2 ± 2.71	3.41 ± 0.46	2.03 ± 0.43
			18.7 ± 2.58	4.24 ± 0.92	11.15 ± 3.01
Yu et al. (2015b) ; Rats, Fischer, male; n = 8–9	0 (air control) or 2.46 mg/m ³ [13CD2]-CH2O from [13CD2]PFA; nose-only exposure; 6 hrs/d for 7, 14, 21, or 28 consecutive d; postexposure recovery for 6, 24, 72, and 168 hrs. Sacrificed immediately after exposure at indicated time points; tissues collected.	Nasal epithelium			
			Air control	2.84 ± 0.54	ND
			7 d	2.51 ± 0.63	0.35 ± 0.17
			14 d	3.09 ± 0.98	0.84 ± 0.17
			21 d	3.34 ± 1.06	0.95 ± 0.11
			28 d	2.82 ± 0.76	1.05 ± 0.16
			6 hrs PE	2.80 ± 0.58	0.83 ± 0.33
			24 hrs PE	2.98 ± 0.70	0.80 ± 0.46
			72 hrs PE	2.99 ± 0.63	0.63 ± 0.12
			168 hrs PE	2.78 ± 0.48	0.67 ± 0.20

Abbreviations: CH2O, formaldehyde; D2, deuterium; MS, mass spectrometry; PE, postexposure; PFA, paraformaldehyde; ND, not detected; N2-hm-dG, N2-hydroxymethyl-deoxyguanine; N6-hm-dA, N6-hydroxymethyl-deoxyadenine; dG-CH2-dG, dG-dG crosslinks; UPLC, ultra-pressure liquid chromatography

^aTissue DNA was extracted, reduced with sodium cyanogen borohydride (NaCNBH₃), digested, and analyzed by nano-UPLC-MS/MS.

^bNasal respiratory epithelium from the right and left sides of the nose and the septum.

^cExogenous N6-hmdA adducts were not detected in any tissues; exogenous N2-hm-dG and dG-dG crosslinks were detected only in nasal tissues.

Unknown contribution of potential interactions with other nasal mucosa elements

Formaldehyde is likely to interact with other components of the nasal mucosa depending on the concentration and duration of exposure. A small amount of inhaled formaldehyde, converted predominantly to methanediol, is expected to penetrate the epithelial cell layer, and react with the basement membrane or with constituents of the *lamina propria*, including components of the connective tissue/extracellular space, mucus gland components, lymphoid components, and vascular components. [Andersen et al. \(2008\)](#) examined gene expression in different tissue compartments of male F344 rats exposed to formaldehyde concentrations ranging from 0.9–18.5 mg/m³ by inhalation exposure. They reported that at low concentrations (0.9–2.5 mg/m³) formaldehyde is likely to react with the extracellular components of the cells at or near the cell membrane, while at higher doses (7.5–18.5 mg/m³) responses are observed in both extracellular

and intracellular sites involving more genes in the response. The gene expression data from this study suggests the possibility for a potential interaction of formaldehyde with other nasal mucosa components.

Removal of inhaled formaldehyde from the POE

The main processes for removing inhaled formaldehyde from the URT involve clearance in the mucus and metabolism to formic acid. Formic acid can enter the 1C pool and may either be oxidized to CO₂ or incorporated metabolically into nucleic acids and proteins carrying the 1C units through THF derivatives. Formate can also be absorbed into circulation, reach the kidneys, and be excreted in urine.

Summary of penetration, metabolism, and removal of inhaled formaldehyde within the URT tissue

In summary, as inhaled formaldehyde enters the URT it interacts with the mucociliary apparatus which is the first line of defense. In nasal mucus, most of the formaldehyde is rapidly converted to methanediol (~99.9%) and a minor fraction remains as free formaldehyde (~0.1%). Inhaled formaldehyde induces mucostasis and ciliastasis in rat nasal mucociliary apparatus extending from the anterior to posterior regions of the nasal cavity depending on the concentration and duration of exposure ([Morgan et al., 1986a](#)). However, as previously noted, uncertainties remain regarding the pattern of induced mucostasis, or the complete lack thereof, at low levels of formaldehyde exposure. Methanediol is assumed to be better able to penetrate tissues, while free formaldehyde reacts with macromolecules. It is assumed that equilibrium is reached rapidly, hence that the methanediol: free formaldehyde equilibrium ratio is maintained ([Fox et al., 1985](#)). However, uncertainties remain regarding the net impact of the transition of inhaled formaldehyde from the mucociliary layer to the underlying epithelium due to the presence of endogenous formaldehyde, which is a component of normal cellular metabolism. In the URT, formaldehyde is predominantly metabolized by glutathione-dependent class III alcohol dehydrogenase (ADH3) and by a minor pathway involving aldehyde dehydrogenase 2 (ALDH2) to formate. Formate can either enter the one-carbon pool leading to protein and nucleic acid synthesis or is further metabolized to CO₂ and eliminated in expired air or excreted in urine unchanged.

Formaldehyde can interact with macromolecules either by noncovalently binding to GSH, THF, or albumin in nasal mucus or covalently forming DPX, DDX, hm-DNA adducts, or protein adducts. In rats and monkeys, formaldehyde exposure results in a concentration-dependent increase in DPX. Metabolic incorporation studies with ¹⁴C-formaldehyde have shown both covalent binding and metabolic incorporation in nasal tissues ([Casanova-Schmitz et al., 1984b](#); [Casanova and Heck, 1987](#)). Distribution patterns in the nasal passages correspond to the tumor incidence locations in rats and to proliferative response patterns in both rats and monkeys. Hence, DPX has been used as a surrogate biomarker of exposure for risk assessment. Inhaled formaldehyde induces a concentration-dependent increase in N²-hm-dG adducts in the nasal passages of monkeys and

rats. Recently, analytical methods have been developed that can distinguish N²-hm-dG adducts formed from exogenous sources from those formed from endogenous sources. Notably, endogenous N²-hm-dG adduct levels are much higher than exogenous monoadduct levels in animals because formaldehyde is known to be produced continuously during normal cellular metabolism. It has been suggested that N²-hm-dG adducts could be used as a marker of exposure in risk assessment. However, this use might be compromised by several methodological issues in the adduct isolation and analysis.

C.1.4. Modifying Factors and Specific Uncertainties Regarding the Toxicokinetics of Inhaled Formaldehyde Within the POE

Many factors could influence the uptake and removal of inhaled formaldehyde at the POE. Distribution and tissue penetration of inhaled formaldehyde could both be significantly modified as a result of changes in environmental factors or tissue alterations induced by prolonged exposure. Similarly, metabolic detoxification of formaldehyde and clearance from the URT are dependent upon a number of cofactors and proteins that may be modified by changes to the environment or by prolonged exposure. Finally, modeling indicates that endogenous formaldehyde has the potential to impact the toxicokinetics of inhaled formaldehyde. This section will not include a description of every potential modifying factor but will attempt to highlight those interpreted to be most important or controversial, particularly those that may be essential to interpreting differences between experimental animals and humans.

Adjustments to account for reflex bradypnea in rodent studies

Reflex bradypnea (RB) is a protective reflex that allows rodents—but not humans—to significantly reduce their inhalation exposures to URT irritants such as formaldehyde. When an irritating concentration of formaldehyde triggers RB via the trigeminal nerve, rodents have an immediate decrease in respiratory rate and minute volume, and thus a marked decrease in formaldehyde exposure. Their RB persists until the exposure ends although the strength of the response in the initial minutes after exposure begins can be much stronger than later in the exposure. [Kane and Alarie \(1977\)](#) showed a maximal response in naïve mice of 13.7% decreased respiration rate from exposure to 0.55 ppm formaldehyde. This increased slightly to 15.6% in mice preexposed for 3 days. Consequently, a rodent study may not be health protective for humans unless the chamber concentrations or minute volume are adjusted to account for the rodents' reduced formaldehyde exposure. However, existing models and dose-response analyses have not accounted for this effect.

Unfortunately, it is not known if or when rodents develop a tolerance to formaldehyde and resume normal breathing. Considering that Chang and Barrow ([Chang and Barrow, 1984](#)) reported that F-344 rats experienced RB throughout 10 days of formaldehyde exposure, it may be appropriate to adjust short-term rodent exposure concentrations to make them health protective for humans. Because a long-term RB study has never been performed for formaldehyde or any

other URT irritant, there is no way of knowing whether similar adjustment is warranted for subchronic and/or chronic rodent studies. This is a significant data gap.

Modification due to effects of exposure on nasal mucosa function

Several events reported to occur after inhalation exposure to formaldehyde have the potential to modify the toxicokinetics of formaldehyde in the URT during subsequent exposure scenarios. Important among these factors are dynamic tissue remodeling, changes in mucociliary clearance, reduction in minute volume, and changes in glutathione levels and glutathione-mediated ADH3 activity.

Functional changes in the respiratory epithelium could have significant effects on the subsequent uptake of inhaled formaldehyde. Squamous metaplasia, a tissue conversion that is an adaptive response that occurs in nasal epithelium exposed to toxic levels of formaldehyde, has been observed in rats exposed to ≥ 2.46 mg/m³ formaldehyde for longer than 18 months. This type of dynamic tissue remodeling of nasal airways can affect formaldehyde dosimetry, as squamous metaplastic tissue is known to absorb considerably less formaldehyde than other epithelial types ([Kamata et al., 1997](#)). This is of critical concern for dosimetric modeling efforts, which typically rely on results from simulations of acute, rather than prolonged, exposure. The highest flux levels of formaldehyde in simulations of the rat nose in ([Kimbell et al., 2001b](#)) are estimated to be in the region just posterior to the nasal vestibule. A consequence of squamous metaplasia is a “push” of the higher levels of formaldehyde flux toward the more distal regions of the nose ([Kimbell et al., 1997b](#)). Uncertainties in the modeling of formaldehyde dosimetry are presented in ([Subramaniam et al., 2008](#)) and are discussed in Appendix C.1.12. A similar concern is raised regarding the observation that exposure affects the integrity and/or function of the mucociliary layer, as discussed previously and further evaluated in Appendix C.7.

Exposure-induced changes to factors involved in the detoxification of formaldehyde could also affect its toxicokinetics during a subsequent challenge. The enzyme ADH3 is central to the metabolism of formaldehyde; however, exposure to formaldehyde in turn alters the activity of ADH3-dependent critical metabolic pathways. For example, transcription of ADH3 correlates with the proliferative states in human oral keratinocytes ([Nilsson et al., 2004](#); [Hedberg et al., 2000](#)). In rodent lung, an increase in ADH3 activity affects other ADH3 substrates involved in protein modification and cell signaling ([Que et al., 2005](#)). Other pathways of ADH3 include oxidation of retinol and long-chain primary alcohols and reduction of S-nitrosoglutathione (GSNO). GSNO can accelerate ADH3-mediated formaldehyde oxidation and, likewise, formaldehyde increases ADH3-mediated GSNO reduction nearly 25-fold. Because GSNO is an endogenous bronchodilator and reservoir of nitric oxide (NO) activity, ADH3-mediated reduction of GSNO can cause a deregulation of NO (Reviewed in ([Thompson et al., 2010](#))).

Similarly, GSH is essential to detoxification of formaldehyde through the major pathway. GSH is present in most cells at levels far in excess of formaldehyde. In humans, the HMGSH levels are high since circulating GSH concentrations are ≈ 50 times higher than formaldehyde ([Sanghani et](#)

[al., 2000](#)). It is estimated that ≈50–80% of formaldehyde in animal cells is reversibly bound to GSH ([Uotila and Koivusalo, 1989](#)) and to a minor extent bound reversibly to tetrahydrofolate ([Heck et al., 1982](#)). Inhaled formaldehyde is similarly expected to undergo detoxification following reversible binding to GSH. GSH levels are unchanged in tissue homogenates following acute exposures but represent a possible adaptive response that may be location-specific and changed with prolonged exposure. For example, repeated exposure to formaldehyde (18.45 mg/m³, 6 hours/day for 9 days) did not affect either the GSH levels or the specific activities of ADH3 and ALDH2 in the nasal mucosa F344 rats ([Casanova-Schmitz et al., 1984a](#)). Interfacial DNA levels can be increased by glutathione depletion. This was tested by Casanova and Heck ([Casanova and Heck, 1987](#)) by exposing rats for 3 hours on two consecutive days to a range (1.11–12.3 mg/m³) of formaldehyde by inhalation, on Day 1 to nonlabeled formaldehyde and on Day 2 to a mixture of [³H] and [¹⁴C]-labeled formaldehyde. Two hours before the exposure on the second day, the animals were injected i.p. with 300 mg/kg phorone, a GSH depleting agent. The authors reported a 90–95% decrease in GSH levels and a significant decrease in metabolic incorporation in nasal respiratory and olfactory mucosa and bone marrow of phorone-treated rats. In contrast, the ³H/¹⁴C ratios of IF DNA were increased in a concentration-dependent manner for both phorone-treated and control groups of rats, albeit the levels were slightly higher in phorone-treated rats compared to control rats. Thus, depletion of GSH appeared to result in more unmetabolized formaldehyde available for covalent binding (crosslink formation) following 3-hour exposure.

Summary of potential modifying factors

The toxicokinetics of formaldehyde may be influenced by certain formaldehyde-related effects, such as mucociliary clearance ([Morgan et al., 1983](#)), reflex bradypnea (rodents only) and reduction in minute volume ([Chang et al., 1981](#); [Chang et al., 1983](#)), and dynamic tissue remodeling ([Kamata et al., 1997](#)), which have the potential to modulate formaldehyde uptake and clearance. For example, during repeated inhalation exposure to formaldehyde, mice but not rats lower their minute volume thereby restricting the intake of the gas ([Chang et al., 1981](#); [Chang et al., 1983](#)), which may impact dosimetric adjustment if extrapolated to humans. Exposure to formaldehyde can also cause a perturbation of ADH3-dependent pathways involved in cell proliferation ([Nilsson et al., 2004](#); [Hedberg et al., 2000](#)), protein modification and cell signaling ([Que et al., 2005](#)), GSNO metabolism, and deregulation of nitric oxide-dependent pathways ([Thompson et al., 2010](#)). In rats exposed by inhalation to formaldehyde, a rapid GSH depletion can result in more free formaldehyde available for covalent binding and lowering metabolic incorporation ([Casanova and Heck, 1987](#)).

Specific uncertainties regarding the potential impact of endogenous formaldehyde

Since formaldehyde is produced through normal cellular metabolism, several uncertainties exist which might impact the metabolism of exogenous formaldehyde in the body. This section covers the sources of endogenous formaldehyde, comparisons about its concentration gradient, its metabolism and reactivity, and the impact of inhaled formaldehyde on endogenous formaldehyde.

Sources of endogenous formaldehyde

Formaldehyde is endogenously produced through normal cellular metabolism from three main sources. As detailed below and outlined in Figure C-5, these sources include: (1) enzymatic reactions, (2) nonenzymatic reactions, and (3) as a metabolic byproduct of cellular metabolism of xenobiotics (e.g., drugs, environmental contaminants) that enter the body.

(1) Enzymatic pathways that generate formaldehyde endogenously as a normal component of cellular metabolism include four metabolic pathways: methylamine deamination, choline oxidation, histone lysine demethylation, and amino acid metabolism (serine, glycine, methionine). Formaldehyde can also be generated through endogenous generation from exogenous sources (e.g., methanol). These enzymatic sources are summarized in Figure C-5.

Methylamine is endogenously produced through amine catabolism, which upon deamination carried out by the enzyme semicarbazide-sensitive amino oxidase (SSAO) gives rise to formaldehyde. Choline oxidation is another endogenous metabolic process by which formaldehyde is generated. Choline is converted to glycine through several intermediary steps (choline → betaine → dimethylglycine (DMG) → sarcosine → glycine. The last two steps in this pathway are catalyzed by dimethylglycine dehydrogenase (DMGDH) and sarcosine dehydrogenase (SDH), respectively, using flavin adenine dinucleotide (FAD) as a cofactor. During these two steps the dehydrogenases nonenzymatically condense tetrahydrofolate (THF) with formaldehyde generating 5, 10-methylene-THF (5, 10-CH₂-THF), also known as “active formaldehyde.”

The other mechanism of endogenous formaldehyde production is through histone lysine demethylation, which is carried out by two classes of enzymes near the nucleus in a cell. One is a FAD-dependent amine oxidase, also known as lysine-specific demethylase 1 (LSD1/KDM1). The other one belongs to the Jumonji C terminal (JmjC) domain-containing histone demethylase (JHDM1/KDM2A). The LSD1 and JHDM1 enzymes act, respectively, on dimethyl lysine and trimethyl lysine converting them to monomethyl- and dimethyl lysine with the liberation of formaldehyde as an intermediary product ([Shi et al., 2004](#)). Formaldehyde can also be generated from methanol by either enzymatic or nonenzymatic pathways.

(2) Formaldehyde can also be formed nonenzymatically by the spontaneous reaction of methanol with hydroxyl radicals, wherein intracellular hydrogen peroxide is converted to the hydroxyl radical through the Fenton reaction ([Cederbaum and Qureshi, 1982](#)). Another mechanism of nonenzymatic production of formaldehyde is through lipid peroxidation of polyunsaturated fatty acids (PUFA) ([Slater, 1984](#); [Shibamoto, 2006](#)). It is known that a certain level of oxidative stress and lipid peroxidation occurs in every individual, and these oxidative processes are likely to contribute to endogenous formaldehyde production ([Zararsiz et al., 2006](#); [Ozen et al., 2008](#)).

(3) Formaldehyde may also be produced intracellularly during microsomal cytochrome P450 enzyme-catalyzed oxidative demethylation of *N*-, *O*-, and *S*-methyl groups of xenobiotics ([ATSDR, 2008](#)) that enter the body through dietary, environmental, or medicinal exposures, as shown in Figure C-5. [Dhareshwar and Stella \(2008\)](#) estimated that formaldehyde released from

prodrugs is ≈ 2 –100 mg. However, the authors point out that in humans with endogenous blood levels of ≈ 2 –3 $\mu\text{g/g}$ of blood total formaldehyde (Heck et al., 1985), the fraction of formaldehyde released from xenobiotics may contribute a small fraction to the endogenous pool (Dhareshwar and Stella, 2008).

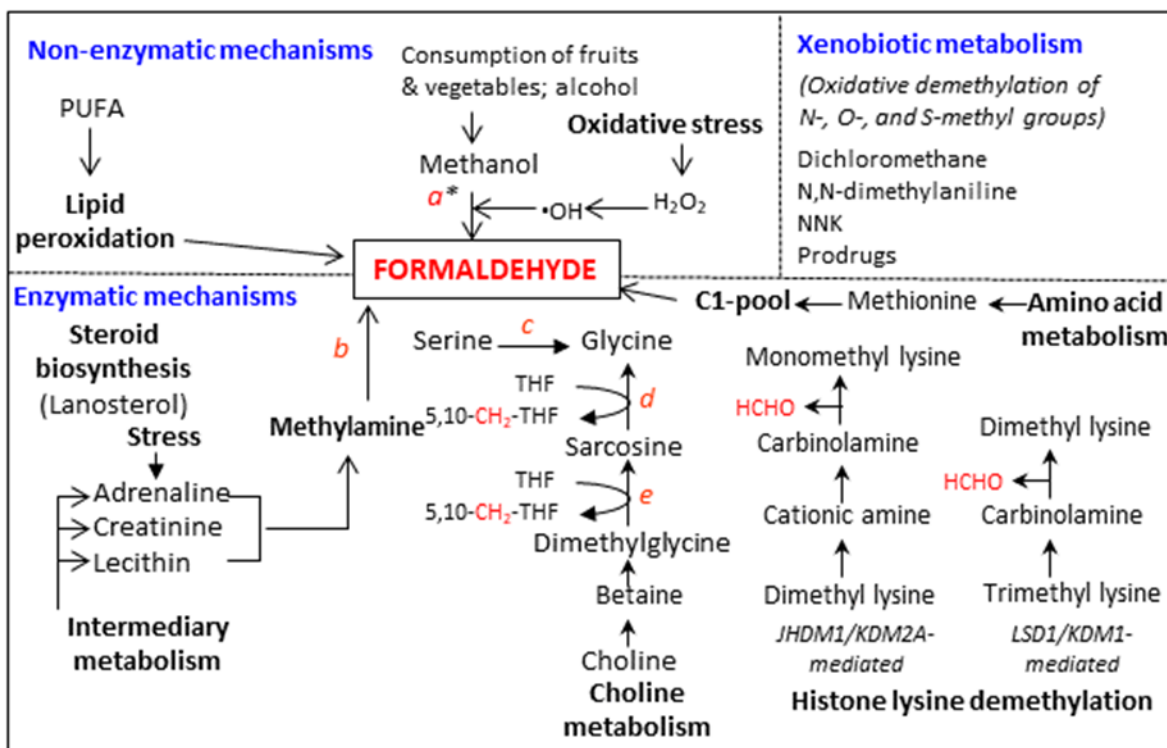


Figure C-5. Endogenous and dietary sources of formaldehyde production.

Formaldehyde is generated in the body through (a) Enzymatic mechanisms - involving (i) Steroid biosynthesis – from lanosterol, (ii) Intermediary metabolism – from methylamine (Yu and Zuo, 1996), (iii) Choline metabolism (Binzak et al., 2000), (iv) Stress – through adrenaline (Yu et al., 1997), (v) histone lysine demethylation (Shi et al., 2004) and (vi) Methanol metabolism (enzymatic) (Skrzydłowska, 2003); (b) Nonenzymatic mechanisms – (i) Methanol oxidation (Cederbaum and Qureshi, 1982) (ii) Lipid Peroxidation of polyunsaturated fatty acids or PUFA (Shibamoto, 2006) and (iii) Oxidative Stress (Slater, 1984); (c) Xenobiotic metabolism – demethylation of chemicals (ATSDR, 2008) and prodrugs (Dhareshwar and Stella, 2008).

Abbreviations: DMG: dimethyl glycine; C1: one carbon; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; THF: tetrahydrofolate; LSD1/KDM1, lysine (K)-specific demethylase 1; JHDM1/KDM2A, JumonjiC-domain containing histone demethylase 1.

Enzymes: *a*, alcohol dehydrogenase-1 (ADH1) in primates and ADH1 and catalase in rodents; *b*, semicarbazole-sensitive amine oxidase; *c*, serine hydroxymethyl transferase; *d*, sarcosine dehydrogenase; *e*, dimethylglycine dehydrogenase.

The presence of comparatively high levels of endogenous formaldehyde in cells of the URT presents an important uncertainty to evaluating the toxicokinetics of inhaled formaldehyde. Once inhaled formaldehyde interacts with aqueous matrices such as mucus and is hydrated, the biochemical interactions of inhaled formaldehyde and endogenous formaldehyde are assumed to be

very similar, given that there are no differences in chemical structure. However, other than in the nucleus (i.e., the experiments detailing DNA adducts), no data are available to inform where and to what extent endogenous and exogenous formaldehyde may be available to participate in these reactions.

Although much is unknown regarding the impact of endogenous formaldehyde on inhaled formaldehyde uptake and metabolism as outlined in the sections above, uncertainties relevant to interpreting the potential for biological differences between inhaled formaldehyde and endogenous formaldehyde are important to specify. Several of these uncertainties, which are essential to consider when comparing the distribution and macromolecular binding of endogenous formaldehyde versus inhaled formaldehyde, are outlined below.

Potential heterogeneity due to concentration gradients and interindividual differences

Endogenous formaldehyde is known to be produced within all cells of the URT. The specific levels of endogenous formaldehyde within each type of cell, or even within the various components of the nasal tissue (e.g., the respiratory mucosa lining the maxilloturbinates; the squamous epithelium lining the luminal surface of the nasal vestibule), have not been experimentally defined and are likely to vary across individuals.

In contrast to endogenous formaldehyde, which is likely homogeneously distributed within a tissue type, decreasing concentrations of inhaled exogenous formaldehyde reach deeper into the nasal mucosa. ([Georgieva et al., 2003](#)) modeled the penetration of inhaled formaldehyde across nasal tissue in the rat using a distributed parameter model; their results predict a sharp concentration gradient vertically across the tissue down to the basement membrane where the concentration levels off at steady state at a thickness of 11 μ m from the mucus layer. Therefore, interactions of the exogenous formaldehyde are more enriched in the epithelial cells and associated cellular/extracellular components along the apical surface of the respiratory mucosa. These differences in distribution can complicate comparisons of the relative contributions of exogenous and endogenous formaldehyde to macromolecular binding. Thus, measured differences in these levels from tissue isolates do not necessarily reflect the comparative levels in the target cells. However, it would be methodologically arduous to isolate select portion(s) of the respiratory mucosa for comparison, and as such, it does not appear that any studies have done so.

Possible differences in metabolism and reactivity of endogenous formaldehyde

As compared to exogenous formaldehyde, for which it is unknown how quickly it may be detoxified by the normal cellular machinery, the production and subsequent detoxification of endogenous formaldehyde appears to be kept under strict control. As mentioned earlier, the majority of endogenous formaldehyde is reversibly bound to GSH at any time ([Sanghani et al., 2000](#)).

The regulation of endogenous formaldehyde appears to be imperfect, given the presence of endogenous N²-HOCH₂-dG (dG) adducts ([Swenberg et al., 2011](#)). The endogenous adduct levels

reported by ([Swenberg et al., 2011](#)) are about the same as the exogenous dG adduct levels resulting from a single 6-hour exposure to ≈ 10 ppm formaldehyde. (Given that endogenous formaldehyde is present continuously, the equivalent continuous exposure to exogenous formaldehyde that would result in the same dG levels must be somewhat less than 10 ppm.) Some insight into the implications of this observation is obtained by comparing the endogenous levels reported by ([Heck et al., 1982](#)) with results from the toxicokinetic modeling of exogenous formaldehyde levels in the rat nasal tissue. ([Georgieva et al., 2003](#)) modeled the penetration of inhaled formaldehyde across nasal tissue using a distributed parameter model that was calibrated with data on DPX levels induced by exposure to formaldehyde and which used the DNA-binding constant determined in vitro by Heck and Keller ([Heck and Keller, 1988](#)). These authors predicted an exogenous level in nasal tissue of around $17\ \mu\text{M}$ from a 6-ppm exposure. This value is roughly 25 times lower than the total endogenous level of $12.6\ \mu\text{g/g}$ or $420\ \mu\text{M}$ reported by ([Heck et al., 1982](#)) in rat nasal tissue. This large difference is at odds with the above observation of similar dG adduct levels for endogenous and exogenous formaldehyde in the ([Swenberg et al., 2011](#)) experiment. The apparent discordance may suggest that much, but not all, of the endogenous formaldehyde (measured by ([Heck et al., 1982](#))) must be bound or sequestered in a way that reduces its ability to react with DNA, in comparison with exogenous formaldehyde.

Impact of inhaled formaldehyde on the function of endogenous formaldehyde

Although formaldehyde inhalation does not appear to result in a measurable change in the total level of formaldehyde in the nasal tissue of rats ([Heck et al., 1982](#)), it has yet to be determined whether exposure results in any changes to the normal functions of endogenous formaldehyde. For example, in the study by ([Lu et al., 2011](#)), rats exposed to ^{13}C -formaldehyde showed a concentration-dependent increase in the exogenous hm-dG adduct levels, and the corresponding endogenous N^2 -hm-dG adduct levels were highly variable at different exposure concentrations in the nasal tissues. In addition to the potential “compartmentalization” differences mentioned above, the endogenous DNA adduct levels, reflective of endogenous formaldehyde, do not appear to be static. Possible effects of exogenous formaldehyde exposure on metabolism and distribution processes of endogenous formaldehyde cannot be conclusively ruled out. However, no appreciable changes in the number of adducts formed as a result of interactions of endogenous formaldehyde with cellular constituents have been noted, even in the presence of formaldehyde exposure ([Yu et al., 2015b](#)).

C.1.5. Conclusions Regarding the Toxicokinetics of Inhaled Formaldehyde Within the POE

Within the POE, a majority of inhaled formaldehyde is rapidly retained in the URT of humans and experimental animals, irrespective of species differences in the anatomy, physiology, and breathing patterns. Following inhalation, formaldehyde levels are successively reduced as formaldehyde from the air penetrates through the various components of the nasal mucosa. Formaldehyde levels are reduced through interactions with components of the mucus and through

mucociliary clearance; through reactions with cellular materials at the plasma membrane of the respiratory epithelium; via interactions with GSH and other macromolecules in the intracellular and extracellular space; through localized metabolism and conjugation reactions; and through reversible interactions with intracellular materials. This results in the formation of a gradient of formaldehyde across the tissue space, with the greatest formaldehyde concentration at the apical surface of the mucosa, and the lowest levels of formaldehyde at deeper components of the tissue, such as the nasal associated lymphoid tissues (NALT) and blood vessels. In the URT, formaldehyde is metabolized by cytosolic ADH3 (major) and mitochondrial ALDH2 (minor) enzymes to formate which is further metabolized to CO₂ and eliminated in expired air, enters the 1C pool leading to metabolic incorporation, or is excreted in urine unchanged.

C.1.6. Toxicokinetics of inhaled formaldehyde beyond the portal of entry

Consistent with the previously described concentration gradient of inhaled formaldehyde within the POE, multiple studies report that very little inhaled formaldehyde reaches the vasculature of the respiratory tract to allow for absorption into the systemic circulation. Similarly, there is very little evidence that inhaled formaldehyde is distributed to tissues such as the bone marrow, liver, or brain. Studies examining the potential for direct interactions of inhaled formaldehyde with cellular macromolecules at distal sites have also not reported any evidence of these effects, despite observing that endogenous formaldehyde elicits such effects. Although the evidence is not entirely conclusive, and some uncertainties remain to be explored, the currently available data support an overall conclusion that appreciable amounts of inhaled formaldehyde are not distributed outside of the URT.

C.1.7. Levels of Endogenous and Inhaled Formaldehyde in Blood and Distal Tissues

Using the detection methods employed by ([Heck et al., 1982](#)), two studies from the same group reported endogenous levels of total formaldehyde in blood to be 2.61 ± 0.14 µg/g of blood in unexposed human subjects ([Heck et al., 1985](#)), 2.24 ± 0.07 and 2.71 ± 0.29 µg/g of blood in control F344 ([Heck et al., 1985](#)) and SD rats ([Kleinnijenhuis et al., 2013](#)), respectively, and 2.42 ± 0.09 µg/g of blood in unexposed rhesus monkeys ([Casanova et al., 1988](#)), providing relatively consistent measurements across species with an average blood level of ≈ 2.5 µg/g (≈ 0.1 mM) (see Table C-7). Levels of endogenous formaldehyde higher than in blood were also detected in other distal tissues of rats, although the nasal tissue contained the highest levels ([Heck et al., 1982](#)). The blood formaldehyde levels were not significantly changed when tested during exposure or shortly after exposure to formaldehyde concentrations ranging from 2.3 to 7.4 mg/m³ across the three species, with varying durations of exposure ([Heck et al., 1985](#); [Casanova et al., 1988](#)). The lack of increase in blood formaldehyde levels could also be due to the metabolism of formaldehyde in human erythrocytes, which are known to contain the formaldehyde metabolizing enzymes ADH3 ([Uotila and Koivusalo, 1987](#)) and ALDH2 ([Inoue et al., 1979](#)).

The tissue levels of endogenous formaldehyde determined experimentally by (Heck et al., 1982) may be highly uncertain. (Campbell Jr et al., 2020) assessed these values to be 20× lower based upon their modeling estimates and attributed this discrepancy to the potential for the Heck et al. measurement methodology to overestimate tissue formaldehyde levels. This is addressed again in Appendix C.1.12 in a discussion of model-derived estimates of the effects of endogenous formaldehyde on inhaled formaldehyde dosimetry.

EPA notes that while these data indicate that inhaled formaldehyde is not absorbed into the systemic circulation, a rough bounding calculation based on the human data indicates that the (Heck et al., 1985) experiment lacks the sensitivity needed to reach this conclusion. This bounding calculation assumes that the 2.3 mg/m³ of inhaled formaldehyde completely mixes with the blood, and because of its high solubility, it has a volume of distribution equal to that of all body water [0.57 L/kg of body weight; (Guyton, 1991)]. Using these parameters, the Heck et al. (1985) experiment is estimated to result in an increased blood formaldehyde concentration of 0.016 µg/g⁴. This quantity is one-half the experimental error of 0.03 µg/mL. Hence, even if all of the 2.3 mg/m³ of inhaled formaldehyde completely mixes with the blood, under the experimental protocol above for human exposure, formaldehyde blood concentration would increase by 0.016 µg/g, a quantity that cannot be detected by the (Heck et al., 1985) experiment.⁵ Moreover, this quantity is two orders of magnitude lower than the endogenous blood levels. Hence, these results are consistent with a lack of ¹⁴C radiolabel increases in the plasma of rats exposed to ¹⁴C formaldehyde (Heck et al., 1983), as well as a lack of increase in total formaldehyde calculated following exposure of rats to ¹³C formaldehyde (Kleinnijenhuis et al., 2013). Altogether, the data argue that the amount of inhaled formaldehyde absorbed into the blood is not likely to be significant, even if one assumes that only 5% of the endogenous formaldehyde in blood is not sequestered.

A similar trend was observed in distal tissues. Heck et al. (1983) exposed rats to a range of ¹⁴C-formaldehyde concentrations (6.14–29.48 mg/m³ for 6 hours) and observed that the ratio of tissue distribution relative to plasma radioactivity (µmole equivalents/g tissue) was not correlated with the exposure concentration, except in the esophagus (Heck et al., 1983). Mucociliary transport from the nose and trachea may have led to these relatively higher esophageal levels. Overall, these data also indicate that tissue distribution of formaldehyde levels were independent of the exposure concentration and duration of exposure.

Overall, the published data demonstrate no significant increase in formaldehyde levels in blood following formaldehyde inhalation. These data also report no significant differences in tissue and blood formaldehyde levels between preexposed and naïve animals. Such observations were

⁴Heck et al. (1985) air concentration = 1.9 ppm (2.34 mg/m³); t = 40/60 h; Inhalation Rate = 10–15 cubic m/day. Assuming 10 m³/24 hours, we get 10/24 m³/h. Formaldehyde inhaled = 1.9 × 1.23 × (10/24) × 40/60 h = 0.649 mg. Body water = 40 kg for a 70-kg man (Guyton, 1991); concentration of HCHO = HCHO inhaled/body water in mg/kg = 0.649/40 = 0.0162 mg/kg or µg/g.

⁵Even if one were to assume that formaldehyde stays only in the blood stream, this concentration increases to 0.12 µg/g of blood, which is still within the experimental error.

obtained from short-term experimental animal studies based on ¹⁴C-radiolabeling by GC-MS. The use of only this approach is problematic because there is no distinction as to whether the formaldehyde measured in these studies is free, reversibly or irreversibly bound, measured as formate, or part of the one-carbon pool. Nevertheless, taken together with the bounding calculations and relative activity calculations described above, the lack of significance of exogenous formaldehyde reaching distal tissues appears to hold even given the uncertainty.

Table C-7. Summary of blood and tissue levels of total^a formaldehyde in humans and experimental animals following inhalation exposure to formaldehyde

Reference and species	Exposure and analysis	Observations	
Heck et al. (1985) Human volunteers Male, <i>n</i> = 4; female, <i>n</i> = 2 24–44 years old	2.34 ± 0.07 mg/m ³ CH ₂ O (source not specified); 40 min exposure in a walk-in chamber; venous blood collected before and after exposure; Total CH ₂ O measured as PFPH derivative by GC-MS/SIM	<i>Total^a formaldehyde (μg/g of blood)</i>	
		Before exposure: After exposure:	2.61 ± 0.14 2.77 ± 0.28
Casanova et al. (1988) Monkeys, rhesus Male, <i>n</i> = 4; 200–250 g	7.37 mg/m ³ CH ₂ O (from PFA); 6 hrs/d, 4 d/wk, 4 wks; chamber inhalation; whole-body exposure; pre- and postexposure blood collected; Total CH ₂ O measured as PFPH derivative by GC-MS/SIM	<i>Total^a formaldehyde (μg/g of blood)</i>	
		Before exposure: 0 min. after exposure 40 min. after exposure:	2.42 ± 0.09 1.84 ± 0.15 2.04 ± 0.40
Heck et al. (1985) Rats, Fischer Male, <i>n</i> =4, Mean±SE = 232 ± 22 g	17.69 ± 2.95 mg/m ³ CH ₂ O (source not specified); 2-hrs exposure; chamber inhalation; nose-only; controls–no exposure; Total CH ₂ O measured as PFPH derivative by GC-MS/SIM	<i>Total^a formaldehyde (μg/g of blood)</i>	
		Before exposure: After exposure:	2.24 ± 0.07 2.50 ± 0.07
Kleinnijenhuis et al. (2013) Rats, Sprague Dawley Male, <i>n</i> = 10 12 wks-old	12.3 mg/m ³ ¹³ CH ₂ O (19.3% in aqueous solution: source not specified); 6-hrs exposure, Nose-only chamber; Blood samples collected before, during and after exposure; analyzed by HPLC-MS/MS after derivatizing with 2,4-DNPH	<i>Total^a formaldehyde (mg/L of blood^b)</i>	
		Before Exposure: During Exposure (3 hrs): During Exposure (6 hrs): After Exposure (~6.2 hrs): After Exposure (6.5 hrs):	2.71 ± 0.29 2.63 ± 1.12 2.01 ± 0.48 2.11 ± 0.35 1.81 ± 0.22
Heck et al. (1982) Rats, Fischer Male, <i>n</i> = 8 200–250 g	7.37 mg/m ³ ¹³ CH ₂ O from PFA; 6 hrs/d; 10-d exposure; chamber inhalation; CH ₂ O measured as PFPH derivative by GC/MS	Rat tissue levels (mean ± SE) of total^a CH₂O	
		<i>Tissue</i>	<i>Unexposed</i>
			<i>Exposed</i>
			μg/g
		Nasal mucosa	12.6 ± 2.7
		Liver	6.03 ± 0.5
		Testes	8.40 ± 3.0
		Brain	2.91 ± 0.42

Supplemental Information—Formaldehyde (Inhalation)

Reference and species	Exposure and analysis		Observations		
Heck et al. (1983) Rats, Fischer Male, $n = 3$; 180–250 g	Two groups: (a) <i>preexposure</i> ; (b) <i>naïve</i> ; On Days 1–9: group a) received 18.42 mg/m ³ ; CH ₂ O (from PFA); whole body exposure, 6 hrs/d; group b): no exposure. On day 10: groups a and b received ¹⁴ C-CH ₂ O (from PFA) for 6 hrs, nose-only exposure. Tissue homogenates counted with LSC for ¹⁴ CO ₂ trapped in ethanolamine in 2-methoxy-ethanol counted for radioactivity		Animals Exposed	Equivalents of ¹⁴ C in tissues (Mean ± SE)	
				Nasal mucosa	Plasma
			naïve rats	2,148 ± 255	76 ± 11
			preexposed	2,251 ± 306	79 ± 7
				Not significant	Not significant
Heck et al. (1983) Rats, Fischer, Male, $n = 12$	Naïve rats: dosed with 6.14, 12.28, 18.42 or 29.48 mg/m ³ ¹⁴ C-CH ₂ O (from PFA); 6-hrs nose-only; sacrificed immediately after exposure; tissue homogenates counted with LSC.	Tissue	(DPM/g tissue)/(DPM/g plasma) ^c	Tissue	(DPM/g tissue)/(DPM/g plasma) ^c
		Esophagus	4.94 ± 1.23	Spleen	1.59 ± 0.50
		Kidney	3.12 ± 0.47	Heart	1.09 ± 0.09
		Liver	2.77 ± 0.25	Brain	0.37 ± 0.06
		Intestine	2.64 ± 0.48	Testes	0.31 ± 0.05
		Lung	2.05 ± 0.36	RBC	0.30 ± 0.08

CH₂O, formaldehyde; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; HPLC-MS/MS, high performance liquid chromatography/tandem mass spectroscopy; PFA, paraformaldehyde; SIM, selected ion monitoring; DNPH, dinitrophenyl hydrazine; PFPH, pentafluorophenyl hydrazine; DPM, disintegrations per minute; ND, not detected; UPLC, ultraperformance liquid chromatography; NaCNBH₃, sodium cyanogen borohydride.

^aIncludes free and reversibly bound formaldehyde ([Heck et al., 1982](#)).

^bCalculated concentration in blood and corrected for stability.

^cValues (Mean ± SD) are ratios of concentrations (radioactivity) in tissues relative to plasma immediately after a 6-hour exposure to ¹⁴C-formaldehyde averaged for four concentration groups ($n = 12/\text{concentration}$).

Covalent binding of formaldehyde to macromolecules beyond POE

Formaldehyde has been shown to interact with macromolecules in blood or blood cells, but not in other distal organs as described below.

Evidence of covalent binding of formaldehyde to blood proteins

Formaldehyde has also been shown to covalently bind to serum proteins such as the amino acid valine in hemoglobin (Hb) forming N-methylvaline adducts in workers in plywood and laminate factory workers with occupational exposure ([Bono et al., 2006](#)). Also, with human serum albumin (HSA) it forms formaldehyde-HSA complexes ([Thrasher et al., 1990](#)). However, N⁶-formyllysine, another formaldehyde-induced protein adduct that also occurs endogenously, was not detectable in blood cells or in distal tissues (liver, lung, and bone marrow) in rats exposed to exogenous ¹³C-labeled formaldehyde ([Edrissi et al., 2013a](#)).

Evidence of DPX in the blood cells of formaldehyde exposed workers

DPXs have also been reported in the peripheral blood lymphocytes (PBLs) of formaldehyde-exposed workers ([Shaham et al., 1996](#); [Shaham et al., 1997](#); [Shaham et al., 2003](#)). [Shaham et al. \(1996\)](#) observed a statistically significant increase in DPX levels in PBLs compared to unexposed subjects and reported a linear relationship between years of exposure and the amount of DPX.

Lack of experimental evidence of endogenous and exogenous DNA monoadducts and DNA-protein crosslinks in blood and distal tissues

According to the available adduct studies, inhaled formaldehyde does not reach systemic tissues in concentrations sufficient to elicit detectable interactions of formaldehyde with DNA. In the bone marrow of monkeys ([Moeller et al., 2011](#)), and in the bone marrow, liver, lung, spleen, thymus, and blood of rats ([Lu et al., 2010a](#)), DNA monoadducts were formed by interactions with endogenous formaldehyde, but adducts formed from exogenous formaldehyde were not found (see Table C-8). It is important to note that ([Moeller et al., 2011](#)) observed 6–8 times higher endogenous N²-hm-dG adducts in the bone marrow compared to the nasal tissues of monkeys. Although there were some limitations with the experimental methods, including a possible overestimation of endogenous adducts due to reasons discussed (see Section C.1.3), the data support a general lack of systemic distribution of inhaled formaldehyde.

As described for the POE tissues, efforts have been made to differentiate covalent binding from metabolic incorporation in bone marrow. Male F344 rats were exposed to a mixture of ³H- and ¹⁴C-labeled formaldehyde for 6 hours at 0.37–18.42 mg/m³ 1 day after exposure to nonradioactive formaldehyde with the same exposure range ([Casanova-Schmitz et al., 1984b](#)). The authors extracted IF DNA from bone marrow (femur) and determined the ³H/¹⁴C ratios of different phases of DNA (i.e., AQ DNA and IF DNA). As previously described, a sample that contains adducts and crosslinks should have a higher ratio than a sample that primarily contains metabolically incorporated formaldehyde. In contrast to results in respiratory mucosa, bone marrow from the distal femur did not show an increased ³H/¹⁴C ratio in the IF DNA, AQ DNA, or proteins phase as a function of formaldehyde concentration (see Figure C-6). Therefore, the authors concluded that radiolabeled metabolites of formaldehyde reached the distal site (femur bone marrow) and were subsequently metabolically incorporated into macromolecules. In total, these data suggest that the labeling of bone marrow macromolecules was likely due to metabolic incorporation rather than due to covalent binding ([Casanova-Schmitz et al., 1984b](#)).

Recently [Lai et al. \(2016\)](#) developed an ultrasensitive mass spectrometry method which distinguishes unlabeled DPX from ¹³CD₂-labeled DPX induced respectively, from endogenous and exogenous formaldehyde. The authors demonstrated that inhalation exposure of stable isotope labeled (¹³CD₂) formaldehyde to rats (18.45 mg/m³; 6 hours/day; 1–4 days) and monkeys (2.5 mg/m³; 6 hours/day; 2 days) induced exogenous DPX in POE tissues such as nasal passages in both species, but not in distal tissues, such as bone marrow and peripheral blood monocytes (rats and monkeys) and liver (monkeys), although endogenous DPX were detectable in all tissues (see Table

C-9). These observations further confirm the lack of experimental evidence of formaldehyde distribution to distal tissues.

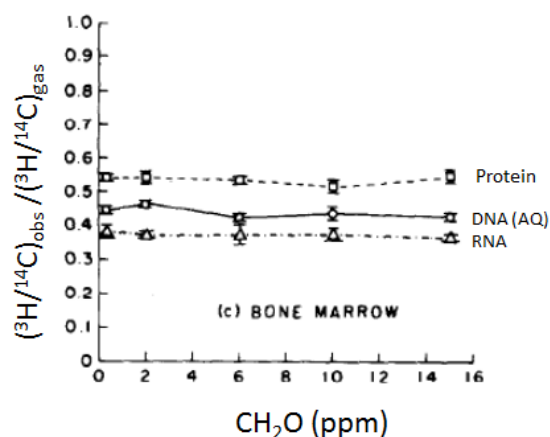


Figure C-6. $^3\text{H}/^{14}\text{C}$ ratios in macromolecular extracts from rat bone marrow following 6-hour exposure to ^{14}C - and ^3H -labeled formaldehyde (0.3, 2, 6, 10, and 15 ppm, corresponding to 0.37, 2.46, 7.38, 12.3, 18.42 mg/m^3 , respectively).

Source: Adapted from [Casnovaschmitz et al. \(1984\)](#).

Table C-8. Summary of endogenous and exogenous DNA monoadducts in distal tissues of monkeys and rats following inhalation exposure of $^{13}\text{CD}_2$ -labeled formaldehyde

Reference and design	Exposure and analysis ^a		CH_2O conc.	Observations	
Moeller et al. (2011) ; Monkeys, cynomolgus; n = 3	2.3 and 7.5 mg/m^3 [$^{13}\text{CD}_2$]- CH_2O from PFA; 6 hrs/d; for 2 d; whole-body exposure; sacrificed immediately after exposure; necropsied within 3 hrs; nasal mucosa and bone marrow collected; tissue DNA extracted, reduced with NaCNBH_3 , digested, and analyzed by nano-UPLC/MS.		(mg/m^3)	Bone marrow	
				Endogenous adducts	Exogenous adducts
				DNA adducts/ 10^7 dG	
			2.34	17.5 ± 2.6	ND
			7.5	12.4 ± 3.6	ND
Yu et al. (2015b) ; Monkeys, cynomolgus;	0 (air control), 2.4 or 7.5 mg/m^3 [$^{13}\text{CD}_2$]- CH_2O from [$^{13}\text{CD}_2$]PFA; nose-only exposure; 6 hrs/d for 2 consecutive d; Sacrificed immediately after exposure; Tissue DNA was	Distal tissue		$\text{N}^2\text{-hm-dG}/10^7$ dG	
		Air control	0	10.18 ± 1.35	ND
		Scrapped bone marrow	7.5	11.00 ± 2.01	ND
		Air control	0	5.65 ± 2.12	ND
		Saline extrusion bone marrow	7.5	4.41 ± 1.00	ND
		Air control	0	3.64 ± 1.09	ND
		White blood cells	7.5	3.79 ± 1.19	ND

Supplemental Information—Formaldehyde (Inhalation)

Reference and design	Exposure and analysis ^a		CH ₂ O conc.	Observations	
	extracted, reduced with NaCNBH ₃ , digested, and analyzed by nano-UPLC-MS/MS				
Lu et al. (2010a) ; Rats, Fisher; Male, n = 5–8	12.3 mg/m ³ [1 ³ CD ₂]-CH ₂ O from [1 ³ CD ₂]PFA; 6 hrs/d, 1 or 5 d; nose-only exposure; Sacrificed immediately after exposure. Lung, liver, spleen, bone marrow, thymus, and blood collected; tissue DNA extracted, reduced with NaCNBH ₃ , digested, and analyzed by nano-UPLC-MS/MS	Adduct	N ² -hm-dG/10 ⁷ dG ^a		
		Duration	1 day		5 days
		Tissue	Endogenous	Exogenous	Endogenous Exogenous
		Lung	2.39 ± 0.16 ^b	ND ^c	2.61 ± 0.35 ND
		Liver	2.66 ± 0.53	ND	3.24 ± 0.42 ND
		Spleen	2.35 ± 0.31	ND	2.35 ± 0.59 ND
		Bone marrow	1.05 ± 0.14	ND	1.17 ± 0.35 ND
		Thymus	2.19 ± 0.36	ND	1.99 ± 0.30 ND
		Blood ^d	1.28 ± 0.38	ND	1.10 ± 0.28 ND
		Adduct	N ⁶ -hm-dA/10 ⁷ dA ^a		
		Duration	1 day		5 days
		Distal Tissue	Endogenous	Exogenous	Endogenous Exogenous
		Lung	2.62 ± 0.24	ND	2.47 ± 0.55 ND
		Liver	2.62 ± 0.46	ND	2.87 ± 0.65 ND
		Spleen	1.85 ± 0.19	ND	2.23 ± 0.89 ND
		Bone marrow	2.95 ± 1.32	ND	2.99 ± 0.08 ND
		Thymus	2.98 ± 1.11	ND	2.48 ± 0.11 ND
		Blood ^d	3.80 ± 0.29	ND	3.66 ± 0.78 ND
		Adduct	dG-CH ₂ -dG/10 ⁷ dG ^a		
		Duration	1 day		5 days
		Distal Tissue	Endogenous	Exogenous	Endogenous Exogenous
		Lung	0.20 ± 0.04 ^e	ND	0.20 ± 0.03 ND
		Liver	0.18 ± 0.05	ND	0.21 ± 0.08 ND
		Spleen	0.15 ± 0.06	ND	0.16 ± 0.08 ND
		Bone marrow	0.09 ± 0.01	ND	0.11 ± 0.03 ND
		Thymus	0.10 ± 0.03	ND	0.19 ± 0.03 ND

Supplemental Information—Formaldehyde (Inhalation)

Reference and design	Exposure and analysis ^a			CH ₂ O conc.	Observations	
		Blood ^d	0.12 ± 0.09	ND	0.10 ± 0.07	ND
Yu et al. (2015b); Rats, Fischer	0 (air control), 2.4 or 7.5 mg/m ³ [¹³ CD ₂]-CH ₂ O from [¹³ CD ₂]PFA; nose-only exposure; 6 hrs/d for 2 consecutive d; Sacrificed immediately after exposure; tissues collected. Tissue DNA was extracted, reduced with NaCNBH ₃ , digested, and analyzed by nano-UPLC-MS/MS	Formaldehyde exposure duration	Rat bone marrow		Rat white blood cells	
			N ² -OHMe-dG (adducts/10 ⁷ dG)			
			Endogenous ^f	Exogenous	Endogenous ^f	Exogenous
		Air control	3.58 ± 0.99	ND	2.76 ± 0.66	ND
		7 d	3.37 ± 1.56	ND	2.62 ± 1.12	ND
		14 d	2.72 ± 1.36	ND	2.26 ± 0.46	ND
		21 d	2.44 ± 0.96	ND	2.40 ± 0.47	ND
		28 d	3.43 ± 2.20	0.34 ^g	2.49 ± 0.50	ND
		28 d + 6 hrs PE	2.41 ± 1.14	ND	2.97 ± 0.58	ND
		28 d + 24 hrs PE	4.67 ± 1.84	ND	2.57 ± 0.58	ND
		28 d + 72 hrs PE	5.55 ± 0.76	ND	1.75 ± 0.26	ND
		28 d + 168 hrs PE	2.78 ± 1.94	ND	2.61 ± 1.22	ND
		Distal tissue	N ² -OHMe-dG (adducts/10 ⁷ dG)			
			Air control		28-day exposure	
			Endogenous	Exogenous	Endogenous	Exogenous
		Thymus	0.78 ± 0.04	ND	0.63 ± 0.06	ND
		TBLN	3.46 ± 1.24	ND	3.01 ± 0.71	ND
		Lymph nodes	2.99 ± 0.85	ND	2.80 ± 1.38	ND
		Trachea	3.18 ± 0.72	ND	2.63 ± 0.92	ND
		Lung	2.29 ± 0.24	ND	2.13 ± 0.26	ND
		Spleen	2.18 ± 0.19	ND	1.83 ± 0.25	ND
		Kidneys	2.17 ± 0.60	ND	1.99 ± 0.09	ND
		Liver	1.97 ± 0.38	ND	1.80 ± 0.02	ND
		Brain	2.13 ± 0.17	ND	2.35 ± 1.00	ND

Abbreviations: PFA, paraformaldehyde; UPLC, ultra-pressure liquid chromatography; MS, mass spectrometry; N²-hm-dG, N²-hydroxymethyl-deoxyguanosine; N⁶-hm-dG, N⁶-hydroxymethyl-deoxyadenosine; dG-CH₂-dG, dG-dG crosslink; TBLN, tracheal bronchial lymph nodes; ND, not detected

^aThe limit of detection for dG monoadducts, dA monoadducts, and dG-dG crosslinks was ≈240, ≈75, and ≈60 atto mol, respectively.

^b*n* = 4–5 tissues.

^cNot detectable in 200 µg of DNA.

Supplemental Information—Formaldehyde (Inhalation)

^d60–100 µg of DNA was typically used for analysis of white blood cells isolated from blood.

^e*n* = 3.

^fNo statistically significant difference was found using the 2-sided Dunnett's test (multiple comparisons with a control).

^gThe amount of exogenous N2-hm-dG adducts that was found in only 1 bone marrow sample analyzed by AB SCIEX Triple Quad 6500.

Table C-9. Summary of endogenous and exogenous DNA-protein crosslinks in distal tissues of monkeys and rats following inhalation exposure of ¹³CD₂-labeled formaldehyde

Reference and design	Exposure and analysis	Tissue or Cell Type	Exposure duration (days)	Formaldehyde (mg/m ³)	Endogenous dG-Me-Cys (crosslink/ 10 ⁸ dG)	Exogenous dG-Me-Cys (crosslink/ 10 ⁸ dG)
Lai et al. (2016) ; Monkeys, cynomolgus; N = 4-5 in PBMC and Bone Marrow; N = 6 in liver	0 (air control) or 7.4 mg/m ³ [¹³ CD ₂]-CH ₂ O from PFA; 6 hrs/d; for 2 d; whole-body exposure; PBMC, bone marrow and liver collected; tissue DNA extracted; dG-Me-Cys purified on HPLC and analyzed by nano-LC/ESI/MS-MS.	PBMC	2	0	1.34 ± 0.25	ND
			2	7.4	1.57 ± 0.58	ND
		Bone Marrow	2	0	2.30 ± 0.30	ND
			2	7.4	1.40 ± 0.46	ND
		Liver	2	0	15.46 ± 1.98	ND
			2	7.4	11.80 ± 2.21	ND
Lai et al. (2016) ; Rats, F344; N = 4-5	0 (air control) or 18.5 mg/m ³ [¹³ CD ₂]-CH ₂ O from PFA; 6 hrs/d; for 1, 2, 4 d; whole-body exposure; PBMC, and bone marrow collected; tissue DNA extracted; dG-Me-Cys purified on HPLC and analyzed by nano-LC/ESI/MS-MS.	PBMC	4	0	4.98 ± 0.61	ND
			1	18.5	3.26 ± 0.73	ND
			2	18.5	3.00 ± 0.98	ND
			4	18.5	7.19 ± 1.73	ND
		Bone Marrow	4	0	1.64 ± 0.49	ND
			1	18.5	1.80 ± 0.47	ND
			2	18.5	1.84 ± 0.61	ND
			4	18.5	1.58 ± 0.38	ND

Abbreviations: PFA, paraformaldehyde; LC, liquid chromatography; MS, mass spectrometry; HPLC, high performance liquid chromatography; CH₂O, formaldehyde; DPX, DNA-protein crosslinks; dG-Me-Cys, deoxyguanosine-methyl-cysteine; PBMC, peripheral blood mononuclear cell; ESI, electron spray ionization.

C.1.8. Conjugation, Metabolism, and Speciation of Formaldehyde Outside the POE

Were inhaled formaldehyde to reach the blood or distal tissues, the same factors described for POE effects, specifically those regarding metabolism, reactivity, and the role of endogenous formaldehyde, would be relevant to other tissues. However, the majority of formaldehyde that would have reached these systemic sites is expected to be in the form of methanediol which is not reactive with macromolecules.

C.1.9. Elimination Pathways of Exogenous and Endogenous Formaldehyde

Elimination pathways of endogenous and exogenous pathways may not be different since all tissues contain surplus GSH and NAD⁺. Endogenous formaldehyde is oxidized by ADH3 to formate which is either eliminated as CO₂ in the exhaled breath or used in the cellular macromolecular synthesis or excreted in urine. Similarly, the majority of inhaled formaldehyde is metabolized in the URT by conversion to formate. Further, part of it may be metabolized to CO₂ or utilized in the 1C pool. Since the available evidence does not show significant amounts of exogenous formaldehyde being transported into blood, the subsequent clearance of any exogenous formaldehyde that does reach the blood should be similar to the handling of endogenous formaldehyde.

Excretion of formaldehyde

Inhalation exposure to formaldehyde has not been shown to cause significant changes to the tissue levels of formaldehyde in the nasal mucosa, the blood, or in the distal tissues. Thus, it is not expected that formaldehyde and formaldehyde metabolite content in excretion products would be altered by exposure. The data supporting this expectation are consistent in human and animal studies.

Formate levels have been detected in both unexposed as well as formaldehyde-exposed individuals. [Gottschling et al. \(1984\)](#) examined urinary formic acid levels of 35 veterinary medicine students working in an anatomy lab before exposure and within 2 hours following a 1-, 2-, or 3-week exposure to a mean formaldehyde concentration of <0.615 mg/m³. The authors did not observe significant changes in the pre- and postexposure levels of formic acid. Since co-exposure to methanol may also contribute to the metabolism and excretion of formate, the fact that no significant increase in urinary formate was seen even with that co-exposure further supports the conclusion that the formaldehyde exposure does not significantly increase formate excretion.

[Heck et al. \(1983\)](#) determined the relative contributions of various elimination pathways in F344 rats following inhalation exposure to 0.77 and 16.1 mg/m³ of ¹⁴C-formaldehyde. As shown in Table C-10, the percentages of radioactivity in various fractions appear to be similar between the two dose groups tested. Within 70 hours after a 6-hour formaldehyde exposure, nearly 40% of radioactivity from inhaled ¹⁴C-formaldehyde appeared to be eliminated via expiration, probably as ¹⁴CO₂ (note that about 97% of inhaled formaldehyde is taken up by the URT); and ≈17 and 5% of radioactivity was eliminated in the urine and feces, respectively. Nearly 40% of radioactivity remained in the carcass, which is presumably due to both covalent binding and metabolic incorporation. Thus, in one form or another, 40% of the ¹⁴C from inhaled formaldehyde is not eliminated and is expected to persist in the tissue(s) for some time. Overall, the authors concluded that, in rats, the relative elimination pathways for the remaining 60% of the ¹⁴C are independent of exposure concentration and followed the pattern of elimination in the order of expired air > urine > feces.

Although not specifically demonstrated following exposure, assumptions based on the known distribution and metabolism of formaldehyde and its detoxification products allow for inferences to be drawn regarding how inhaled ^{14}C reaches these elimination points. Approximately one-third of inhaled formaldehyde is estimated to be removed in the URT mucus ([Schlosser, 1999](#)). It is expected that the majority of this formaldehyde would be removed from the URT via mucociliary clearance and excreted in urine in various forms. A large amount of inhaled formaldehyde penetrating the mucociliary layer of the URT is metabolized in the nasal cavity, giving rise to formate, which can be excreted in urine. Part of this formate may also be further oxidized and eliminated in the exhaled breath as CO_2 . Some formaldehyde is incorporated into the 1C pool.

Table C-10. Summary of excretion study following exposure to formaldehyde by inhalation in rats

Reference and species	Treatment and analysis	Observations		
Heck et al. (1983) Rats, Fischer Male, n = 4, mean $\pm$ SD = 210 g	0.77 and 16.1 mg/m ³ HCHO for 6 hrs; rats sacrificed 70 hrs after removal from exposure chamber; tissues, urine, feces collected; exhaled $^{14}\text{CO}_2$ trapped in a solution of 5 M ethanolamine in 2-methoxyethanol and % radioactivity measured in LSC.	% Radioactivity (Mean $\pm$ SD) in various fractions		
		Source of radioactivity	Air borne CH_2O	
			0.77 mg/m ³	16.1 mg/m ³
		Expired air:	39.4 $\pm$ 1.45	41.9 $\pm$ 0.8
		Urine:	17.6 $\pm$ 1.2	17.3 $\pm$ 0.6
		Feces:	4.2 $\pm$ 1.5	5.3 $\pm$ 1.3
		Tissues ^a and carcasses:	38.9 $\pm$ 1.2	35.2 $\pm$ 0.5

^aNasal mucosa, trachea, esophagus, lung, kidney, liver, intestine, spleen, heart, plasma, erythrocytes, brain, testes.

Levels of endogenous formaldehyde in exhaled human breath

It is important to understand the relative amount of formaldehyde that is produced by the body and released in expired breath versus the amount of formaldehyde in ambient air. Table C-11 summarizes six studies that attempted to measure endogenous formaldehyde in exhaled breath. All studies performed prior to 2010 are limited by their analytical methods, which are subject to interference from other ions and isotopes that have the same m/z ratio ($m/z = 31$) as formaldehyde (e.g., methanol, ethanol, and nitric oxide). Also, it was not possible to differentiate between exogenous and endogenous formaldehyde in exhaled breath because the study subjects inhaled room air containing formaldehyde ($\approx 11 \mu\text{g}/\text{m}^3$ formaldehyde).

Table C-11. Measured levels of formaldehyde, methanol and ethanol in room air and exhaled breath

Study	Analytical method	Sample	Formaldehyde c (m/z 31) $\mu\text{g}/\text{m}^3$	Methanol $\mu\text{g}/\text{m}^3$	Ethanol $\mu\text{g}/\text{m}^3$
(Moser et al., 2005) ^a N = 344	PTR-MS DL: NR	Room air:	"Negligible"	"Negligible"	"Negligible"
		Exhaled breath:	5.24 (median) 1.49–89 (range)	198	NR
(Kushch et al., 2008) N = 370	PTR-MS DL: NR	Room air:	NR	NR	NR
		Exhaled breath:	6.39 (median, nonsmokers) 5.53 (median, 81 smokers)	241 (median, nonsmokers)	NR
(Cap et al., 2008) ^b N = 34	SIFT-MS DL: 3.68 $\mu\text{g}/\text{m}^3$ or better	Room air:	11.79 $\pm$ 1.84	NR	NR
		Exhaled breath:	2.46 (mean) 1.23 (median) 0–14.74 (range) 0 and 3.68 in 2 smokers	365 (mean) 232 (median) 125–2,848 (range)	549 (mean) 101 (median) 33–12,604 (range)
(Turner et al., 2008) N = 5	SIFT-MS DL: 6.14 $\mu\text{g}/\text{m}^3$ or better	Room air:	ND	NR	NR
		Exhaled breath:	ND	617 (mean)	549 (mean)
(Wang et al., 2008) N = 3	SIFT-MS DL: NR	Room air:	11.05 $\pm$ 3.68	54 $\pm$ 11	124 $\pm$ 63
		Exhaled breath:	6.51 (mean) 4.91–8.6 (range)	329 (mean)	185.46 (mean)
(Riess et al., 2010) N = 8 (nonsmokers) N = 2 (smokers)	<i>Acac</i> method DL: <0.62 $\mu\text{g}/\text{m}^3$ ^d	Charcoal filtered air:	0	NR	NR
		Exhaled breath:	<0.62 (nonsmokers), ND <0.62 (2 smokers), ND	NR	NR
	PTR-MS ^e DL: $\approx$ 0.62 $\mu\text{g}/\text{m}^3$	Charcoal filtered air:	0	NR	NR
		Exhaled breath:	1.84 (mean; 0.86–2.82), nonsmokers. 1.23–2.82, 2 smokers	NA	NA

Abbreviations: DL = Detection Limit; NR = Not Reported; ND = Not Detected; NA = Not Applicable; PTR-MS = Proton Transfer Reaction Mass Spectrometry; SIFT-MS = Selected Ion Flow Tube Mass Spectrometry.

^aAuthors reported room air concentrations for 179 chemicals were "negligible." No smoker data were provided.

^bSmoker data and formaldehyde ambient concentration provided by Dr. Španěl (personal communication).

^cValues of formaldehyde in parts per billion (ppb) are converted as $\mu\text{g}/\text{m}^3 = \text{ppb} \times 30 \text{ (m.w.)} / 24.45$ or $\text{ppb} \times 1.23$.

^dThe *acac* method's limit of detection is 0.062 μg formaldehyde/ m^3 , but the authors calculated a detection limit of 0.62 $\mu\text{g}/\text{m}^3$ due to a slight periodically fluctuating background noise signal.

^eAfter subtraction for methanol and NO product ions.

[Riess et al. \(2010\)](#), employed the acetyl acetone (*acac*) method⁶ to measure formaldehyde. This method is superior to the PTR-MS method used in previous studies because it has a lower limit of detection, exhibits no interference from other exhaled chemicals, and possesses the ability to measure formaldehyde in dry or humid atmospheres. In addition, volunteers inhaled formaldehyde-free air. For comparison, ([Riess et al., 2010](#)) used both the *acac* method and the PTR-MS method and observed mean exhaled formaldehyde concentrations of 1.84 µg/m³ in nonsmokers and 1.23–2.82 µg/m³ in smokers by the PTR-MS method, but no detectable formaldehyde in any subjects (including smokers) at baseline by the formaldehyde-specific *acac* method (see Table C-11). A concentration of 5.13 µg/m³ was detected by the *acac* method in a single smoker who was asked to smoke two cigarettes immediately before the measurement. This smoker's formaldehyde level declined below the level of detection within 30 min. Formaldehyde levels were 1.47 to 2.09 µg/m³ in subjects asked to consume methanol-rich hard fruit liquor within 48 hours of the test (recall that methanol is metabolized by alcohol dehydrogenase to formaldehyde throughout the body). So, even when formaldehyde levels were intentionally elevated, very little endogenous formaldehyde was expelled in exhaled breath and these elevations were transient.

In summary, [Riess et al. \(2010\)](#), the only study to date which avoided the limitations of previous studies, demonstrated that if endogenous formaldehyde exists in exhaled breath, it is usually below their level of detection of <0.62 µg/m³.

C.1.10. Conclusions Regarding the Toxicokinetics of Inhaled Formaldehyde Outside of the POE

In summary, the published data demonstrate that endogenous formaldehyde blood levels across species are approximately 0.1 mM and these levels do not change with exogenous formaldehyde exposure, arguing that inhaled formaldehyde is not absorbed into blood. One limitation of these studies is that these detection methods did not provide a clear distinction on the nature of formaldehyde (e.g., free, reversibly, or irreversibly bound, measured as formate, or part of the 1C pool). Formaldehyde inhalation studies show metabolic incorporation, but not covalent binding (e.g., hm-DNA adducts and DPXs) in bone marrow of rats which conclusively show that exogenous formaldehyde is not transported to distal tissues. Formaldehyde is likely to be metabolized in a similar way in distal tissues since the enzymes required for metabolism are expressed in all tissues. Endogenous levels of formaldehyde in exhaled breath analyzed by different research groups are often limited due to the lack of specificity in analytical methods and confounding by the presence of formaldehyde in room air in these studies. Based on a recent improved method, endogenous formaldehyde concentrations in exhaled air have been detected to be lower than the study's detection limit of 0.62 µg/m³ outside of exceptional circumstances (just after smoking two cigarettes or ingesting something with a high level of methanol).

⁶The *acac* method entails the cyclization of 2, 4-pentanedione (*acac*), ammonium acetate, and formaldehyde to form dihydropyridine 3, 5-diacetyl-1, 4-dihydrolutidine (DDL), which fluoresces at 510 nm after excitation at 412 nm.

C.1.11. Toxicokinetics Summary

Formaldehyde is an endogenous chemical produced intracellularly by enzymatic and nonenzymatic pathways during normal cellular metabolism, and a relatively small fraction of free formaldehyde is produced from metabolism of xenobiotics. Studies in experimental animals using direct and indirect measurements and modeling studies in human subjects have clearly shown that a majority of inhaled formaldehyde is rapidly absorbed in the URT despite anatomical and physiological differences across species. Inhaled formaldehyde develops a concentration gradient with an anterior to posterior distribution in the nasal cavity. High concentrations of formaldehyde are distributed to squamous, transitional, and respiratory epithelia; less formaldehyde uptake occurs in the olfactory epithelium, and very little or no formaldehyde reaches the lower respiratory tract, except possibly at very high exposure concentrations and/or during periods of high exertion with oronasal breathing. Studies in rats show that a single exposure to high levels of formaldehyde or repeated exposure to varying concentrations does not appreciably change the tissue levels of formaldehyde over the endogenous levels in the nasal mucosa.

Inhaled formaldehyde entering the nasal cavity interacts with the mucociliary apparatus which is the first line of defense. The majority of formaldehyde is rapidly converted to methanediol ($\approx 99.9\%$), with a minor fraction ($\approx 0.1\%$) remaining as free formaldehyde in the nasal mucus. A rapid equilibrium is assumed such that the 99.9:0.1% ratio is maintained at all times. Methanediol penetrates the tissues while free formaldehyde reacts with macromolecules. Uncertainties remain about the distribution of formaldehyde to underlying epithelium owing to the presence of endogenous formaldehyde, which is a component of normal cellular metabolism. Formaldehyde is metabolized to formate predominantly by ADH3 and by a minor pathway involving mitochondrial ALDH2. Formate can either enter the one-carbon pool leading to protein and nucleic acid synthesis or is further metabolized to CO_2 and eliminated in expired air or excreted in urine unchanged.

Formaldehyde can interact with macromolecules either noncovalently (GSH, THF) or covalently (DPX, DDX, hm-DNA monoadducts, protein adducts). In rats and monkeys, DPXs show a dose-response to formaldehyde inhalation exposure in the nasal cavity where DPX distribution corresponds to tumor sites (rats) and cell proliferation (rats and monkeys), suggesting that DPX may be a good biomarker of exposure. Formaldehyde also induces a concentration-dependent increase in DNA monoadducts (e.g., N^2 -hm-dG adducts) in the nasal passages of monkeys and rats which can be distinguished from endogenous adducts using improved analytical methods. Higher levels of endogenous N^2 -hm-dG adducts are detectable than exogenous monoadducts, except at the highest inhaled exposure concentrations.

The toxicokinetics of formaldehyde may be influenced by certain formaldehyde-induced effects, such as modifications to mucociliary clearance, reflex bradypnea (rodents only) and reduction in minute volume, and dynamic tissue remodeling (e.g., squamous metaplasia), which have the potential to modulate formaldehyde uptake and clearance. For example, inhaled formaldehyde induces mucostasis and ciliastasis in the rat nasal mucociliary apparatus extending

from anterior to posterior regions of the nasal cavity depending on the concentration and duration of exposure. Thus, at least at higher concentrations (e.g., at low concentrations, formaldehyde does not clearly cause mucostasis), estimates of tissue formaldehyde levels may be more uncertain. Similarly, the differences observed in altered minute volumes in rats and mice during repeated inhalation exposure to formaldehyde may impact dosimetric adjustment if extrapolated to humans.

Endogenous blood formaldehyde levels average around 0.1 mM across different species and inhalation exposure to formaldehyde does not alter blood formaldehyde levels, suggesting that inhaled formaldehyde is not significantly absorbed into blood. Formaldehyde-induced exogenous DNA monoadducts were detectable in nasal tissues but not in distal tissues of experimental animals exposed by inhalation. This argues against systemic transport of formaldehyde to distal tissues. Also, formaldehyde inhalation studies show metabolic incorporation, but not covalent binding in bone marrow of rats, further supporting the lack of transport of formaldehyde (as opposed to metabolites of formaldehyde) to distal tissues.

Analysis of formaldehyde in exhaled breath can be confounded by interfering gases in the analytical techniques used or can be confounded by the presence of formaldehyde in the room air. With improved techniques, endogenous formaldehyde concentrations in exhaled air have been detected to be usually lower than the past detection limit of 0.62 $\mu\text{g}/\text{m}^3$. Overall, no evidence is available to indicate that inhaled formaldehyde is systemically transported.

C.1.12. Modeling Formaldehyde Flux to Respiratory Tract Tissue

Formaldehyde is highly reactive and water soluble, thus its absorption in the mucus layer and tissue lining of the respiratory tract is known to be significant. This absorption is highly regional, and the absorption patterns differ substantially across species. This section first provides the motivation for developing detailed dosimetry models for the regional and species-specific absorption of formaldehyde. It then discusses the computation of inhaled formaldehyde transport in the upper (nose and mouth) and lower (lung and trachea) respiratory tract using fluid dynamic models and evaluates the level of confidence in these predictions. Finally, a revised dosimetry model that incorporates estimates of endogenous formaldehyde is discussed.

Species differences in anatomy: consequences for gas transport and respiratory tract lesions

The regional dose of inhaled formaldehyde in the epithelial lining of the respiratory tract of a given species depends on the amount absorbed at the airway-tissue interface, water solubility, mucus-to-tissue phase diffusion, and chemical reactions, such as hydrolysis, protein binding, and metabolism, and on the amount of formaldehyde delivered by the inhaled air to the tissue lining. This is a function of the major airflow patterns, air-phase diffusion, and absorption at the airway-epithelial tissue interface. Formaldehyde-induced squamous cell carcinomas (SCC) and other lesions that occur in the rat and monkey nasal passages and in the monkey lower respiratory tract are seen to be localized, with the lesion distribution patterns also showing species-specificity. It has been argued that the main determinant of these patterns and their differences among species is

regional dose ([Morgan et al., 1991](#); [Monticello and Morgan, 1994](#); [Monticello et al., 1996](#); [Bogdanffy et al., 1999](#)).

The anatomy of the respiratory tract, in particular the upper part (Figure C-7), and airflow patterns in this region (Figure C-8) show large differences across species. Furthermore, because of the convoluted nature of the airways (Figure C-7), the uptake of reactive and water-soluble gases such as formaldehyde in the upper respiratory tract (as seen in various simulations, Figure C-9) is highly nonhomogeneous over the nasal surface. Thus, as shown in Figure C-9, the spatial distribution of formaldehyde flux also shows strong species dependence. These observations, when juxtaposed with the localized occurrence of lesions, suggest that regional dose may be important in reducing uncertainty when extrapolating risk-related dose across species. [Kimbell et al. \(1993\)](#); [Kepler et al. \(1998\)](#), and [Subramaniam et al. \(1998\)](#) developed anatomically realistic finite-element representations of the noses of F344 rats, rhesus monkeys, and humans, and used them in physical and computational models ([Kimbell et al., 2001a](#); [Kimbell et al., 2001b](#)); Figures C-7, C-8, C-9). This assessment uses dosimetry derived from these representations.

Formaldehyde dosimetry in the lower human respiratory tract (i.e., in the trachea and lung) may also be important to consider. The upper respiratory tract is generally a good scrubber of formaldehyde; as a result, there is less penetration into the lungs. However, the extent of this scrubbing varies among species. The rat upper respiratory tract is extremely efficient with only about 3% fractional penetration to the lower respiratory tract ([Morgan et al., 1986a](#)); however, penetration to the lung appears to be higher in the rhesus monkey (Figure C-9). Accordingly, while frank effects were seen only in the upper respiratory tract in rodents, DPX lesions induced by exposure to 6 ppm formaldehyde were also present in the major bronchiolar region of the rhesus monkey whose respiratory tract morphology is somewhat similar to the human (see Figure C-7). Another factor is that humans are oronasal breathers, with a significant fraction of the population breathing normally through the mouth ([Niinimaa et al., 1981](#)), while rats are obligate nose-only breathers. Oronasal breathing implies a much higher dose to the lower respiratory tract, particularly at higher activity profiles [see Figure C-11 and ([Niinimaa et al., 1981](#))]. For all these reasons, the cancer dose-response assessment based upon nasal tumors observed in the F344 rat includes an additional exercise involving the human lung, even though the lung is not identified as a target organ in the hazard assessment. The dose-response section evaluates the extent to which human risk estimates increase when formaldehyde dose to the lower human respiratory tract is also considered. The dosimetry modeling for this purpose uses an idealized single-path model of the lower respiratory tract developed by ([Overton et al., 2001](#)).

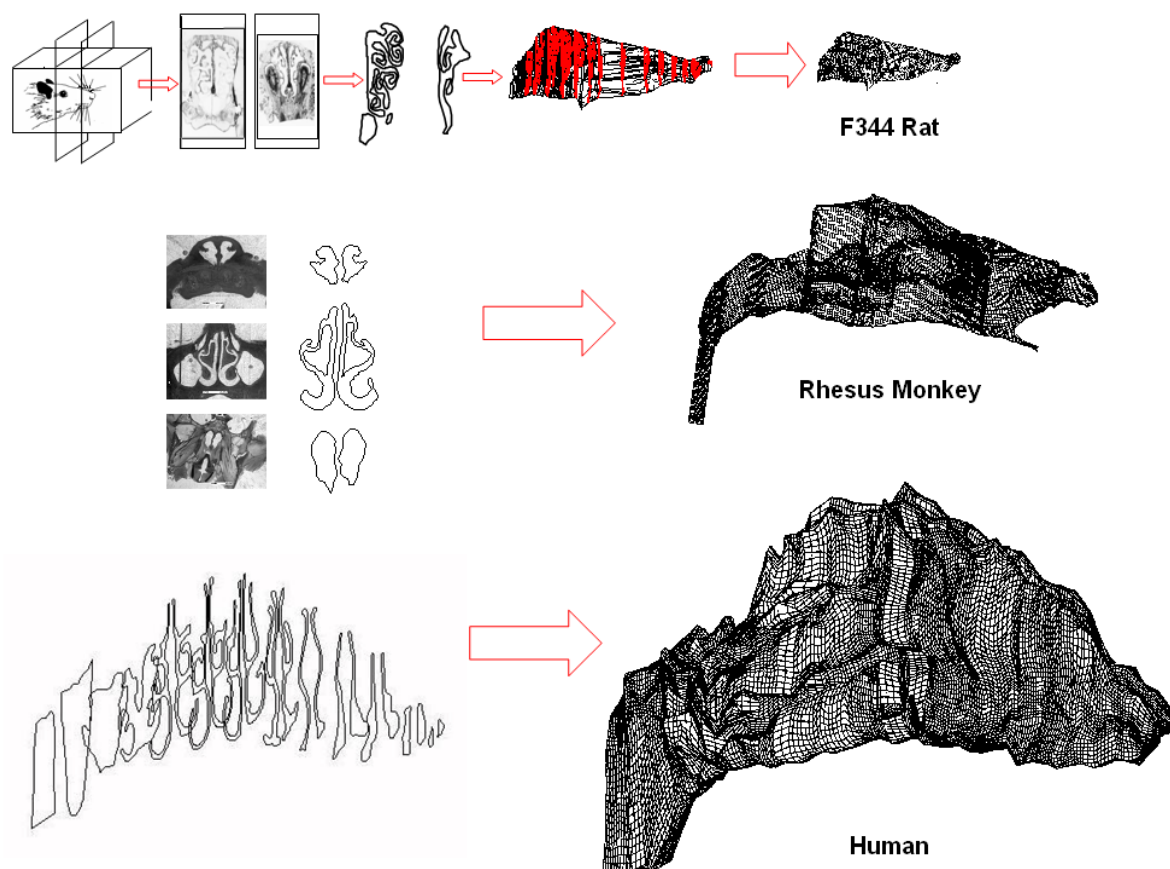


Figure C-7. Reconstructed nasal passages of F344 rat, rhesus monkey, and human.

Note: The nostril is to the right, and the nasopharynx is to the left. The right side shows the finite element mesh. The left-hand side shows tracings of airways obtained from cross sections of fixed heads (F344 rat and rhesus monkey) and magnetic resonance image sectional scans (humans). Aligned cross sections were connected to form a three-dimensional reconstruction and finite-element computational mesh. Source: Adapted from [Kimbell et al. \(2001b\)](#). Additional images provided courtesy of Dr. J.S. Kimbell, CIIT Hamner Institutes.

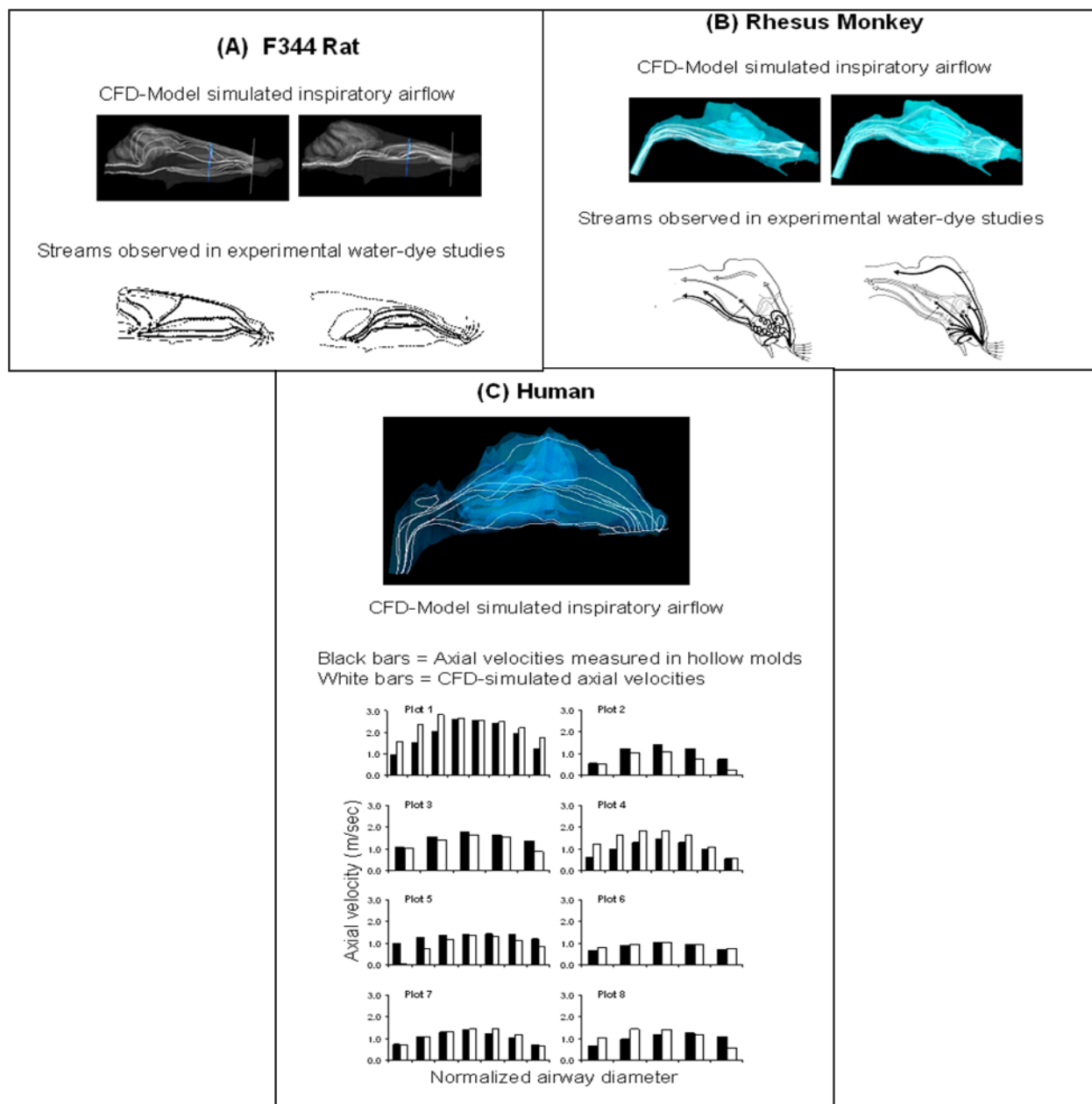


Figure C-8. Illustration of interspecies differences in airflow and verification of CFD simulations with water-dye studies.

Note: Panels A and B show the simulated airflow pattern versus water-dye streams observed experimentally in casts of the nasal passages of rats and monkeys, respectively. Panel C shows the simulated inspiration airflow pattern, and the histogram depicts the simulated axial velocities (white bars) versus experimental measurements made in hollow molds of the human nasal passages. Dye stream plots were compiled for the rat and monkey over the physiological range of inspiration flow rates. Modeled flow rates in humans were 15 L/min.

Source: Adapted from [Kimbell et al. \(2001b\)](#).

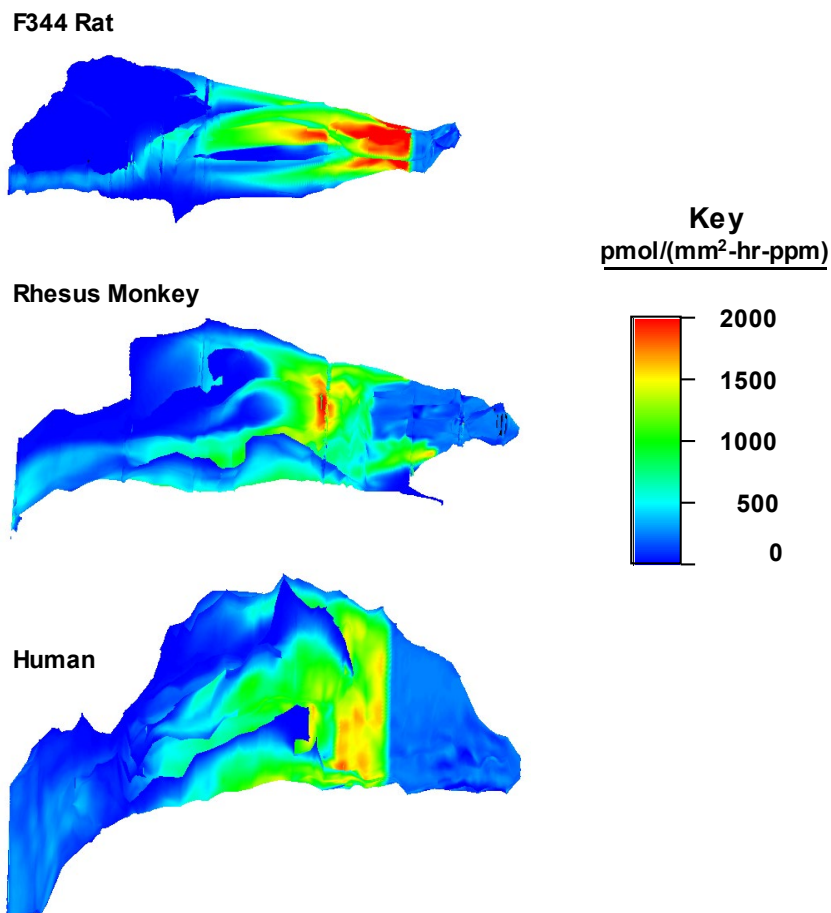


Figure C-9. Lateral view of nasal wall mass flux of inhaled formaldehyde simulated in the F344 rat, rhesus monkey, and human.

Note: This is a rendering of a three-dimensional surface. Nostrils are to the right. Simulations were exercised in each species at steady-state inspiration flow rates of 0.576 L/min in the rat, 4.8 L/min in the monkey, and 15 L/min in the human. Flux was contoured over the range from 0–2,000 $\text{pmol}/(\text{mm}^2\text{-hour-ppm})$ in each species. Source: [Kimbell et al. \(2001b\)](#).

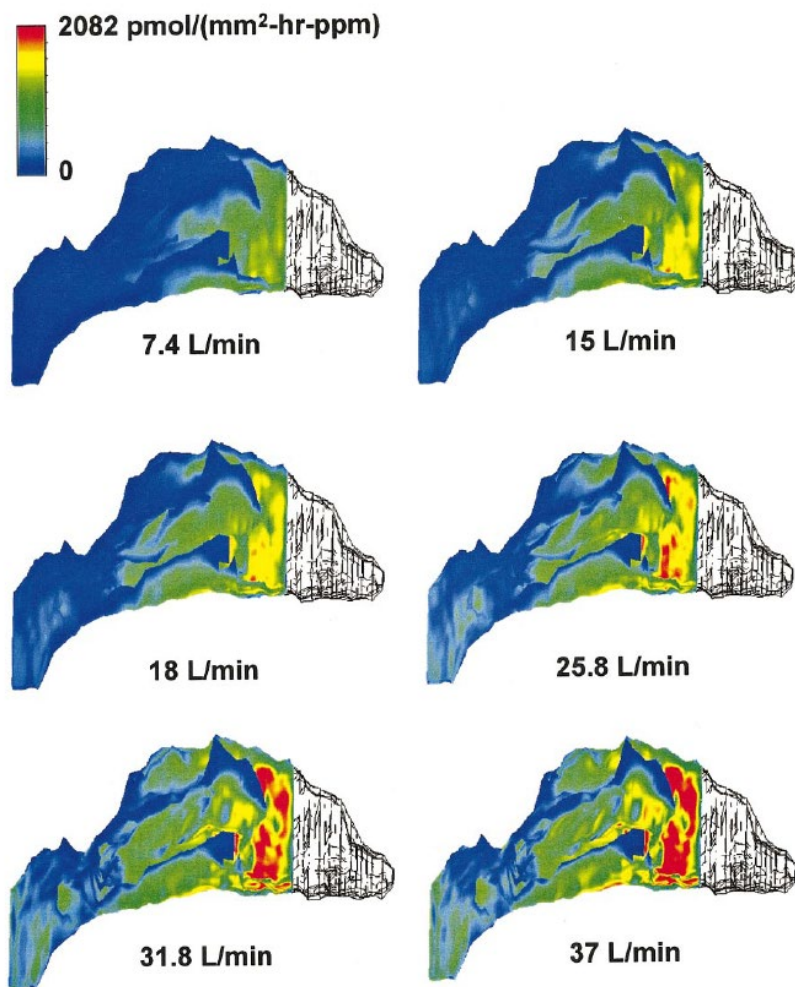


Figure C-10. Lateral view of nasal wall mass flux of inhaled formaldehyde simulated at various inspiratory flow rates in a human model.

Note: This is a rendering of a three-dimensional surface, showing the right lateral view. Uptake is shown for the nonsquamous portion of the epithelium. The front portion of the nose (vestibule) is lined with keratinized squamous epithelium and is expected to absorb relatively much less formaldehyde.

Source: [Kimbell et al. \(2001a\)](#).

Modeling formaldehyde uptake in nasal passages

Anatomical reconstruction and tissue types: The dose-response modeling results evaluated and used in this document are based on several published computational models for air flow and formaldehyde uptake in the nasal passages of a F344 rat⁷, rhesus monkey, and human, and in the human lung ([Subramaniam et al., 1998](#); [Overton et al., 2001](#); [Kimbell et al., 1993](#); [Kimbell et al., 2001b](#); [Kepler et al., 1998](#)). The anatomical reconstructions for both computational and physical

⁷This strain of the rat is considered anatomically representative of its species and widely used experimentally, most notably in bioassays sponsored by the National Toxicology Program.

models were based on tracings of airways obtained from cross sections of fixed heads (F344 rat and rhesus monkey) and magnetic resonance image sectional scans (human).

Formaldehyde-induced nasal SCCs in rats are observed to arise only from respiratory or transitional epithelial cells in F344 rats and thought to be associated with the transformation of these cell-types to a squamous epithelial type due to exposure to formaldehyde ([Morgan et al., 1986a](#)). Therefore, the dosimetry calculations in ([Kimbell et al., 2001b](#)) focused on predicting the wall mass flux of formaldehyde (rate at which mass of formaldehyde is transported to unit area of the nasal or lung lining prior to disposition within the body—mass/[area-time]) to regions lined by respiratory or transitional epithelium and excluding squamous epithelial cells. An additional distinction was made regarding these regions. Formaldehyde hydrolyses in water and reacts readily with a number of components of nasal mucus and was therefore assumed to be absorbed at a higher rate by epithelial lining coated with mucus. The approximate locations of mucus-coated and nonmucus coated respiratory/transitional epithelial cells were mapped onto the reconstructed nasal geometry of the computer models. Types of nasal epithelium overlaid onto the geometry of the models were assumed to be similar in characteristics across all three species (rat, monkey, and human) except for thickness, surface area, location, and the extent of the nasal surface not coated by mucus. These characteristics were estimated from the literature or by direct measurements ([Conolly et al., 2000](#); [CIIT, 1999](#)).

The fluid dynamics modeling in the respiratory tract comprises two steps: (1) model airflow through the airway lumen (solution of Navier-Stokes equations) and (2) using these solutions of the airflow field as input, model formaldehyde flux to the respiratory tract lining (solution of convective-diffusion equations). The local formaldehyde flux at the airway-to-epithelial tissue interface was assumed to be proportional to the air-phase formaldehyde concentration adjacent to the nasal lining. The proportionality constant is the mass transfer coefficient for the tissue phase, specified as boundary conditions on the solutions, and takes different values in the model depending on whether the tissue is coated with a mucus layer (k_m) or not (k_{nm}). Epithelium not coated with mucus was considered similar to epidermal tissue, and a value available from the literature for such tissue was used for k_{nm} . On the other hand, Kimbell et al. determined k_m empirically for the rat by fitting the overall nasal uptake predicted by the CFD model to the average experimental values obtained by ([Morgan et al., 1986a](#)). The values of k_m and k_{nm} depend only on the solubility and diffusivity of the gas in the tissue, the thickness of the tissue, and the reaction rate of the gas ([Hanna et al., 2001](#)). Tissue thickness varies across species, but because formaldehyde is highly reactive and soluble, the primary kinetic determinant of interspecies differences in the net mass transfer rate is likely the difference in air-phase resistance and not tissue thickness. Therefore, ([Kimbell et al., 2001b](#)) assumed that values for the tissue phase mass transfer coefficients were the same for the human. EPA judges this assumption to be reasonable. The air-phase resistance (which is the inverse of the air-phase mass transfer coefficient) on the other hand would vary substantially between the rat and human on account of the substantial interspecies

variations in airway geometry and airflow discussed earlier. Details of the boundary conditions for air flow and mass transfer, are provided in ([Kimbell et al., 1993](#); [Kimbell et al., 2001b](#); [Kimbell and Subramaniam, 2001](#)) and ([Subramaniam et al., 1998](#)).

For the rat, minute volumes were allometrically scaled to 0.288 L/minute for a 315 g rat ([Mauderly, 1986](#)), and simulations were carried out at the steady-state unidirectional inspiratory rate of 0.576 L/min. For the human, simulations were carried out at the steady-state unidirectional inspiratory rate of 15, 18, 50, and 100 L/min, corresponding to half of the values for the minute volumes associated with the activity patterns of sleeping, sitting, and light and heavy exercise, respectively ([ICRP, 1994](#)). Because formaldehyde is highly water soluble and reactive, ([Kimbell et al., 2001b](#)) assumed that uptake occurred only during inspiration. Thus, for each breath, flux into nasal passage walls (rate of mass transport in the direction perpendicular to the nasal wall per mm² of the wall surface) was assumed to be zero during exhalation, with no backpressure to uptake built up in the tissues. [Overton et al. \(2001\)](#) estimated the error due to this assumption to be small, roughly an underestimate of 3% in comparison to cyclic breathing. Inspiratory airflow was assumed to be constant in time (steady state). [Subramaniam et al. \(1998\)](#) considered this to be a reasonable assumption during resting breathing conditions based on a value of 0.02 obtained for the Strouhal number. Unsteady effects are insignificant when this number is much less than one. However, this assumption may not be reasonable for light and heavy exercise breathing scenarios.

[Kimbell et al. \(2001b\)](#) partitioned the nasal surface by flux to facilitate the use of local formaldehyde dose in dose-response modeling. Each of the resulting 20 “flux bins” was comprised of elements of the nasal surface that receive a particular interval of formaldehyde flux per ppm of exposure concentration ([Kimbell et al., 2001b](#)). These elements were not necessarily contiguous. The spatial coordinates of elements comprising a particular flux bin were fixed for all exposure concentrations, with formaldehyde flux (pmol/(mm²-hour) in a bin scaling linearly with exposure concentration (ppm), and therefore often expressed in terms of flux per ppm, that is, pmol/(mm²-hour-ppm).

Mass flux was estimated for the rat, monkey, and human over the entire nasal surface and over the portion of the nasal surface that was lined by nonsquamous epithelium (lateral wall mass flux shown in Figure C-9). Formaldehyde flux was also estimated for the rat and monkey over the areas where cell proliferation measurements were made ([Monticello et al., 1989](#); [Monticello et al., 1991](#)) and over the anterior portion of the human nasal passages that is lined by nonsquamous epithelium. Maximum flux estimates for the entire upper respiratory tract were located in the mucus-coated squamous epithelium on the dorsal aspect of the dorsal medial meatus near the boundary between nonmucus and mucus-coated squamous epithelium in the rat, at the anterior or rostral margin of the middle turbinate in the monkey, and in the nonsquamous epithelium on the proximal portion of the mid-septum near the boundary between squamous and nonsquamous epithelium in the human (see ([Kimbell et al., 2001a](#))) for tabulations of comparative estimates of formaldehyde flux across the species, for tabulations of comparative estimates of formaldehyde flux

across the species. The rat-to-monkey ratio of the highest site-specific fluxes in the two species was 0.98. In the rat, the incidence of formaldehyde-induced SCCs in chronically exposed animals was high in the anterior lateral meatus [ALM, ([Monticello et al., 1996](#))]. Flux (per ppm of inhaled concentration) at this site in the rat was similar to that predicted near the anterior or proximal aspect of the inferior turbinate and adjacent lateral walls and septum in the human, with a rat-to-human ratio of 0.84.

Formaldehyde Uptake in The Lower Respiratory Tract

Unlike the nasal passages, the human lower respiratory tract lends itself to a more simplified or idealized rendering. The one-dimensional (known as a “single-path” model) rendering of the human lung anatomy by Weibel ([1963](#)), which captures the geometry of the airways in an average or homogeneous sense for a given lung depth, is generally considered adequate unless the fluid dynamics at locations of airway bifurcations need to be explicitly modeled. Such an idealization of lung geometry has been successfully used in various models for the dosimetry of ozone and particulate and fibrous matter.⁸ The single-path model was used to calculate formaldehyde uptake in the human lower respiratory tract ([Overton et al., 2001](#); [CIIT, 1999](#)). These authors applied a one-dimensional equation of mass transport to each generation of an adult human symmetric, bifurcating Weibel-type respiratory tract anatomical model. In order to achieve consistency with the inhaled output from the CFD model of the upper respiratory tract in ([Subramaniam et al., 1998](#); [Overton et al., 2001](#)) augmented their model with an idealized upper respiratory tract and constrained their one-dimensional version of the nasal passages to have the same inspiratory air-flow rate and uptake during inspiration as the CFD simulations.

⁸Such idealized representations are likely to be inappropriate for considering susceptible individuals, such as those with chronic obstructive pulmonary disease.

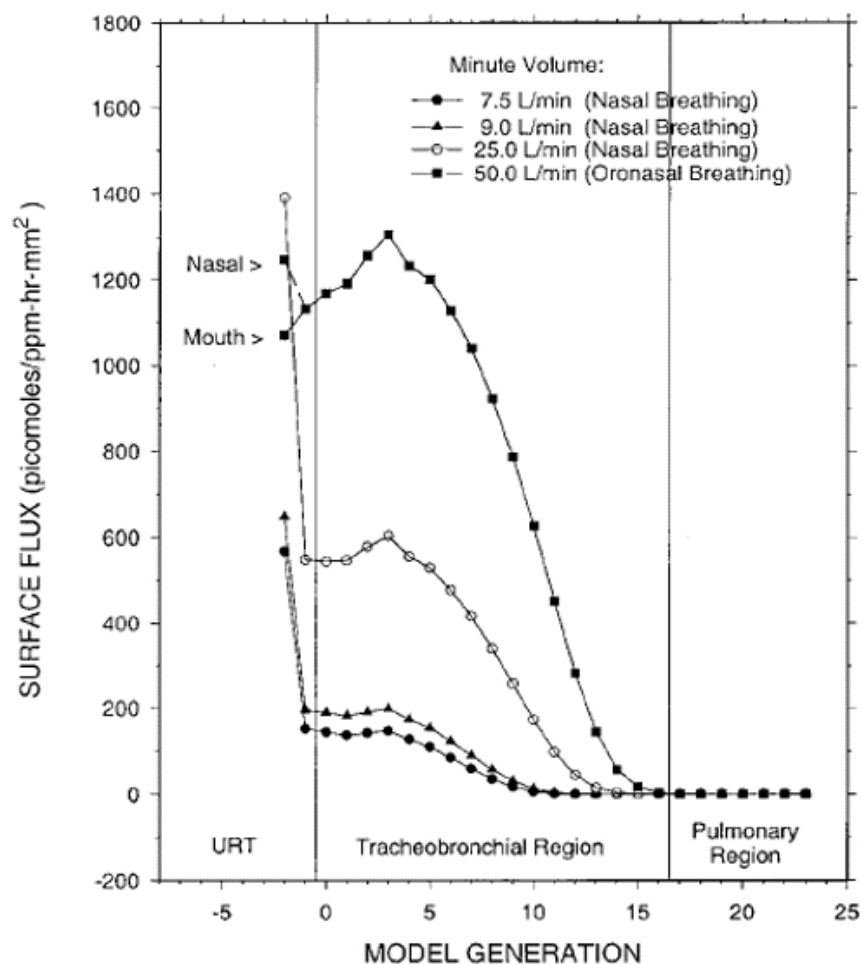


Figure C-11. Single-path model simulations of surface flux per ppm of formaldehyde exposure concentration in an adult male human.

Source: [Overton et al. \(2001\)](#).

The primary predictions (Figure C-11) of the model were: more than 95% of the inhaled formaldehyde is retained; formaldehyde flux in the lower respiratory tract increases for several lung airway generations relative to flux in posterior-most segment of the nose; with further increase in lung depth, formaldehyde flux decreases rapidly resulting in almost zero flux to the alveolar sacs. ([Overton et al., 2001](#)) also modeled uptake at high inspiratory rates. At a minute

volume of 50 L/minute⁹ formaldehyde flux in the mouth cavity is comparable (but a bit less) to that occurring in the nasal passages (Figure C-11).¹⁰

Level of confidence in formaldehyde uptake simulations

As mentioned earlier, the computational fluid dynamics simulations involved two steps, and the confidence in each step is addressed separately below.

Confidence in predicted airflow profiles

To verify the CFD simulations of nasal airflow profiles, the authors constructed physical models from the finite-element reconstructions used in the computational models. The simulated streamlines of steady-state inspiration airflow predicted by the CFD model agreed reasonably well with experimentally observed patterns of water-dye streams made in casts of the nasal passages for the rat and monkey as shown in panels A and B in Figure C-8. The airflow velocity predicted by CFD model simulations of the human also agreed well with measurements taken in hollow molds of the human nasal passages (panel C, Figure C-8) ([Subramaniam et al., 1998](#); [Kimbell et al., 1993](#); [Kimbell et al., 1997b](#); [Kepler et al., 1998](#)). However, the accuracy and relevance of these comparisons are limited. Because the airflow profiles were verified by only a simple video analysis of dye streak lines observed in the physical molds this method can be considered reasonable for only the major airflow streams. For the human, axial airflow velocities were also measured experimentally in a physical cast, and these compared well with CFD simulations (panel C, Figure C-8). However, the physical model used for the velocity measurements corresponds to that of a different individual than the one for which the CFD simulations were carried out.

Another verification comes from measuring pressure gradients across the nasal cavity. Plots of pressure drop versus volumetric airflow rate predicted by the CFD simulations compared well with measurements made in rats in vivo ([Gerde et al., 1991](#)) and in acrylic casts of the rat nasal airways ([Cheng et al., 1990](#)) as shown in Figure C-12. This latter comparison remains qualitative due to differences among the simulation and experiments as to where the outlet pressure was measured and because no tubing attachments or other experimental apparatus were included in the simulation geometry. The simulated pressure drop values were somewhat lower, possibly due to these differences.

⁹ Note: the oronasal switch occurs at about 35 L/min ([Niinimaa et al., 1981](#)).

¹⁰ Mouth breathers form a large segment of the population. Furthermore, at concentrations of formaldehyde where either odor or sensory irritation becomes a significant factor, humans are likely to switch to mouth breathing even at resting inspiration. ([Overton et al., 2001](#)) did not model uptake in the oral cavity at minute volumes less than 50 L/min. However, since 0.55 of the inspired fraction is through the mouth for the normal nasal breathing population ([Niinimaa et al., 1981](#)) at an inspiratory rate of 50 L/min, we can make an indirect inference from their result at this heavy breathing rate that average flux across the human mouth lining would be comparable to the average flux across the nasal lining computed in ([Kimbell et al., 2001b](#); [Kimbell and Subramaniam, 2001](#)) for mouth breathing conditions at resting or light exercise inspiratory rates.

[Kimbell et al. \(2001a\)](#) examined the extent to which their results were subject to errors in mass balance and applied ad-hoc corrections to compensate for these errors. Because airflow and uptake were simulated separately, they each contributed separately to the mass balance error; however, the error component due to airflow was minimal (< 0.4%). The percent overall uptake of formaldehyde was defined as $100\% \times (\text{mass entering nostril} - \text{mass exiting outlet}) / (\text{mass entering nostril})$, and its mass balance error was calculated as $100\% \times (\text{mass entering nostril} - \text{mass absorbed by airway walls} - \text{mass exiting outlet}) / (\text{mass entering nostril})$. For the rat, monkey, and human the mass balance errors associated with simulated formaldehyde uptake from air into tissue were less than 14% at resting minute volumes, and therefore, not a major concern, but these errors increased to 27% at the highest human inspiratory rate corresponding to exercise conditions. Kimbell ([2001a](#)) corrected for these errors by evenly distributing the lost mass over the entire nasal surface in their simulation results.

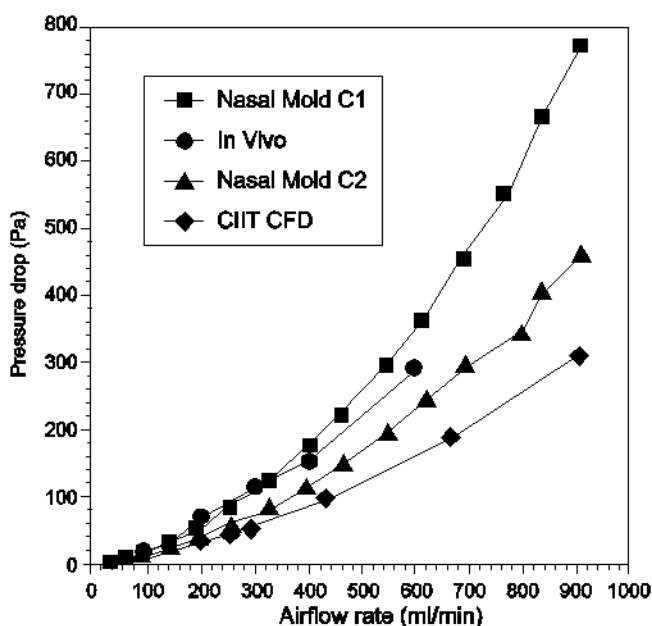


Figure C-12. Pressure drop versus volumetric airflow rate predicted by the CIIT CFD model compared with pressure drop measurements made in two hollow molds (C1 and C2) of the rat nasal passage ([Cheng et al., 1990](#)) or in rats in vivo ([Gerde et al., 1991](#)).

Source: [Kimbell et al. \(1997b\)](#).

Confidence in modeled flux estimates

Unlike the verification of the airflow simulations, it was not possible to evaluate the regional formaldehyde flux calculations directly; however, there are several indirect qualitative and quantitative lines of evidence that provide general confidence in the flux profiles predicted by

([Kimbell et al., 2001b](#); [Kimbell and Subramaniam, 2001](#)) for the F344 rat nasal passages when the flux is averaged over gross regions of the nasal lining. This evidence is listed below.

In [Kimbell et al. \(2001b\)](#), the tissue-phase mass-transfer boundary conditions were set by fitting overall (whole nose) formaldehyde uptake at various exposure concentrations to the experimental data in [Morgan et al. \(1986a\)](#). Since this was the only data set available, it was not possible to independently verify the model results for overall uptake. However, results from earlier work by ([Kimbell et al., 1993](#)) are informative for this purpose because in this case the model was not calibrated by fitting model predictions to experimental data; instead, this model assumed an infinite sink for absorption at the nasal lining on account of the highly reactive and soluble nature of formaldehyde. [Kimbell et al. \(1993\)](#) predict 99% uptake of inhaled formaldehyde in the rat nose, which is slightly above the upper end of the range of 91–98% observed by ([Morgan et al., 1986a](#)). The utility of those simulations is however limited because the posterior portion of the nose was not included in the model, and the assumption of infinitely absorbing nasal walls makes the boundary condition less realistic than that used in ([Kimbell et al., 2001b](#)). Calculations based upon ([Kimbell et al., 1993](#)) are compared with various experimental observations below.

[Morgan et al. \(1991\)](#) showed general qualitative correspondence between the main routes of flow and lesion distribution induced by formaldehyde in the rat nose and hypothesized that the localized nature of the lesions must be related to the regional uptake of formaldehyde. This was borne out by ([Kimbell et al., 1993](#)) who described similarities in patterns of computed regional mass flux and lesion distribution due to formaldehyde. These authors reported on correlations in patterns in the coronal section immediately posterior to the vestibular region (as discussed earlier, the vestibular region is protected by keratinized epithelium and is therefore not likely to significantly absorb formaldehyde); simulated flux levels over regions where lesions were seen, such as the medial aspect of the maxilloturbinate and the adjacent septum, were an order of magnitude higher than over other regions where lesions were not seen, such as the nasoturbinate.¹¹

A reasonable level of confidence in flux predictions by ([Kimbell et al., 1993](#)) is also attained indirectly by comparing experimental data on formaldehyde-DPX concentration in the F344 rat with modeled results in ([Cohen Hubal et al., 1997](#)); these authors used flux estimates generated by the CFD model in ([Kimbell et al., 1993](#)) in a physiologically based pharmacokinetic (PBPK) model for formaldehyde-DPX concentration in the F344 rat. This hybrid CFD-PBPK model was calibrated by optimizing model predictions of DPX concentrations against DPX collected over the entire nose in separate experiments by ([Casanova et al., 1989](#); [Casanova et al., 1991](#)) on F344 rat noses exposed to formaldehyde at 0.3, 0.7, 2.0, 6.0, and 10 ppm. The nasal regions were then separated into two

¹¹This 1993 CFD model differed somewhat from the subsequent model by ([Kimbell et al., 2001b](#)) used in this assessment. In the 1993 model, the limiting mass-transfer resistance for the gas was assumed to be in the air phase; that is, the concentration of formaldehyde was set to zero at the airway lining. Furthermore, this same boundary condition was used on the nasal vestibule as well, while in the more recent model, the vestibule was considered to be nonabsorbing. Unfortunately, ([Kimbell et al., 2001b](#)) did not report on correspondences between flux patterns and lesion distribution.

categories depending upon whether tumor incidence was high or low in a region, and model predictions of DPX concentrations were compared with the experimental data considered only from the high-tumor region, including additional DPX data from the high-tumor region at 15-ppm exposure concentration which had not been used in model calibration. The predictions are seen to compare well with experimental values (Figure C-13). Such a comparison is not available for the simulation of uptake patterns in the human.

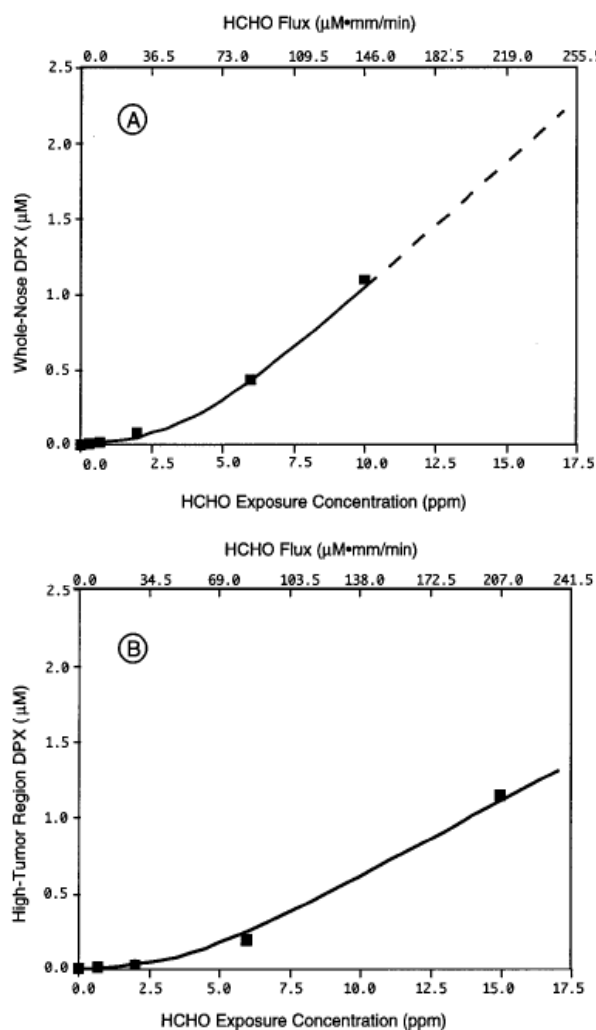


Figure C-13. Formaldehyde-DPX dosimetry in the F344 rat.

Panel A: calibration of the PBPK model using data from high and low tumor incidence sites. Panel B: model prediction compared against data from a high tumor incidence site. Dashed line in panel A shows the extrapolation outside the range of the calibrated data.

Source: [Cohen Hubal et al. \(1997\)](#).

Effect of reflex bradypnea on dosimetry

A source of uncertainty in the modeled human flux estimates arises because the value of the tissue-phase mass-transfer coefficient used as a boundary condition in human simulations is the same as that obtained from calibration of the rat model. As explained earlier, qualitatively this appears reasonable; however, EPA is unable to quantitatively evaluate the impact of this uncertainty.

The CFD simulations do not model reflex bradypnea, a protective reflex observed in rodents. As discussed at length in Appendix C-2, it is reasonable to expect a range of 25% ([Chang et al., 1983](#)) to 45% ([Barrow et al., 1983](#)) decrease in minute volume in F344 rats at the exposure concentration of 15 ppm. Explicit omission of this effect in the modeling is, however, not likely to be a source of major uncertainty in the modeled results for uptake of formaldehyde in the rat nose for the following reason: the CFD model for the F344 rat was calibrated to fit the overall experimental results for formaldehyde uptake in the F344 rat at a 15 ppm exposure concentration by adjusting the mass transfer coefficient used as boundary condition on the absorbing portion of the nasal lining. Thus, any reflex bradypnea occurring in those experimental animals is implicitly factored into the value used for the boundary condition. Nonetheless, some error in the localized distribution of uptake patterns may be expected, even if the overall uptake is reproduced correctly.

Modeling Interindividual Variability in the Nasal Dosimetry of Reactive and Soluble Gases

[Lorenti Garcia et al. \(2009\)](#) used computational fluid dynamics to study human variability in the nasal dosimetry of reactive, water-soluble gases in 5 adults and 2 children, aged 7 and 8 years. The authors considered two model categories of gases, corresponding to maximal and moderate absorption at the nasal lining. We focus here only on the “maximal uptake” simulations in ([Lorenti Garcia et al., 2009](#)); note that this term for the simulations does not correspond to regions of maximum flux but rather characterizes the gas category. In this case, the gas was considered so highly reactive and soluble that it was reasonable to assume an infinitely fast reaction of the absorbed gas with compounds in the airway lining. Although such a gas could be reasonably considered as a proxy for formaldehyde, these results cannot be fully utilized to inform quantitative estimates of formaldehyde dosimetry (and does not appear to have been the intent of the authors either). This is because the same boundary condition corresponding to maximal uptake was applied on the vestibular lining of the nose as well as on the respiratory and transitional epithelial lining on the rest of the nose. This is not appropriate for formaldehyde as the lining on the nasal vestibule is made of keratinized epithelium which is considerably less absorbing than the rest of the nose ([Kimbell et al., 2001a](#)).

[Lorenti Garcia et al. \(2009\)](#) concluded that overall uptake efficiency, and average and maximum flux levels over the entire nasal lining did not vary substantially between adults (1.6-fold difference in average flux and much less in maximum flux), and the mean values of these quantities were comparable between adults and children. These results are also in agreement with

conclusions reached by ([Ginsberg et al., 2005](#)) that overall extrathoracic absorption of highly and moderately reactive and soluble gases [corresponding to Category 1 and 2 reactive gases as per the scheme in ([U.S. EPA, 1994](#))] is similar in adults and children. On the other hand ([Lorenti Garcia et al., 2009](#)) state that their models predicted significant interhuman variability in flux levels at specific points on the nasal wall; Figure 6A of their paper (reproduced here as Figure C-14) indicates a 3- to 5-fold difference among the individuals in the study when flux was plotted as a function of distance from the nostrils normalized by the length of the septum. This observation needs to be accompanied by a caveat: because similar fluxes may correspond to different regions in individuals, it is possible that this spread in values overestimates the actual variability in local flux in these individuals.

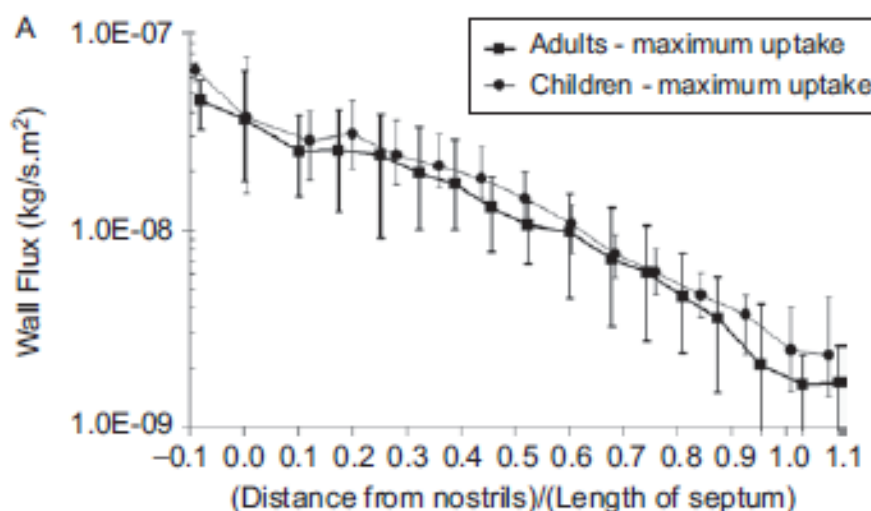


Figure C-14. Flux of highly reactive gas across the nasal lining as a function of normalized distance from the nostril for 5 adults and 2 children.

While the sample size in this study is too small to consider the results representative of the population as a whole, various comparisons with the characteristics of other study populations add to the strength of this study; for example, the surface area to volume ratio among the five adults ranged from 0.87 to 1.12 mm⁻¹ which compared well with a result of 1.05 ± 0.23 obtained from measurements in 40 adult Caucasians ([Yokley, 2009](#)), and the surface area ranged from 16,683 to 23,219 cm² which compared well with a result of $18,300 \pm 2,200$ cm² obtained from measurements in 45 adults ([Guilmette et al., 1997](#)). It is useful to note here that the nasal anatomy reconstructed for modeling the dosimetry of formaldehyde in the human nose in ([Kimbell et al., 2001b](#); [Kimbell and Subramaniam, 2001](#)) and discussed earlier was that of one of the individuals in the ([Lorenti Garcia et al., 2009](#)) study.

Models Estimating the Effects of Endogenous Formaldehyde on Dosimetry Predictions in Nasal Tissues

[Schroeter et al. \(2014\)](#) developed a hybrid toxicokinetic fluid dynamic model for predicting the uptake of inhaled formaldehyde that incorporates the production of endogenous formaldehyde in nasal tissue. EPA reviewed their calculations in detail. In this model, the predicted formation of endogenous formaldehyde leads to a baseline tissue level of formaldehyde in the nasal tissue. When formaldehyde is also inhaled, absorbed formaldehyde, along with endogenous formaldehyde, contributes to increasing predicted nasal tissue levels of formaldehyde (see Figure 4 of their paper).

However, these authors also interpret their results as showing a decrease in "percent net nasal uptake" of inhaled formaldehyde at low concentrations. EPA reviewed these calculations and finds them nontransparent and confusing to the reader. Due to endogenous formaldehyde production, the model of Schroeter et al. (2014) predicts a net desorption of formaldehyde at zero exposure. When formaldehyde is inhaled, the exhaled breath will represent the joint contributions of exhaled endogenous formaldehyde and an unabsorbed fraction of inhaled formaldehyde. Thus, what the authors term "percent net nasal uptake" [calculated as (inhaled formaldehyde - exhaled formaldehyde)/inhaled formaldehyde] conflates absorption of inhaled formaldehyde and release of endogenous formaldehyde. A more transparent understanding of their results can be obtained from Table 5 of the paper which provides estimates of formaldehyde flux into nasal tissue at different concentrations of inhaled formaldehyde. As an example, EPA examined the values shown for average tissue flux for humans. EPA found that for concentrations 0.1 ppm and below, the average human flux estimates (pmol/mm²-hr), were precisely linear with exposure concentrations (ppm):

Flux = -a + b×Conc, where a=1.54 pmol/mm²-hr, b=1,500 pmol/mm²-hr-ppm.

This can be understood as a positive contribution of inhaled formaldehyde in the amount of 1,500 pmol/mm²-hr per ppm superimposed on a negative (-1.54) flux out of the tissue due to loss of endogenously formed formaldehyde and is just the pattern that would be expected when the absorbed amount of inhaled formaldehyde is constant in the lower concentration range. Similar relationships can be fit to their results for other species as well as predictions for maximum flux rates. Schroeter et al. (2014) plotted flux versus exposure concentration on a log-log scale; EPA notes that the apparent "non-linearities" at low concentrations seen in Figure 5 of that paper are to be expected when a relationship with an intercept is plotted in this manner. In summary, any external exposure is predicted to cause some increase in the tissue concentration in this model, since a nonzero air concentration produces positive flux in the opposite direction of the efflux of endogenous formaldehyde. Therefore, the modeling results do not lead to an inference of nonlinearities in the flux of inhaled FA into nasal tissue at low concentrations. While the analysis of ([Schroeter et al., 2014](#)) represents an important first step towards incorporating the presence of endogenous formaldehyde into models estimating the flux (or uptake) of inhaled formaldehyde, several uncertainties in the underlying assumptions have yet to be addressed:

Supplemental Information—Formaldehyde (Inhalation)

- The tissue levels of formaldehyde predicted by the model of ([Schroeter et al., 2014](#)) appear to be orders of magnitude in excess of the levels that would be consistent with the observed DPX levels ([Heck et al., 1983](#)) and formaldehyde-DNA binding rate ([Heck and Keller, 1988](#)).
- Endogenous formaldehyde levels were calculated based on blood concentrations. But ([Heck et al., 1982](#)) measured 12.6 µg/g total formaldehyde in rat nasal tissues and only 2.24 µg/g in rat blood ([Heck et al., 1985](#)). Based on DNA-adduct measurements, it appears that the majority of formaldehyde is bound to GSH in a manner that reduces its interaction with DNA and, presumably, other key macromolecules (see Appendix C.1.4). The extent of GSH-binding could significantly reduce diffusion across the epithelial cell membrane (i.e., between blood and nasal tissue), in which case blood concentrations may not correlate well with tissue concentrations.
- Since nasal tissue levels of formaldehyde are higher than blood levels, it is likely that these levels are produced by endogenous metabolism in situ, rather than entering the mucosa via diffusion from a “blood” layer at a specific depth from the mucosa-air surface, the latter being the assumption used by ([Schroeter et al., 2014](#)).
- While ([Schroeter et al., 2014](#)) did not report exhaled breath levels, their results indicate that uptake will exactly balance desorption in humans at about 1.23 µg/m³ (1.0 ppb), from which one might assume this is the level their model would predict in exhaled breath. In the study of ([Riess et al., 2010](#)), exhaled breath levels for nonsmokers were found to be below a detection limit of 0.62 µg/m³, which corresponds to 0.5 ppb at 20°C. While this is within a factor of two, an acceptable level of error for such an extrapolation, it is a further indication that the assumed level of free endogenous formaldehyde in the ([Schroeter et al., 2014](#)) model is too high.

Despite these limitations, the efforts by ([Schroeter et al., 2014](#)) highlight the fact that at sufficiently low levels of exogenous formaldehyde, the contribution of endogenous formaldehyde could become significant; accounting for this contribution would address a critical uncertainty for interpreting the uptake of inhaled formaldehyde. Additional studies addressing the potential contribution of endogenous formaldehyde are warranted. As a sensitivity analysis, EPA notes in Section 5.2.1 that the unit risk estimate for nasal cancers based on rat studies are not appreciably altered if calculated using the revised formaldehyde estimates from ([Schroeter et al., 2014](#)).

[Campbell Jr et al. \(2020\)](#) modified the original model by ([Andersen et al., 2010](#)) using exogenous and endogenous formaldehyde adduct data from ([Leng et al., 2019](#)) (28-day study of 6 hours/day exposures), ([Yu et al., 2015b](#)) (28-day study of 6 hrs/day exposures), and ([Lu et al., 2010a](#); [Lu et al., 2011](#)) (a single 6-hour exposure). The following major changes were made to the original model:

- a) The model simulates observed data for formaldehyde-induced DNA mono-adducts (N2-hydroxymethyl-dG). The previous models simulated formaldehyde-induced DNA-protein cross-links (DPX).
- b) A zero-order term (VMMUC) was used to account for tissue clearance of inhaled formaldehyde. This is a restriction on uptake from the air phase to the tissue compartment.

- c) The rate of production of endogenous formaldehyde (K_p) was increased to nearly double the original rate set by ([Andersen et al., 2010](#)). The maximum rate of formaldehyde oxidase metabolism (V_{max}) was increased by over a factor of 10.

There are some notable observations from the data used in the modeling. [Leng et al. \(2019\)](#) showed no exogenous formaldehyde-induced DNA adducts in the nose at concentrations up to 0.3 ppm and no increase in endogenous formaldehyde-induced DNA adducts up to 0.3 ppm. ([Lu et al., 2010a](#); [Lu et al., 2011](#)) observed an increase in exogenous formaldehyde adducts in rat nasal tissue starting at 0.7 ppm but no increase in endogenous adducts between 0.7 ppm–15 ppm (although there does appear to be a perturbation in the mean and variance of endogenous adducts in this range). The data at and above 0.7 ppm was used to re-optimize the cellular metabolic parameters. The data up to 0.3 ppm by ([Leng et al., 2019](#)) (which did not observe increased adducts) was used to visually optimize the parameter defining the lower limit on uptake (VMMUC). Because of the abrupt change in observed adduct levels between 0.3 ppm and 0.7 ppm there is model uncertainty within that concentration range and below the limit of detection.

Key results from this work add to our characterization of uncertainties related to endogenous formaldehyde levels and formaldehyde dose-response at low exposures. First, the model estimated a non-zero value for VMMUC, indicating that the inhalation rate must exceed the tissue clearance rate for formaldehyde to be absorbed by the tissue. The model was calibrated with the restriction that formaldehyde absorption in the nose occurs only at exposure concentrations above 0.3 ppm in the rat. Secondly, ([Campbell Jr et al., 2020](#)) assessed steady-state concentration of free endogenous formaldehyde to be 20 times lower than the value determined experimentally by ([Heck et al., 1982](#)) and 15 times lower than assessed by ([Andersen et al., 2010](#)). In ([Campbell Jr et al., 2020](#)), the estimate for free endogenous levels decreased from 0.31 mM to 0.020 mM and the basal concentration of endogenous formaldehyde bound to sulfhydryl increased from 0.057 to 0.12mM (2 times higher). [Campbell Jr et al. \(2020\)](#) attributed this discrepancy to the potential for the ([Heck et al., 1982](#)) measurement methodology to overestimate tissue formaldehyde levels.

The original model ([Andersen et al., 2010](#)) did not adequately fit these new data, and ([Campbell Jr et al., 2020](#)) justified changes to the ([Andersen et al., 2010](#)) model parameters for cellular metabolism on the grounds that data from ([Heck et al., 1982](#)) are biased due to the method used to measure tissue formaldehyde. However, it is possible that the cause of this model/data discrepancy is inadequate model structure rather than a bias in the original data. As a result, there is inherent model uncertainty in the revised model for cellular metabolism.

Extrapolation of results in ([Campbell Jr et al., 2020](#)) to humans is not possible because the data and the model are specific to rats.

C.2. REFLEX BRADYPNEA

Reflex bradypnea (RB) is a protective reflex that allows laboratory rodents to minimize their exposure to upper respiratory tract (URT) irritants such as aldehydes, ammonia, isocyanates,

and pyrethroids ([Gordon et al., 2008](#)). This reflex is initiated by stimulation of trigeminal nerve endings in the mucosa of the URT and the eyes. It is associated with the chemosensitive part of the nociceptive system—the common chemical sense that detects noxious airborne exposures ([Nielsen, 1991](#)).

C.2.1. The signs of reflex bradypnea

RB is manifest by immediate decreases in the metabolic rate, CO₂ production, and demand for oxygen. This is followed by rapid decreases in body temperature (i.e., hypothermia; as much as 11°C in rats and 14°C in mice; Figure C-15), activity, heart rate, blood pressure, respiratory rate (breaths/minute; Figure C-16), and minute volume (see Figure C-17). RB also results in decreased blood *p*O₂ and *p*CO₂ and increased blood pH ([Pauluhn, 2008, 2018](#); [OECD, 2009](#); [Jaeger and Gearhart, 1982](#); [Gordon et al., 2008](#); [Chang and Barrow, 1984](#)). Thus, the physiological effects and signs of RB may be misinterpreted as, for example, chemical-induced behavioral or developmental effects.

RB is regulated by a complex feedback response ([Yokley, 2012](#)). [Gordon et al. \(2008\)](#) demonstrated that the extent of RB depends on the concentration of the irritant (see Figure C-15). For example, after several hours of exposure to an isocyanate, mice exhibited concentration-dependent changes with those in the high concentration group presenting a mean body temperature of 23°C and approximately 90% decreases in respiratory rate and minute volume.

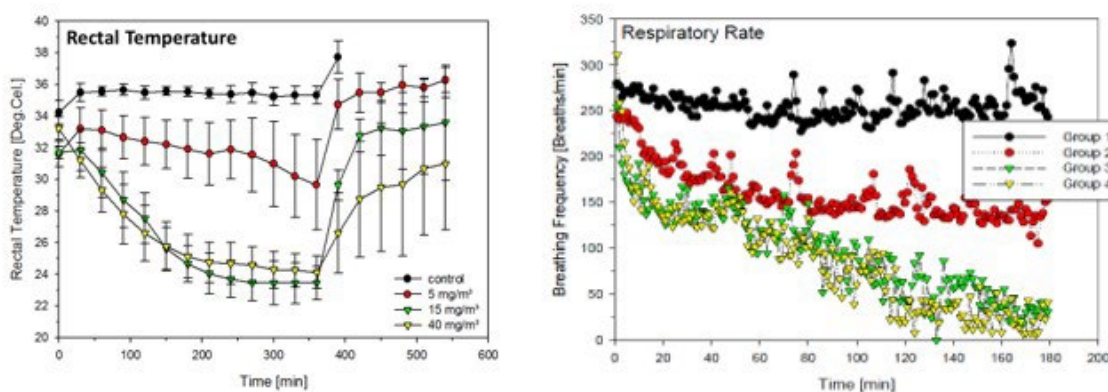


Figure C-15. Signs of Reflex Bradypnea. Left Panel: Concentration-related hypothermia in mice exposed to an isocyanate for 360 minutes. Note the gradual recovery in body temperature after exposure ceased. Right panel: Concentration-related decreases in respiratory rate in mice exposed to an isocyanate. Note the correlation between the curves for rectal temperature and respiratory rate over the course of 180 minutes.

Source: [Gordon et al. \(2008\)](#).

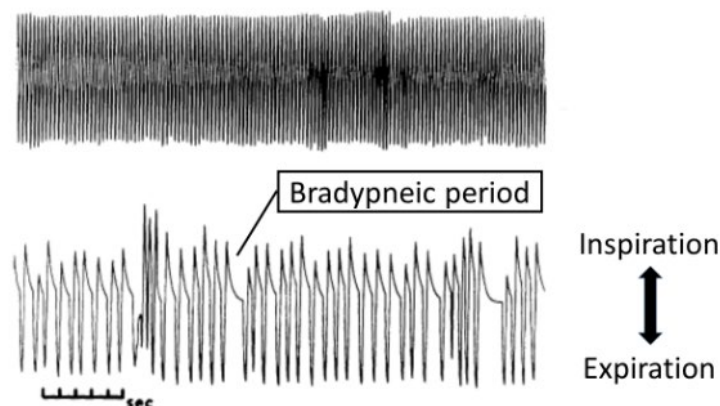


Figure C-16. An oscillograph that compares the respiratory cycle for mice exposed to an URT irritant (lower tracing) to an air control group (upper tracing). The exposed animals have a characteristic pause before exhaling—a bradypneic period—which results in a net decrease in the respiratory rate (breaths/minute). Because the exposed group has a slightly greater tidal volume (height of the tracings) but a much lower respiratory rate, the net result is a lower minute volume and reduced exposure to the irritant.

Source: [Kane and Alarie \(1977\)](#).

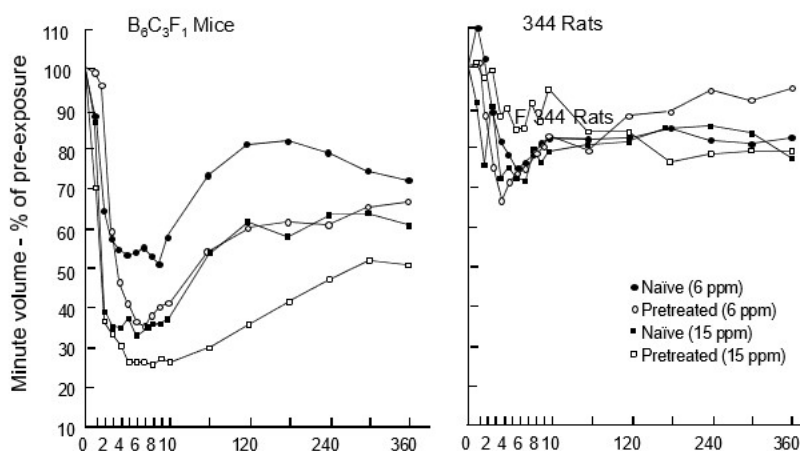


Figure C-17. Formaldehyde effects on minute volume in naïve and formaldehyde-pretreated male B6C3F1 mice and F344 rats. Pretreated animals were exposed to 6.9 or 17.6 mg/m³ formaldehyde 6 hrs/d for 4 d. Note that the mice had a greater response than the rats, and the pretreated animals had a greater response than the naïve animals.

Source: Redrawn from [Chang et al. \(1983\)](#).

Figure C-17 demonstrates that the onset of RB after formaldehyde inhalation is immediate, with a marked decrease in minute volume in mice and rats minutes after exposure begins. Because

reduced respiration lessens exposure to an irritating chemical, the toxicity is reduced and the animal's survival is enhanced. This is important for the survival of rodents living in burrows and confined spaces that may not be able to avoid exposure. Figure C-15 (left panel) demonstrates that the effects of RB are reversible, but it can take several minutes to several hours for all physiological parameters to return to preexposure conditions, depending on the extent of hypothermia ([Pauluhn, 2008, 2018](#); [OECD, 2009](#); [Jaeger and Gearhart, 1982](#); [Barrow et al., 1983](#)).

The physiological signs of RB in rodents can be striking, but they are not signs of toxicity and, as such, are not considered appropriate for defining an animal POD. Also, the signs of RB are not relevant to humans since humans cannot experience RB. RB can only occur in small animals such as mice and rats that can, because of their small size, rapidly lower their core body temperatures when their metabolic rate reflexively decreases. Even a mild decrease in body temperature can lessen the toxicity and metabolic activation of many chemicals, but it can also slow the excretion of toxicants. Overall, the protection from cellular toxicity afforded by RB-induced hypothermia outweighs the undesirable effect of a slower excretion rate ([Gordon et al., 2008](#)). Even though RB has been reported in the literature since the 1960s, it is largely unknown to most toxicologists. None of the rodent inhalation studies of formaldehyde, except for a few RB-specific studies, attempted to identify or measure RB, including measures of body temperature and respiration. As RB likely occurred in most, if not all, rodent inhalation toxicity studies involving high level exposures to formaldehyde, this uncertainty is acknowledged and discussed in the assessment, and for particular health outcomes it is specifically considered during study evaluation (e.g., see description below regarding behavioral effects, since RB can affect activity).

C.2.2. Irritation, reflex bradypnea, and the RD₅₀

A test for assessing sensory irritation was developed by Yves Alarie in the 1960s. In an Alarie test, rodent respiration is measured before, during, and after exposure to one or more concentrations of an irritant, and then respiratory depression (RD) is statistically quantified. RD is followed by a subscript that gives the percentage of respiratory depression (e.g., RD₀, RD₂₀, RD₅₀, RD₇₀, etc.) The most commonly reported value in Alarie tests is the RD₅₀—the concentration of an irritating chemical that causes a 50% depression in the respiratory rate ([Kane et al., 1979](#)).

“Irritation” refers to two distinct processes. The first process is sensory irritation of nerve endings. URT irritation of the trigeminal nerve, which humans perceive as a burning or stinging sensation, is what triggers RB in rodents. The second process relates to an inflammatory response elicited by an irritating chemical, which is manifested by histopathologic changes such as local redness, edema, pruritus, and cellular alterations. Sensory irritation may prevent histopathologic damage through avoidance or through RB in rodents. [Bos et al. \(2002\)](#) found no correlation between chemical concentrations that cause sensory irritation (as measured by the Alarie test) and concentrations that induce histopathological changes. For a variety of irritants, the lowest concentration that induces nasal histopathologic lesions can range from 0.3 times the RD₅₀ to more than 3 times the RD₅₀.

Alarie tests are useful for (1) identifying chemicals which are URT sensory irritants, (2) quantifying irritating concentrations, and (3) ranking chemicals for their irritancy potential. [Alarie \(1981\)](#) proposed using 0.03 times the RD₅₀ values to predict threshold limit values (TLVs: typically used to define workplace exposures that can be repeatedly encountered without adverse effects) for a variety of irritants. More recently, [Nielsen et al. \(2007\)](#) proposed the use of animal RD₅₀ and RD₀ values along with human data in a weight-of-evidence approach to predict acute or short-term TLVs, the RD₀ being a threshold or NOEL for decreased respiratory rate.

Tables C-12 and C-13 present formaldehyde RD values from several Alarie studies for mice and rats, respectively.¹² No RD values exist for female mice or rats. Across the literature, there is fairly good agreement on RD₅₀ values for various strains of mice:

Table C-12. Formaldehyde respiratory depression (RD) values for several mouse strains and exposure durations

Study	Mouse strain	Exposure (min)	RD ₅₀ (mg/m ³)	RD ₁₀ (mg/m ³)	RD ₀ (mg/m ³)
Kane and Alarie (1977)	♂ Swiss-Webster	10	3.8	0.5 ^a	0.31 ^a
Nielsen et al. (1999)	♂ BALB/c	10	4.9	0.4	
Barrow et al. (1983)	♂ B6C3F1	10	5.4	0.9*	0.49*
Chang et al. (1981)	♂ B6C3F1	10	6.0	–	–
de Ceaurriz et al. (1981)	♂ Swiss OF ₁	5	6.5	–	–

^aValue derived from a graph.

Figure C-17 shows that rats are less responsive to URT irritants than mice, which is why rats have higher RD₅₀ values than mice.

Table C-13. Formaldehyde respiratory depression (RD) values for several rat strains and exposure durations.

Study	Rat strain	Exposure (min)	RD ₅₀ (mg/m ³)	RD ₁₀ (mg/m ³)	RD ₀ (mg/m ³)
Casseo et al. (1996a)	♂ Wistar	30	12.3	–	–
Barrow et al. (1983)	♂ F-344	10	16.1	1.2 ^a	–
Gardner et al. (1985)	♂ Crl-CD	15	17.0	–	–
Chang et al. (1981)	♂ F-344	10	39.0	–	–

^aValue derived from a graph.

¹²Several studies cited in Tables C-12 and C-13 tested formalin, which means the animals were co-exposed to formaldehyde and methanol. Considering that methanol's mouse RD₅₀ of 54,963 mg/m³ (41,514 ppm) is 10,000 times greater than formaldehyde's mouse RD₅₀, methanol was likely to have a negligible impact on the formaldehyde RD values ([Nielsen et al., 2007](#)).

C.2.3. Tolerance

Nearly all rodent studies that assessed RB are acute Alarie tests lasting no more than a few minutes or hours. There are no long-term studies that investigated whether-or-when rodents develop a tolerance to formaldehyde or other irritants and eventually begin to breathe normally. Mouse studies are a particular concern because mice have a greater RB response than rats and are able to sustain bradypnea and hypothermia for a longer period than rats. The bulleted short-term (4 days to 4 weeks) studies below examined the potential for rodents to develop tolerance to formaldehyde and cyfluthrin. The formaldehyde studies show no sign of tolerance over 10 days of exposure at concentrations as high as 18 mg/m³, but what happens after 10 days remains unknown.

- [Kane and Alarie \(1977\)](#) observed a progressive decrease in respiratory rate (i.e., a progressively greater RB response) over 4 days of formaldehyde exposure in Swiss-Webster mice exposed to an RD₅₀ of 3.8 mg/m³. A similar lack of tolerance was also seen in mice exposed to acrolein (an aldehyde) at an RD₅₀ of 3.9 mg/m³.
- [Chang et al. \(1983\)](#) exposed mice and rats to 6.9 or 17.6 mg/m³ formaldehyde (two of the concentrations used in the Battelle carcinogenicity study) 6 hours/day for 4 days. On day 4, both mice and rats showed concentration-related decreases in respiratory rate and minute volume, but the decreases in mice were markedly greater (see Figure A-20).
- [Chang and Barrow \(1984\)](#) observed no tolerance in F-344 rats exposed to 18 mg/m³ formaldehyde for 10 days. Tolerance was observed in rats exposed over 4 days to a very high formaldehyde concentration of 34 mg/m³, likely due to destruction or downregulation of sensory trigeminal nerve endings or receptors, respectively.
- [Pauluhn \(1998\)](#) exposed Wistar rats 6 hours/day, 5 days/week for 4 weeks to cyfluthrin, a pyrethroid URT irritant, at the acute RD₅₀ concentration of 47 mg/m³. Mean decreases in respiratory rate were 45% at week 2 and 55% at week 4, that is, there was no sign of tolerance. Since formaldehyde and cyfluthrin are both URT irritants, it is likely that similar results might be seen with formaldehyde.

C.2.4. Reflex bradypnea and interpreting health effects data

Current testing guidelines do not require examination of RB-related endpoints and reduced inhaled rodent exposure may complicate interpretations regarding inferences of potential human risk. For example, Battelle's carcinogenicity study illustrates an apparent role of RB in long-term studies. The study authors observed a disparity in formaldehyde-induced squamous metaplasia and inflammation between B6C3F1 mice and F-344 rats. Both species were identically exposed in whole-body chambers at analytical concentrations of 0, 2.5, 6.9, or 17.6 mg/m³. At comparable concentrations, nasal lesions were much less severe in mice than in rats. In fact, incidences of squamous cell carcinoma were similar in rats exposed at 6.9 mg/m³ and in mice exposed at 17.6 mg/m³—a difference in concentration of more than 2-fold ([Kerns et al., 1983](#)). Kerns et al. reasoned this 2-fold difference between mice and rats may be due to “their physiological responses to

formaldehyde inhalation,” that is, due to RB. To support their hypothesis, they cited a 4-day Alarie test by ([Chang et al., 1983](#)) described in the bullet above, in which the reduction in minute volume was 2-fold greater in mice than in rats when exposed at 17.6 mg/m³ (see Figure C-17). In other words, the rats exposed at 6.9 mg/m³ and the mice exposed at 17.6 mg/m³ may have had similar lesion incidences because they were exposed to approximately the same inhaled “dose” of formaldehyde due to RB.

The hypothesis offered by ([Kerns et al., 1983](#)) that mice in the Battelle study inhaled about half as much formaldehyde as rats at 17.6 mg/m³ due to RB is logical and compelling, but there are no long-term RB data to support it at this time. Thus, although it might be considered appropriate to adjust a rodent POD to account for potential decreases in respiration (thus inferring that use of the exposure levels and corresponding results of that study may not be health protective for humans), this approach was not applied in this assessment. Overall, the lack of a long-term study to determine whether-or when rodents eventually develop tolerance to formaldehyde or any other URT irritant represents a significant data gap.

The potential impact of reflex bradypnea on behavioral studies

The normal physiological effects of RB can complicate the interpretation of behavioral studies in rodents. Hypothermia causes reduced peripheral nerve conduction velocity due to an apparent reduced flux of potassium and chloride ions across axon membranes. Hypothermia also causes prolonged synaptic delay time at neuromuscular junctions. A progressive decrease in body temperature results in ataxia, loss of fine motor control and reflexes, a reduction in cerebral blood flow and brain function, and eventually a loss of consciousness ([OECD, 2009](#); [Mallet, 2002](#)). Thus, what appear to be chemically induced behavioral effects may actually be partly attributable to RB-induced hypothermia. Thus, the irritant effects were considered during evaluations of behavioral studies (see Appendix B.2.7), including a preference for studies that allowed for a recovery time of at least 2 hours after exposure before testing, given the recovery parameters discussed above.

The potential impact of reflex bradypnea on developmental toxicity studies

Pregnant dams are protected by RB, but their fetuses are not. Fetuses can experience developmental delays or defects due to impaired placental transfer of O₂ (hypoxia) and CO₂ (hypercapnia), fetal hypothermia, and malnutrition. Fetuses are more sensitive to the effects of hypothermia as compared to adults ([OECD, 2009](#)).

When dams experience RB, their fetuses may experience hypoxia due to (1) reduced maternal respiration and (2) a left shift in maternal oxyhemoglobin affinity caused by an increase in blood pH (respiratory alkalosis). Normal oxygen exchange to the fetus requires a gradient between maternal and fetal oxyhemoglobin affinities. When pregnant dams experience RB, their blood pH becomes more alkaline, resulting in a left shift in maternal oxyhemoglobin affinity. A maternal left shift results in the affinities of maternal and fetal oxyhemoglobin being indistinguishable, which impairs oxygen exchange to the fetus (hypoxia) and removal of CO₂ (hypercapnia). Rossant and

Cross ([Rossant and Cross, 2001](#)) describe hypoxia as a normal regulator of placental development in both humans and mice.

When Holzum et al. (1994¹³) exposed pregnant rats to cyfluthrin, they observed concentration-related decreases in fetal weights (see Figure C-18); Holzum et al. also observed concentration-related decreases in placental weights. Clearly, further studies on the impact of formaldehyde and other URT irritants on the placenta and fetus are needed, but the results of Holzum et al. show how RB has the potential to delay fetal growth. It should be noted that reductions in maternal feeding and metabolism during periods of RB can result in reduced fetal glucose levels. It is also important to emphasize that RB-induced developmental effects caused by fetal hypoxia, hypercapnia, hypothermia, and malnutrition are not relevant to humans.

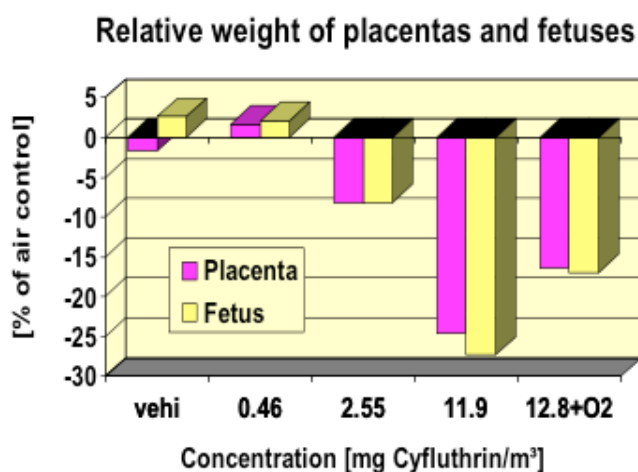


Figure C-18. The potential impact of reflex bradypnea on fetal development.

This graph shows concentration-related decreases in placental and fetal weights in pregnant dams exposed to cyfluthrin, a pyrethroid insecticide. Note that the decrements in fetal and placental weights were lessened in the 12.8 mg/m³ group when the dams were provided with oxygen-rich air (39% O₂).

Source: Holzum et al. (1994). Graph generated by Jürgen Pauluhn (Bayer Healthcare AG, Germany).

C.2.5. Summary

Reflex bradypnea (RB) is a protective response observed in rodents exposed to formaldehyde and other upper respiratory tract irritants. The most notable signs of RB are concentration-related decreases in body temperature, respiratory rate (breaths/minute), and minute volume. Even though the effects of RB can be striking, they are not relevant to humans. It is likely that RB occurred in most, if not all, rodent inhalation toxicity studies testing high levels of formaldehyde exposure, but the extent of RB in these studies cannot be ascertained since it was not

¹³https://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-128831_13-Feb-01_b.pdf.

measured. In comparative studies, mice exhibit RB at a lower formaldehyde concentration than rats and had a more pronounced and more sustained RB response than rats.

Because rodents experiencing RB have reduced minute volumes, they inhale less formaldehyde and thus are expected to experience less toxicity than if they were breathing normally. Several studies demonstrate that mice and rats do not develop tolerance to formaldehyde over as much as 10 days of exposure; however, there are no long-term studies that show whether or when rodents eventually develop a tolerance to formaldehyde. This is a significant data gap. Thus, while RB is considered during study evaluation and during evidence synthesis and integration, adjustments are not applied to account for the potential impact of RB on long-term rodent health endpoints considered for use in dose-response analysis.

C.3. GENOTOXICITY

The evaluations of genotoxic effects of formaldehyde exposure included primary sources from peer-reviewed literature and secondary sources of peer-reviewed reports by other federal agencies and non-federal institutions (see Appendix B.2.10 for details). In general, the following criteria were considered for making judgments about evidence for the genotoxic and/or mutagenic potential of formaldehyde. These include but are not limited to: (a) nature and type of tests, (b) degree of response, (c) number and performance of test strains, (d) dose/concentration levels, (e) biological significance, (f) strength of evidence (conflicting evidence in the same assay system for the same end point), and (g) evaluation of the study results across the same end points. Studies of genotoxicity in exposed humans were consistently evaluated using a structured set of criteria (see Appendix B.3.10).

The terms genotoxicity and mutagenicity differ depending on the effect seen on DNA. Genotoxicity refers to potentially harmful effects caused either directly or indirectly to the genetic material by chemical or physical agents, and these effects are not necessarily persistent and transmissible and may or may not be associated with mutagenicity. Mutagenicity refers to the induction of permanent, transmissible changes in the amount, chemical properties, or structure of the genetic material. Mutations may involve a single gene or gene segment, a block of genes, parts of chromosomes, or whole chromosomes and result in either structural and/or numeric changes. Since mutagenicity is considered a subset of genotoxic effects, the term “genotoxic effects” will be generally used throughout the rest of the document unless the assay determines specific mutations.

A variety of genotoxic effects have been demonstrated in both in vitro and in vivo test systems as a result of exposure to formaldehyde (a summary table by genotoxic endpoint is presented in Appendix C.3.7). Note that no single genotoxicity or mutagenicity test/system or study is able to detect the entire spectrum of formaldehyde-induced genotoxic events. Therefore, genotoxic endpoints are briefly discussed for cell free systems, prokaryotic organisms, nonmammalian organisms, in vitro mammalian systems, in vivo experimental animals, and humans [reviewed in ([NTP, 2010](#); [Ma and Harris, 1988](#); [Liteplo and Meek, 2003](#); [IARC, 1995, 2006](#); [Conaway](#)

[et al., 1996](#); [Auerbach et al., 1977](#); [ATSDR, 2008](#)). In addition, the overall weight of evidence for formaldehyde-induced mutations is considered in the context of the current EPA cancer guidelines ([U.S. EPA, 2005](#)). Note that all studies from the available database have been depicted in several of the following tables, but only the studies most relevant to this discussion are briefly described in the text.

C.3.1. Genotoxicity of Formaldehyde in Cell-Free Systems

Formaldehyde or formalin¹⁴ has been shown to form both hydroxymethyl DNA (hmDNA) adducts and DNA-protein crosslinks (DPX) following treatment of various cell-free systems with formaldehyde or formalin (see Table C-14). The formation of DNA-DNA crosslinks was observed in calf thymus DNA ([Chaw et al., 1980](#)) and duplex DNA ([Huang et al., 1992](#); [Huang and Hopkins, 1993](#)). Furthermore, DNA-protein crosslinks were seen in plasmid DNA, calf thymus histones, and other acellular systems ([Lu et al., 2008](#); [Lu, 2009](#); [Lu et al., 2010b](#); [Kuykendall and Bogdanffy, 1992](#)). The formation of hmDNA adducts was observed following in vitro reaction of formalin in solution with free DNA ribonucleoside ([Kennedy et al., 1996](#)), deoxyribonucleosides and nucleotides ([Mcghee and von Hippel, 1975a, b](#); [Cheng et al., 2003](#); [Cheng et al., 2008](#)), calf thymus DNA ([Von Hippel and Wong, 1971](#); [Fennell, 1994](#); [Beland et al., 1984](#)), human placental DNA ([Zhong and Hee, 2004](#)), and isolated rat liver nuclei ([Heck and Casanova, 1987](#); [Fennell, 1994](#)). [Cheng et al. \(2008\)](#) also reported that nitrosamines which form formaldehyde during their metabolism via formation of α -esters can react in vitro with deoxyribonucleosides or calf thymus DNA and form the hmDNA adducts. Studies have shown that N⁶-hydroxymethyl-deoxyadenosine (N⁶-hmdAdo) was the predominant adduct formed followed by N²-hydroxymethyl-deoxyguanosine (N²-hmdGuo) and N⁴-hydroxymethyl-deoxycytidine (N⁴-hmdCyd) when formaldehyde was reacted with calf thymus DNA ([Cheng et al., 2008](#); [Beland et al., 1984](#)) or human placental DNA ([Zhong and Hee, 2004](#)).

Table C-14. Summary of genotoxicity of formaldehyde in cell-free systems

Test system	Dose and agent ^a	Results ^b	Duration; method	Reference
DNA-DNA crosslinks				
Calf thymus DNA	0.17 mM 37% HCHO	+	40 d; RP-HPLC	Chaw et al. (1980)
Duplex DNA	25 mM HCHO	+	9 d; DPAGE	Huang et al. (1992)
Duplex DNA	25 mM HCHO	+	9 d; DPAGE	Huang and Hopkins (1993)
DNA-protein crosslinks				
Lysine or Cysteine and dG	50 mM 20% HCHO in H ₂ O	+	48 hrs; RP-HPLC/LC_MS	Lu et al. (2010a)

¹⁴Studies that used formalin often contained 10–15% methanol as a stabilizing agent. Although formaldehyde is a metabolic product of methanol, it is not genotoxic in in vitro reactions.

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose and agent ^a	Results ^b	Duration; method	Reference
Histone 4	50 mM 20% HCHO in H ₂ O	+	10 min; LC-MS	Lu et al. (2008)
Plasmid DNA, calf thymus histones	0.0015 mM HCHO	+	1 hr; filter binding assay	Kuykendall and Bogdanffy (1992)
Calf thymus DNA	0.5 mM HCHO	+	4 hrs; ESI-MS/MS	Lu (2009)
DNA adducts				
Guanosine	2,400 mM 37% HCHO	+	48 hrs	Kennedy et al. (1996)
Deoxyguanosine	2,300 mM formalin ^c	+	20 hrs	Cheng et al. (2003)
Guanosine	0.001 mM HCHO	+	90 hrs	Cheng et al. (2003)
DNA nucleosides/ nucleotides	50 mM formalin	+	72–120 hrs	McGhee and von Hippel (1975a)
DNA nucleosides/ nucleotides	300 mM formalin	+	72–120 hrs	McGhee and von Hippel (1975a)
Calf thymus DNA	0.001 mM formalin	+	90 hrs	Cheng et al. (2003)
Calf thymus DNA	0.167 mM formalin	+	48 hrs	Beland et al. (1984)
Calf thymus DNA	0.4 mM formalin	+	4 hrs	Fennell (1994)
Calf thymus DNA	200 mM formalin	+	20 hrs	Von Hippel and Wong (1971)
Calf thymus DNA or deoxyribonucleosides	50 mM β -acetates of NDMA; NNK and NNAL ^d	+	1 or 90 hrs	Cheng et al. (2008)
Human placental DNA	3.34 mM formalin	+	20 hrs	Zhong and Hee (2004)
Rat - Hepatic nuclei	0.1 mM HCHO (¹⁴ C and ³ H) aqueous solution	+	0.5 hr	Heck and Casanova (1987)
Rat - Hepatic nuclei	0.4 mM ¹⁴ C-HCHO	+	4 hrs	Fennell (1994)

^alowest effective concentration for positive results; highest concentration tested for negative or equivocal results.

^b+ = positive, all experiments performed without exogenous activation.

^cFormalin – all experiments with formalin contained 37% formaldehyde plus 10–15% methanol.

^dthese nitrosamines are precursors to formaldehyde.

Abbreviations: HCHO, formaldehyde; NDMA, N-nitrosodimethylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; DPAGE, denaturing polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography; LC-ESI-MS, liquid chromatography electrospray ionization mass spectrometry; LSC, liquid scintillation counting; MS, mass spectrometry; NMR, nuclear magnetic resonance; RP-HPLC, reverse phase high performance liquid chromatography; UV, ultraviolet.

C.3.2. Genotoxicity of Formaldehyde in Prokaryotic Organisms

A number of reports describe the mutagenicity of formaldehyde in bacterial test systems (*Salmonella typhimurium* and *Eschericia coli*) using reverse and forward mutation assays as well as assays with specific *E. coli* strains for detecting deletions, insertions and point mutations (see Table C-15).

Formaldehyde was mutagenic in the reverse mutation assay in all of the studies with the *Salmonella* strains TA102 and TA104, and most of the studies with TA100 strains with and without metabolic activation and in strains TA2638 and TA2638a without metabolic activation. Mixed results were reported with TA97, TA98, and TA1537 strains, while most of the studies with the TA1535 and TA1538 strains were negative with or without metabolic activation ([Wilcox et al., 1990](#); [Sarrif et al., 1997](#); [Rydén et al., 2000](#); [O'Donovan and Mee, 1993](#); [Müller et al., 1993](#); [Marnett et al., 1985](#); [Le Curieux et al., 1993](#); [Jung et al., 1992](#); [Dillon et al., 1998](#)).

With respect to forward mutations, formaldehyde has been shown to induce these types of mutations both in *S. typhimurium* ([Temcharoen and Thilly, 1983](#)) as well as in *E. coli* strains ([Temcharoen and Thilly, 1983](#); [Bosworth et al., 1987](#)). Temcharoen and Thilly (1983) showed that formaldehyde induced both toxicity and mutagenicity in the *Salmonella* strain TM677 (8-azaguanine sensitive), both with or without metabolic activation. On the other hand, ([Bosworth et al., 1987](#)) reported formaldehyde to be mutagenic in *E. coli* strain D494 uvrB, a more sensitive strain to base-pair substitutions. Furthermore, formaldehyde has been shown to induce diverse mutations in a forward mutation assay in *E. coli* strains GP120, GP120A, 7-2, and 33694, which contained a xanthine guanine phosphoribosyl transferase (*gpt*) reporter gene ([Crosby et al., 1988](#)). In this study, formaldehyde tested at two different concentrations (4 and 40 mM) produced point mutations (41%), deletions (18%), and insertions (41%) at low concentrations of exposure, while the high-dose exposure resulted predominantly in point mutations (92%). The point mutations at low-dose exposure were transversions at GC base pairs, while at high-dose exposure they were transition mutations at a single AT base pair in the *gpt* gene ([Crosby et al., 1988](#)).

[Wang et al. \(2007b\)](#) have also shown that formaldehyde causes dose-dependent increases in microsatellite instability in *E. coli*. Exposure to 2.5 mM formaldehyde caused a 2- to 24-fold induction in mutation frequencies of the complementary dinucleotide repeat microsatellites (GpT) and (ApC) compared to in untreated controls. It is possible that microsatellite instability could change the conformation of DNA to a Z-DNA structure, making the DNA not amenable for DNA repair.

Table C-15. Summary of genotoxicity of formaldehyde in prokaryotic systems

Test system	Dose ^a (µg/ plate)	Agent ^b	Results ^{c,d}		Comments	Reference
			−S9	+S9		
Reverse mutation						
S. typhimurium TA100	10, 25	35% HCHO sol.	+	+	PP method; values visually determined from graph; (T) at 37.5 (−S9) and 50 (+S9) µg/plate	Orstavik and Hongslo (1985)
	12	37% HCHO with 10% methanol	(+)	(+)	PI method	Schmid et al. (1986)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose ^a (µg/ plate)	Agent ^b	Results ^{c,d}		Comments	Reference
			–S9	+S9		
	15, 7.5	HCHO/mL	+	+	Suspension method	Sarrif et al. (1997)
	30	37% HCHO with 10–15% methanol	+	+	PI method; values visually determined from graph. Methanol tested '–ve' up to 500 µg/plate (–S9 or +S9) in the same study.	Connor et al. (1983)
	30	HCHO (form not specified)	(+)	ND	PP method	Takahashi et al. (1985)
	39	37% HCHO with 10–15% methanol	– (T)	– (T)	PI method	De Flora (1981)
	50	35% HCHO	+	+	PP method; dose range 6.25–50 µg/plate only provided	Dillon et al. (1998)
	75	HCHO (form not specified)	–	+	PI method; –S9 data <2-fold compared to control	Sarrif et al. (1997)
	80	37% HCHO with 10% methanol	(+)	+	PP method	Schmid et al. (1986)
	90	HCHO (form not specified)	–	ND	PP method; (T): >90 µg/plate	Marnett et al. (1985)
	100, 50	37% aq.sol. HCHO	+, +	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	100	HCHO (form not specified)	+	–	PP method; (T) ≥200 µg/plate	Sarrif et al. (1997)
	150	37% HCHO	+	ND	PP method; discrepancy in mutagenic data observed between author's report and the graph from the citation (150 vs. ≈30 µg/plate)	Fiddler et al. (1984)
	333.3, 10	37% HCHO	–	+	PP method; (T): NR	Haworth et al. (1983)
	500, 20	37% HCHO in distilled water	(+)	+	PP method	Connor et al. (1985a)
S. typhimurium TA102	10	HCHO/mL	+	ND	Fluctuation test; (T) at 30 µg/mL	Le Curieux et al. (1993)
	17.2	HCHO (in water)	+	ND	PP method	Rydén et al. (2000)
	25	HCHO (form not specified)	+	ND	PI method; (T) >100 µg/plate	Wilcox et al. (1990)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose ^a (µg/ plate)	Agent ^b	Results ^{c,d}		Comments	Reference
			–S9	+S9		
	50	HCHO (form not specified)	(+)	(+)	PP method; values visually determined from graph	De Flora et al. (1984)
	50	35% HCHO	+	+	PP method; '+' with rat S9 and '±' with mouse S9; Authors show a dose range 6.25–50 µg/plate	Dillon et al. (1998)
	90	HCHO (form not specified)	+	ND	PP method; (T): >90 µg/plate	Marnett et al. (1985)
	200, 100	37% aq.sol. HCHO	+, +	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	200	HCHO (in water)	+	ND	PI method; (T) at 600 mg/plate	Watanabe et al. (1996)
	5000	HCHO (form not specified)	(+)	(+)	PI method; (+) by 1 lab and '–ve' by 2 labs	Jung et al. (1992)
	5,000	HCHO (form not specified)	(+)	(+)	PI method; reported '(+)' by 1 lab and '–ve' by 2 labs	Müller et al. (1993)
S. typhimurium TA104	50	35% HCHO	+	+	PP method; Authors show a dose range 6.25–50 µg/plate.	Dillon et al. (1998)
	90	HCHO (form not specified)	+	ND	PP method; (T): >90 µg/plate	Marnett et al. (1985)
S. typhimurium TA1535	39	formalin	– (T)	– (T)	PI method	De Flora (1981)
	100	37% aq.sol. HCHO	–, –	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	100	HCHO (form not specified)	–	–	PI method; (T) at 150 µg/plate	Sarrif et al. (1997)
	100	HCHO (form not specified)	–	–	PP method; (T) ≥200 µg/plate	Sarrif et al. (1997)
	333.3	37%HCHO	–	–	PP method; (T): NR	Haworth et al. (1983)
S. typhimurium TA97	50	HCHO (form not specified)	+	ND	PI method; (T) at 100 µg/plate	Sarrif et al. (1997)
	90	HCHO (form not specified)	–	ND	PP method; (T): >90 µg/plate	Marnett et al. (1985)
S. typhimurium TA98	10, 25	35% HCHO sol.	+	+	PP method; values visually determined from graph; (T) at 37.5 (–S9) and 50 (+S9) µg/plate	Oerstavik and Hongslo (1985)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose ^a (µg/ plate)	Agent ^b	Results ^{c,d}		Comments	Reference
			–S9	+S9		
	30	37% HCHO with 10-15% methanol	+	+	PI method; Methanol tested up to 500 mg/plate (–S9 or +S9) was '–ve'. Values visually determined from graph.	Connor et al. (1983)
	30	HCHO (form not specified)	(+)	ND	PP method	Takahashi et al. (1985)
	39	37% HCHO with 10–15% methanol	– (T)	– (T)	PI method	De Flora (1981)
	50, 100	37% aq.sol. HCHO	+, +	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	50, 100	HCHO (form not specified)	+	+	PP method; (T) ≥00 µg/plate	Sarrif et al. (1997)
	75	HCHO (form not specified)	–	+	PI method; –S9 data <2-fold compared to control	Sarrif et al. (1997)
	90	HCHO (form not specified)	–	ND	PP method; (T): >90 µg/plate	Marnett et al. (1985)
	333.3, 10	37% HCHO	–	(+)	PP method; (T): NR	Haworth et al. (1983)
	500	37% HCHO in distilled water	– (T)	(+) (T)	PP method	Connor et al. (1985b)
S. typhimurium TA1537	39	37% HCHO with 10-15% methanol	– (T)	– (T)	PI method	De Flora (1981)
	50, 75	HCHO (form not specified)	+	+	PI method	Sarrif et al. (1997)
	100	37% aq.sol. HCHO	–, –	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	100	HCHO	–	–	PP method	Sarrif et al. (1997)
	333.3	37% HCHO	–	–	PP method; (T): NR	Haworth et al. (1983)
S. typhimurium TA1538	39	formalin	– (T)	– (T)	PI method	De Flora (1981)
	100	37% aq.sol. HCHO	–, –	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
S. typhimurium TA2638	500	HCHO (in water)	+	ND	PI method; (T) at 1000 mg/plate	Watanabe et al. (1996)
S. typhimurium TA2638a	17.2	HCHO (in water)	+	ND	PP method	Rydén et al. (2000)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose ^a (µg/ plate)	Agent ^b	Results ^{c,d}		Comments	Reference
			–S9	+S9		
S. typhimurium UTH8413, UTH8414	500	37% HCHO with 10–15% methanol	– (T)	– (T)	PI method; Methanol tested '–ve' up to 500 µg/plate with/without S9.	Connor et al. (1983)
	500	37% HCHO in distilled water	– (T)	– (T)	PP method	Connor et al. (1985b)
E. coli WP2, WP2uvrA, H/R30R, Hs30R (uvrA)	420	HCHO (form not specified)	+	ND	RM assay	Takahashi et al. (1985)
E. coli NG30 (recA)	63	HCHO (form not specified)	–	ND	RM assay; values visually determined from graph	Takahashi et al. (1985)
E. coli O16 (polA)	52.5	HCHO (form not specified)	–	ND	RM assay; values visually determined from graph	Takahashi et al. (1985)
E. coli K12 (AB1886)/(uvrA); K12 (AB2480)/(recA /uvrA)	150	HCHO (form not specified)	–	ND	RM assay	Graves et al. (1994)
E. coli K12 (AB1157)(WT)	1,875	HCHO (form not specified)	+	ND	RM assay	Graves et al. (1994)
E. coli WP2 (pkM101)	200	HCHO (form not specified)	– (T)	ND	PI method	Wilcox et al. (1990)
	200, 100	37% aq.sol. HCHO	–, +	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	700	HCHO (in water)	+	ND	PI method	Watanabe et al. (1996)
E. coli WP2 uvrA (pkM101)	150	HCHO (form not specified)	+	ND	PI method; dose- response from 10–300 µg/plate	Wilcox et al. (1990)
	200, 50	37% aq.sol. HCHO (form not specified)	+, +	ND	Results by PI & PP methods, respectively	O'Donovan and Mee (1993)
	400	HCHO (in water)	+	ND	PI method	Watanabe et al. (1996)
E. coli (Lac+ reversion) WP3104P	10	HCHO (form not specified)	(+)	ND	RM assay	Ohta et al. (1999)
E. coli (Lac+ reversion) WP3101P, WP3102P, WP3103P,	30	HCHO (form not specified)	–	ND	RM assay	Ohta et al. (1999)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose ^a (µg/ plate)	Agent ^b	Results ^{c,d}		Comments	Reference
			–S9	+S9		
WP3105P, WP3106P						
Forward mutation						
<i>S. typhimurium</i> TM677	0.167, 0.33 mM	37% HCHO with 10–15% methanol	+	+	PP method	Temcharoen and Thilly (1983)
<i>E. coli</i> D494uvrB (pGW1700)	6.0 µg/mL	HCHO (form not specified)	+	ND	Ampicillin FM assay	Bosworth et al. (1987)
Deletions, Insertions and Point mutations						
<i>E. coli</i> GP120, GP120A, 7-2, 33694	4 mM	HCHO (form not specified)	+	ND	gpt FM assay	Crosby et al. (1988)
Microsatellite Instability						
<i>E. coli</i> JM109	2.5 mM	HCHO (form not specified)	+	ND	Mutation frequency analysis and sequencing.	Wang et al. (2007b)

^alowest effective dose for positive results; highest ineffective dose tested for negative or equivocal results.

^bsingle value indicates identical dose/concentration effective for both without (–S9) or with (+S9) metabolic activation; for –S9 assay data showing two signs (+ or –) separated by a comma indicate respectively, use of PI and PP methods.

^c+ = positive; – = negative; (+) = weak positive; ND = test was not done; (T), toxic.

Abbreviations: HCHO, formaldehyde; PI, plate incorporation (or standard plate); PP, pre-incubation plate; FM, forward mutation; RM, reverse mutation; *gpt*, xanthine guanine phosphoribosyl transferase.

C.3.3. Genotoxicity of Formaldehyde in Nonmammalian Systems

Formaldehyde (commercial grade) or formalin (mostly containing 37% formaldehyde and 10–15% methanol) has been tested in several nonmammalian systems including yeast, molds, plants, insects, and nematodes. As summarized in Table C-16, formaldehyde has been shown to cause gene conversion, strand breaks, crosslinks, homozygosis and related damage in yeasts (*Saccharomyces cerevisiae*); forward and reverse mutations in molds (*Neurospora crassa*); micronuclei formation in spiderworts (*Tradescantia pallida*); DNA damage and mutations in several plants; genetic cross-over or recombination, sex-linked recessive lethal mutations, dominant lethal mutations, heritable translocations, and gene mutations in insects (*Drosophila melanogaster*); and recessive lethal mutations in nematodes (*Caenorhabditis elegans*). Formaldehyde failed to show micronuclei formation in newt larvae (*Pleurodeles waltl*) (reviewed in reviewed in ([NTP, 2010](#); [IARC, 2006, 2012](#))). DNA protein crosslinks were observed in *Saccharomyces cerevisiae* and *E. coli* ([Wilkins and Macleod, 1976](#); [Magaña-Schwencke and Ekert, 1978](#); [Magaña-Schwencke and Moustacchi, 1980](#)).

Some of the nonmammalian studies compared the effects of formaldehyde in wild type and DNA repair-deficient organisms. For example, ([Magaña-Schwencke et al., 1978](#)) showed that

Supplemental Information—Formaldehyde (Inhalation)

excision repair-deficient *Saccharomyces cerevisiae* strains are more susceptible to formaldehyde-induced lethal effects and have reduced capacity to form single strand breaks (SSBs) compared with repair-proficient strains, suggesting that the repair process possibly involves SSB formation. Also, formaldehyde is more mutagenic in repair-deficient *Neurospora crassa* compared to the corresponding repair-proficient strains ([de Serres and Brockman, 1999](#)).

Table C-16. Summary of genotoxicity studies for formaldehyde in nonmammalian organisms

Test system	Concentration ^{a,b}	Results ^c	Comments ^d	Reference
DNA damage				
Various plant and fungal species ^e	1233 mM 3.7% HCHO (at pH 3.0 and 7.0)	+	1.5 hrs, PCR/GE,	Douglas and Rogers (1998)
DNA protein crosslinks				
<i>Saccharomyces</i> (S.) <i>cerevisiae</i>	17 mM HCHO (form not specified)	+	0.25 hrs, DNA extractability; (T) 90 & 60% survival at 33 & 66 mM HCHO with 42 & 95% DNA damage, respectively	Magaña-Schwencke and Ekert (1978)
<i>S. cerevisiae</i>	33 mM HCHO (form not specified)	+		Magaña-Schwencke and Moustacchi (1980)
<i>E. coli</i>	130 mM HCHO (form not specified)	+	10 min; alkaline sucrose gradient centrifugation	Wilkins and Macleod (1976)
DNA repair inhibition				
<i>S. cerevisiae</i>	66 mM HCHO (form not specified)	+	0.25 hrs, ASG; (T) 90 & 60% survival at 33 & 66 mM HCHO with 42 & 95% DNA damage, respectively	Magaña-Schwencke and Ekert (1978)
Dominant lethal mutation				
<i>Drosophila</i> (D.) <i>melanogaster</i>	60 mM 36% HCHO in water	+	larval feeding method, frequency of hatchability	Auerbach and Moser (1953a, 1953b)
<i>D. melanogaster</i>	43 mM HCHO (form not specified)	+	Exposure duration NR, frequency of dominant lethal mutations	Srám (1970)
Forward mutation				
<i>Neurospora</i> (N.) <i>crassa</i> heterokaryon H-59 strain	3 mM formalin	+	3 hrs, frequency of ad-3 mutations	de Serres et al. (1988) ; de Serres and Brockman (1999)
<i>N. crassa</i> heterokaryon H-12 strain	8 mM formalin	(+)	3 hrs, frequency of ad-3 mutations	de Serres et al. (1988) ; de Serres and Brockman (1999)
Gene conversion				

Supplemental Information—Formaldehyde (Inhalation)

Test system	Concentration ^{a,b}	Results ^c	Comments ^d	Reference
S. cerevisiae strain D4	18 mM 30% HCHO solution	+	0.5 hr, frequency of recombinants	Chanet et al. (1975)
Genetic crossing over or recombination				
D. melanogaster	14 mM HCHO (form not specified)	+	larval feeding method	Alderson (1967)
	42 mM HCHO (form not specified)	+	duration of exposure NR, frequency of recombinant	Sobels and van Steenis (1957)
	83 mM HCHO (form not specified)	+	duration of exposure NR, frequency of cross overs	Ratnayake (1970)
Heritable translocation				
D. melanogaster	14 mM HCHO (form not specified)	+	2 hrs, frequency of recombinants	Khan (1967)
	83 mM HCHO (form not specified)	+	duration of exposure NR, frequency of translocations	Ratnayake (1970)
Homozygosis by mitotic recombination or gene conversion				
Saccharomyces cerevisiae	0.62 mM formalin	+	16 hrs, frequency of resistant colonies	Zimmermann and Mohr (1992)
Micronucleus				
Pleurodeles waltl	0.17 mM HCHO (form not specified)	–	168 hrs, Masson's haemalum staining	Siboulet et al. (1984)
Pleurodeles waltl larva	0.33 mM HCHO (form not specified)	–	12 hrs, Masson's haemalum staining	Le Curieux et al. (1993)
Tradescantia pallida	8 mM HCHO (form not specified)	+	6 hrs, acetocarmine staining	Batalha et al. (1999)
Mutation				
Plants (others)	NR	+	NR	Auerbach et al. (1977)
Reverse lethal mutation				
Caenorhabditis elegans	23 mM HCHO from PFA	+	4 hrs, frequency of mutations	Johnsen and Baillie (1988)
Reverse mutation				
Neurospora crassa	10 mM HCHO (form not specified)	+	4 hrs, frequency of mutations	Jensen et al. (1951)
	10 mM formalin	–	3 hrs, frequency of mutations	Kölmark and Westergaard (1953)
	24 mM HCHO (form not specified)	–	0.5 hrs, frequency of mutations	Dickey et al. (1949)
Sex-linked lethal mutation				

Supplemental Information—Formaldehyde (Inhalation)

Test system	Concentration ^{a,b}	Results ^c	Comments ^d	Reference
D. melanogaster	8 mM formalin	+	larval feeding method, frequency of sex linked lethals	Stumm-Tegethoff (1969)
	14 mM HCHO (form not specified)	+	larval feeding method	Alderson (1967)
	14 mM HCHO (form not specified)	+	2 hrs, frequency of progeny	Khan (1967)
	33 mM formalin	+	duration of exposure NR, frequency of eclosions	Kaplan (1948)
	42 mM HCHO (form not specified)	+	Exposure duration NR, frequency of sex-linked lethals	Sobels and van Steenis (1957)
	60 mM 36% HCHO in water	+	larval feeding method, frequency of sex linked lethals	Auerbach and Moser (1953b); Auerbach and Moser (1953a)
	67 mM HCHO (form not specified)	(+)	larval feeding method, frequency of sex linked lethals	Ratnayake (1968)
	73 mM HCHO (form not specified)	+	duration of exposure NR, frequency of sex-linked lethals	Ratnayake (1970)
Single strand breaks				
S. cerevisiae	33 mM HCHO (form not specified)	+	0.25 hrs, ASG; (T) 90 & 60% survival at 33 & 66 mM HCHO with 42 & 95% DNA damage, respectively	Magaña-Schwencke et al. (1978)

^aindicates lowest effective concentration for positive results; highest concentration tested for negative or equivocal results.

^bindicates that the multiple dose/concentration values reported correspond to order of the indicated test result(s) (e.g., without activation; with activation). Identical doses/concentrations for multiple test results are indicated by a single value.

^cindicates + = positive; – = negative; (+) = weak positive.

^dindicates the duration of exposure and the assay used to assess the endpoint, dose-response and toxicity (T) if any.

^eindicates that authors tested the following species: *Agaricus bisporus*, *Glycine max*, *Lycopersicon esculentum*, *Pinus resinosa*, *Pisum sativum*, *Populus x euramericana*, *Vicia faba*, and *Zea mays*.

Abbreviations: ad-3, adenine-3 locus; ASG, alkaline sucrose gradient; HCHO, formaldehyde; NR, not reported; PCR/GE, polymerase chain reaction/gel electrophoresis; PFA, paraformaldehyde.

C.3.4. Genotoxicity of Formaldehyde in in Vitro Mammalian Cells

Formaldehyde has been tested for its genotoxic potential in several mammalian cell culture systems originating from rodents (mice, rats, hamsters) and humans, mostly without metabolic activation. In a majority of these systems, formaldehyde tested positive for: DNA reactivity including DNA adducts, DPXs, and SSBs; cytogenetic changes such as sister chromatid exchanges

(SCEs), chromosomal aberrations (CAs), and micronuclei (MN); cell transformation and mutation induction; and other genotoxic endpoints such as unscheduled DNA synthesis (UDS) and DNA repair inhibition (summarized in Table C-17).

DNA Reactivity and Damage

DNA adducts

Formaldehyde has been shown to form hmDNA adducts in CHO cells ([Beland et al., 1984](#)) and rat and human nasal epithelial cells ([Zhong and Que Hee, 2004](#)) as shown in Table C-17. [Beland et al. \(1984\)](#) first reported hmDNA adducts in CHO cells incubated with 1 mM of radiolabeled formaldehyde. After a 2-hour incubation, small amounts of N⁶-hmdA were detected with concomitant metabolic incorporation of formaldehyde (i.e., into DNA bases). Zhong and Que Hee ([2004](#)) reported three types of hmDNA adducts in human nasal epithelial cells exposed to varying concentrations of formalin (10–500 µg/mL). In this study, the hmDNA adduct levels were in the order of N⁶-hmdA > N²-hmdG > N⁴-hmdC. In HeLa cells exposed to [¹³CD₂]-formaldehyde, ([Lu et al., 2012a](#)) detected both exogenous (¹³C-labeled) and endogenous (unlabeled) N²-hmdG adducts; however, this study detected endogenous but not exogenous N⁶-hmdA adducts.

DNA-protein crosslinks

As summarized in Table C-17, DNA protein crosslinks have been reported in several mammalian cell lines (primary and transformed) from rodents (mice, rats, hamsters) and humans (reviewed in ([IARC, 1995, 2006](#); [Conaway et al., 1996](#))).

The lowest effective concentration of formaldehyde or formalin causing DPX formation varied between different cell lines (see Table C-17). Among the animal cell lines, DPX formation was observed at the in vitro concentrations of 0.125–0.25 mM in CHO cells and 0.01–0.2 mM in V79 cells. Several human cell lines (either primary cells or developed cells lines), including epithelial, fibroblasts, buccallymphoblastoid, lymphoma, and peripheral blood lymphocytes, among others, that were exposed to formaldehyde also formed DPXs ([Li et al., 2004](#); [Emri et al., 2004](#); [Craft et al., 1987](#); [Costa et al., 1997](#)). Selected studies have been briefly described below, although all available and relevant studies are included in Table C-17).

[Craft et al. \(1987\)](#) analyzed DPXs in TK6 human lymphoblastoid cells immediately after a 2-hour exposure (zero time) to 0–600 µM formaldehyde with a significant nonlinear increase in DPXs above 50 µM, which correlated with the onset of cytotoxicity. DPXs were completely repaired within 24 hours after exposure.

DPXs were also detected in Epstein-Barr Virus (EBV)-human Burkitt's lymphoma cells exposed to paraformaldehyde (which depolymerizes to release formaldehyde) at doses that were cytotoxic (>0.003%) ([Costa et al., 1997](#)). [Grafrström et al. \(1986\)](#) reported that the number of DPXs induced by 100 µM formaldehyde in vitro in human bronchial epithelial cells and fibroblasts was similar; although, DPX levels were several-fold higher than SSBs in the epithelial cells. In a different

study, the same authors ([Grafstrom et al., 1984](#)) noted that formaldehyde exposure resulted in the formation of DPXs at similar levels in bronchial epithelial cells and in DNA excision repair-deficient xeroderma pigmentosum (XP) skin fibroblasts, and their removal rate was similar with a half-life of 2–3 hours, suggesting that the DPXs are repaired independently of excision repair. Further, formaldehyde was only moderately cytotoxic to normal bronchial epithelial cells and fibroblasts at concentrations that induced substantial DNA damage. Repair of the formaldehyde-induced DNA SSBs and DPXs appeared to be inhibited by the continued presence of formaldehyde in the culture medium ([Grafstrom et al., 1984](#)).

A linear increase in DPX levels was observed in primary human skin fibroblasts and keratinocytes from 25–100 μM formaldehyde, as indicated by the ability of formaldehyde to reduce DNA migration in the comet assay after methylmethane sulfonate (MMS) pretreatment ([Emri et al., 2004](#)). Similar findings were also reported for primary human peripheral blood lymphocytes (PBLs) and HeLa cells ([LICM, 2006](#)). Peak response for SSBs was seen at 10 μM in both cells, with higher concentrations resulting in crosslink formation ([LICM, 2006](#)). DPX formation was also observed in a whole blood culture after exposure to 25 μM , as indicated by the effect of formaldehyde on DNA migration in the comet assay after γ -radiation ([Schmid and Speit, 2007](#)). The repair of DPXs was complete 8 hours after an exposure to 100 μM formaldehyde, while DPXs formed at $>200 \text{ mM}$ was repaired within 24 hours.

Formaldehyde-induced DPXs are removed either through spontaneous hydrolysis or active repair processes ([Quievryn and Zhitkovich, 2000](#)). Inhibition of specific proteasomes (protein complexes involved in degrading unwanted or damaged proteins) in xeroderma pigmentosum (XP)-A cells inhibited DPX repair, thereby supporting the role of enzymatic degradation ([Quievryn and Zhitkovich, 2000](#)). The average half-life of formaldehyde-induced DPXs in human epithelial cell lines was 12.5 hours (range 11.6 to 13 hours) ([Quievryn and Zhitkovich, 2000](#)), 18 hours in HeLa cells ([LICM, 2006](#)), and 24 hours in human lymphoblasts ([Craft et al., 1987](#)). This difference was primarily due to slower active repair of DPXs, with a $t^{1/2}$ of 66.6 hours for human lymphocytes compared to other human cell lines ([Quievryn and Zhitkovich, 2000](#)).

[Speit et al. \(2000\)](#) hypothesized that single peptides or small peptide chains cross-linked to DNA are critical to formaldehyde-induced mutation. However, these authors did not find significant differences in the induction and repair of DPXs in a normal human cell line (MRC4CV1), nucleotide excision repair (NER)-deficient xeroderma pigmentosum (XP) fibroblast cell line, and a Fanconi anemia (FA) cell line exposed to 125–500 μM formaldehyde for 2 hours. In contrast, these cells showed increased susceptibility to formaldehyde-induced MN formation. It is suggested that the NER pathway affects cytogenetic makers of genotoxicity rather than the cross-link repair ([Speit et al., 2000](#)).

DNA Single Strand Breaks (SSBs)

Formaldehyde has been shown to induce SSBs in a number of mammalian cell systems in vitro (see Table C-17). Certain cell lines seem to be more sensitive for SSB formation than others.

For example, formaldehyde induced SSBs at concentrations ranging from 0.005–0.8 mM in human primary cells including lung/bronchial epithelial cells ([Saladino et al., 1985](#); [Grafstrom et al., 1984](#); [Grafstrom, 1990](#); [Fornace et al., 1982](#)), skin fibroblasts ([Snyder and van Houten, 1986](#); [Grafstrom et al., 1984](#)), lymphocytes ([LICM, 2006](#)), and in human cell lines A549 ([Vock et al., 1999](#)) and HeLa ([LICM, 2006](#)) cells, and rat hepatocytes ([Demkowicz-Dobrzanski and Castonguay, 1992](#)). In many of these studies SSB induction was dose-dependent. However, formaldehyde did not induce SSBs in human foreskin fibroblasts ([Snyder and van Houten, 1986](#)), human skin keratinocytes exposed for 20 hours ([Emri et al., 2004](#)), mouse leukemia cells ([Ross and Shipley, 1980](#); [Ross et al., 1981](#)), hamster CHO cells ([Marinari et al., 1984](#)), and V79 cells ([Speit et al., 2007b](#)).

Formaldehyde induces more DPXs than SSBs in normal human bronchial epithelial cells ([Saladino et al., 1985](#); [Grafstrom, 1990](#)). [Grafstrom et al. \(1984\)](#) examined the kinetics of DNA repair in nucleotide excision repair (NER)-proficient human bronchial epithelial cells and fibroblasts and NER-deficient fibroblasts from XP patients by the alkaline elution technique. They reported comparable levels of DPX in all cell lines, suggesting non-involvement of NER in DPX removal. However, SSB levels are higher than DPX levels in XP cells compared to normal fibroblasts, although both these DNA lesions are repaired at comparable rates, suggesting an additional indirect mechanism of SSB formation possibly involving a different repair pathway. SSBs in HeLa cells induced by 10 μ M formaldehyde were repaired by 90 minutes after cells were washed to remove formaldehyde ([LICM, 2006](#)).

Cytogenetic markers of genotoxicity

Clastogenic effects, including increased MN, CAs, and SCEs, have been reported in a variety of in vitro systems as shown in Table C-17.

Micronucleus (MN) formation

Studies have shown MN formation in V79 lung epithelial cell lines ([Speit et al., 2007b](#); [Merk and Speit, 1998](#)), in human fibroblasts with varying DNA repair backgrounds ([Speit et al., 2000](#)), and in whole blood cultures ([Schmid and Speit, 2007](#)). [Speit et al. \(2000\)](#) reported a higher frequency of MN formation in xeroderma pigmentosum (XP) and Fanconi anemia (FA) cell lines compared to normal human cell lines suggesting the importance of NER and crosslink repair following formaldehyde exposure. In V79 cells, [Speit et al. \(2007b\)](#) observed that MN frequency increased with repeated formaldehyde treatments compared to a single treatment; however, such an increase was not observed if the treatment interval was increased to 24 hours. An increase in micronucleus frequency was observed in mouse erythropoietic cells ([Ji et al., 2014](#)), human A549 lung epithelial cells ([Speit et al., 2011a](#)), human lymphoblasts ([Ren et al., 2013](#)), and human whole blood cultures ([Speit et al., 2011a](#)).

[Schmid and Speit \(2007\)](#) observed a statistically significant increase in MN formation at or above a formaldehyde concentration of 300 μ M in human whole blood cultures treated with formaldehyde 24 hours after the start of the culture and cytochalasin B (CytB) added 20 hours later

(44 hours after the start of the culture). This prompted the conclusion that the level of DPX formation from formaldehyde exposure would need to be high for MN formation and the cells must be exposed after the first mitosis (which is 24 hours). In examining MN formation more closely with Fluorescence In Situ Hybridization (FISH), Schmid and Speit ([2007](#)) found that 81 percent of the time, formaldehyde was inducing a micronuclei that was centromere negative indicating the effect to be clastogenic rather than aneugenic (a centromere contained micronuclei).

Sister chromatid exchanges (SCEs)

Sister chromatid exchanges occur as a result of errors in the replication process, where an exchange in the chromatids between sister chromatids occurs during the anaphase. DPX are likely to cause replication block and might stimulate SCEs in cells. Therefore, evaluation of SCEs is important in assessing the genotoxicity of formaldehyde.

Formaldehyde has been shown to induce SCEs in most of the in vitro studies, both in rodent and human cells. The available studies are summarized in Table C-17. Different cell types responded differently to various concentrations of formaldehyde, particularly at low doses. For example, the lowest effective concentration (LEC) of formaldehyde in Chinese hamster embryo cells was 0.01 mM, for CHO cells it was 0.03 mM, and V79 cells responded at a concentration of 0.06 mM, while human lymphocytes required slightly higher concentrations (0.125 mM) to show any effect. Neuss and Speit ([2008](#)) observed a significant dose-dependent increase in SCE formation in V79 cells and A549 cells following a range of formaldehyde concentrations with 0.1 mM being the LEC when BrdU was added immediately after formaldehyde exposure. However, when BrdU addition was delayed by 4 hours the LEC increased to 0.2 mM suggesting DNA repair. In co-cultivation experiments, the authors first treated A549 cells for 1 hour with 0.05 mM formaldehyde and then co-cultured them with V79 cells with or without changing the culture medium, SCEs were observed in A549 cells in both situations, but in the co-cultured V79 cells, SCEs were observed only when the medium was not changed, suggesting residual availability of formaldehyde in the medium to induce SCEs in V79 cells and that formaldehyde which entered the A549 cells is either utilized or inactivated. Miyachi and Tsutsui ([2005](#)) measured the induction of SCEs in Syrian hamster embryo (SHE) cells at an LEC of 0.01 mM within an hour of formaldehyde exposure. Schmid and Speit ([2007](#)) observed that SCEs were induced by 200 µM in lymphocytes from human whole blood cultures, an effect apparently associated with cytotoxicity as indicated by a concomitant reduction in the proliferative index.

Chromosomal aberrations (CAs)

Several studies have demonstrated formaldehyde-induced CAs in a variety of mammalian cells, such as CHO cells ([Natarajan et al., 1983](#); [Lorenti Garcia et al., 2009](#)), Chinese hamster lung fibroblasts ([Ishidate et al., 1981](#)), Syrian hamster embryo (SHE) cells ([Hikiba et al., 2005](#); [Hagiwara et al., 2006](#)), mouse lymphoma cells ([Speit and Merk, 2002](#)), human PBLs ([Schmid et al., 1986](#); [Dresp and Bauchinger, 1988](#)), and human fibroblasts ([Levy et al., 1983](#)).

[Hikiba et al. \(2005\)](#) used SHE cells to measure the induction of CAs following exposure to a series of formaldehyde concentrations (0, 33, 66, and 99 μ M) for 24 hours and observed the percentages of aberrant metaphases to be 0, 6, 6, and 71, respectively. The aberrations were predominantly chromosome gaps and chromosomal breaks and exchanges. The relative colony-forming efficiency remained high (at least 85%). Dose-dependent increases in chromosomal aberrations were observed when CHO cells were exposed to 0.15 mM of commercial formaldehyde ([Lorenti Garcia et al., 2009](#)). Chinese hamster lung fibroblasts, when exposed to 0.6 mM formalin induced chromosomal aberration within 24 hour of exposure ([Ishidate et al., 1981](#)). Note that formalin was used in this study as a source of formaldehyde.

Dresp and Bauchinger ([1988](#)) exposed human lymphocytes to various concentrations of formaldehyde. A dose-dependent increase in chromosomal aberrations was observed. [Schmid et al. \(1986\)](#) used the same cell lines and exposed them to 0.25 and 0.5 mM formaldehyde containing 10% methanol. Both chromatid breaks and gaps were observed. It should be recognized that the in vitro studies used different forms of formaldehyde, including commercial grade formaldehyde, paraformaldehyde, formalin (formaldehyde containing 10–15% methanol) or methanol-free formaldehyde.

Mutations and cell transformation

Mutations may occur as a result of the misrepair of formaldehyde-induced DNA damage (DPXs, DNA adducts, SSBs, or clastogenic effects) or as a result of replication errors during mitogenesis. The in vitro evidence for formaldehyde-induced mutations, as discussed below, is strengthened by the correlation between these genotoxic and clastogenic events of formaldehyde and the induction of mutations in other test systems. Numerous studies have demonstrated formaldehyde-induced DNA mutations under a variety of experimental conditions (reviewed in ([NTP, 2010](#); [Ma and Harris, 1988](#); [Liteplo and Meek, 2003](#); [IARC, 1995, 2006, 2012](#); [Conaway et al., 1996](#); [Auerbach et al., 1977](#))).

Deletion and point mutations

Several studies demonstrated deletion mutations in cultured mouse lymphoma cells ([Speit and Merk, 2002](#); [Mackerer et al., 1996](#)), CHO cells and V79 lung epithelial cells at the hypoxanthine phosphoribosyl transferase (hprt) locus ([Merk and Speit, 1998, 1999](#); [Graves et al., 1996](#); [Grafström et al., 1993](#)) as well as in human TK6 lymphoblast cells ([Goldmacher and Thilly, 1983](#); [Crosby et al., 1988](#); [Craft et al., 1987](#)) as shown in Table C-17.

[Craft et al. \(1987\)](#) measured the induction of mutations in the thymidine kinase (*tk*) locus or at the ouabain resistance (*Oua^r*) locus in TK6 human lymphoblastoid cells. The mutagenesis at *tk* locus can result from base-pair substitutions, small and large deletions, and chromosome exchange events, while mutations at the *Oua^r* locus require specific base-pair substitutions. Lymphoblastoid cells were exposed to single (0, 15, 30, 50, 125, or 150 μ M for 2 hours) or multiple treatments, that is, 3, 5, or 10 treatments of 50, 30, or 15 μ M, respectively, or 4 treatments of 150 μ M for 2 hours

(treatments were spaced 2–4 days apart) with formaldehyde and mutations analyzed. The authors observed a nonlinear increase in *tk* mutagenesis with single treatment of formaldehyde with an increasing slope at $>125\ \mu\text{M}$. Although multiple treatments caused an increase in *tk* mutagenesis, their combined effect was less than the single treatment of equivalent $C \times t$ ($150\ \mu\text{M} \times 2$ hours). No mutations were observed at the *Oua^r* locus in lymphoblasts that received four treatments of $150\ \mu\text{M}$ for 2 hours. *Tk* mutagenesis followed a similar exposure-response curve as DPX formation in this study ([Craft et al., 1987](#)).

Using the same cell system, ([Crosby et al., 1988](#)) showed that repetitive treatments of $150\ \mu\text{M}$ formaldehyde induced mutants at the X-linked hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus. Of these mutants, 14/30 of them contained partial or complete deletions with most of the partial deletions showing unique deletion patterns, while only a third (5/15) of spontaneous mutants had partial or complete deletions, indicating that formaldehyde can induce large losses of DNA in human lymphoblast cells. This work was followed up by ([Liber et al., 1989](#)), who showed that HPRT mRNA from human lymphoblast mutants (16 formaldehyde-induced and 10 spontaneous, both not showing deletions) contained a preferential AT to CG transversion at a specific site ([Liber et al., 1989](#)).

Formaldehyde has been shown to induce *hprt* mutations in CHO cells involving single-base pair transversions mostly occurring at AT sequences ([Graves et al., 1996](#)). Formaldehyde also induced forward mutations in mouse lymphoma L5178Y *tk $\pm$* cells both in the absence and presence of rat liver S9 (higher concentrations were required for an effect with S9). Both toxicity and mutagenicity were abolished when formaldehyde dehydrogenase (FADH) was incorporated in the exposure medium ([Blackburn et al., 1991](#)), suggesting detoxification of formaldehyde.

A study by Merk and Speit ([Merk and Speit, 1998](#)) indicated that formaldehyde-induced DPXs did not result in direct gene mutations in the *hprt* locus of V79 Chinese hamster cells, suggesting that formaldehyde was not mutagenic. However, the *hprt* mutation assay may be insensitive to deletion mutations ([Merk and Speit, 1998](#)) because the *hprt* locus in the V79 cell line is primarily sensitive to point mutations. Additionally, one study showed the formation of deletion mutations by formaldehyde at the same locus in human lymphoblasts ([Crosby et al., 1988](#)).

In the mouse lymphoma assay (L5178Y cells), Speit and Merk ([Speit and Merk, 2002](#)) demonstrated that a 2-hour exposure to formaldehyde was mutagenic in a concentration-dependent manner. Mutation was mainly attributed to a strong increase in small colony mutants suggestive of CAs. Recombination or deletion of DNA from the *tk* locus was primarily responsible for the loss of heterogeneity, thereby leading to the observed mutant phenotype. This mutagenic finding in the L5178Y cell mouse lymphoma system, which is likely to occur by a clastogenic mechanism rather than by point mutations ([Speit and Merk, 2002](#)), is consistent with that of ([Craft et al., 1987](#)), who demonstrated formaldehyde mutagenicity at the *tk* locus of TK6 cells, and also with the findings of ([Grafstrom et al., 1984](#)), who demonstrated increased SSB formation in formaldehyde-exposed cell lines.

Transformation

Formaldehyde has also been shown to induce cell transformation in mouse embryo fibroblasts ([Ragan and Boreiko, 1981](#); [Frazelle et al., 1983](#); [Boreiko and Ragan, 1983](#)) and hamster kidney cells ([Plesner and Hansen, 1983](#)) as shown in Table C-17. In mouse embryonic C3H/10T^{1/2} cells, a single exposure to formaldehyde (0.003–0.083 mM) for 24 hours did not induce transformation; however, when formaldehyde treatment was followed by continuous treatment with 0.1 µg/mL with the tumor promoter 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), a dose-dependent increase in transformation was observed at low concentrations of 0.003 mM ([Boreiko and Ragan, 1983](#)) or 0.017 mM ([Ragan and Boreiko, 1981](#)) formaldehyde. Ragan and Boreiko ([1981](#)) have also shown that treatment of mouse embryo fibroblasts with varying doses of formic acid (≈2 to 22 mM) or methanol (≈0.11 to 1.1 M) did not induce transformation either alone or following TPA promotion in mouse embryo fibroblasts. The authors concluded that since commercial formalin contains 10% methanol, and use of 105 times higher methanol concentrations (≈2.2 M) in this experiment ruled out the background interference of methanol (precursor to formaldehyde) or formic acid (a metabolic product of formaldehyde) with formaldehyde-induced cell transformation. In a different study using the same cells, the ability of formaldehyde to act as a tumor promoter was tested with repeated applications of formaldehyde following initiation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) by ([Frazelle et al., 1983](#)) who observed a weak tumor promoting activity of formaldehyde. Another study with a 3-hour exposure to formaldehyde (0.003 to 3.33 mM) with metabolic activation using S9 mix in baby hamster kidney (BHK) cells induced dose-dependent increase in transformation ([Plesner and Hansen, 1983](#)).

p53 mutation and cell death

Four cell lines derived from formaldehyde induced rat nasal squamous cell carcinomas (SCCs) from a previous study ([Recio et al., 1992](#)) were analyzed by Bermudez et al. ([1994](#)) for p53 mutations as shown in Table C-17. These cell lines were aneuploid overexpressing transforming growth factor-α and epidermal growth factor, expression of which is a common feature of SCCs and is frequently found in human tumors. Two of these cell lines contained wild type DNA sequences while the two others possessed mutated p53 gene sequences, being point mutations, specifically having transversions at codons 132 (TTC→TTA) and 271 (CGT→CAT) of the *p53* gene. To understand the mechanism of transformed cell lines converting to the tumor phenotype, the authors injected either the wild type cells or cells with mutant p53 sequences into nude mice. They observed that only cell lines expressing the p53 mutation were tumorigenic, suggesting involvement of specific p53 mutations in the tumorigenicity of formaldehyde. [Wong et al. \(2012\)](#) examined signal transduction pathways in response to formaldehyde exposure. The authors studied p53 phosphorylation in human lung epithelial (H460 cells) and fibroblast cells exposed to formaldehyde and compared the role of different protein kinases using specific inhibitors for ATR, ATM, and DNA-dependent protein kinases, measuring Ser15p53 and thr68-CHK1 phosphorylation,

p53 accumulation, and induction of p21. At low doses, formaldehyde-induced DNA-protein crosslinks caused ATR-mediated activation of p53 in human lung fibroblasts and epithelial cells. The S-phase of the cell cycle seems to be specifically sensitive for this effect without the involvement of topoisomerase binding protein 1 (topBP1). For other pathways, such as BER and NER, mismatch repairs were not affected by p53 activation, suggesting that non-DPX adducts, including DNA-peptide and hmDNA adducts, play a minor role in formaldehyde-induced p53 activation.

Other genotoxic endpoints

As summarized in Table C-17, in vitro formaldehyde exposure induces other genotoxic and related effects in mammalian cells such as unscheduled DNA synthesis (UDS) and DNA repair inhibition.

Unscheduled DNA synthesis

UDS, which represents DNA repair activity following excision of DNA damage, has been reported in rat hepatocytes ([Williams et al., 1989b](#)) and SHE cells ([Hamaguchi and Tsutui, 2000](#)) exposed to formaldehyde. UDS was also observed in HeLa cells ([Martin et al., 1978](#)), but not in human bronchial epithelial cells ([Doolittle et al., 1985](#)) upon formaldehyde exposure. These studies suggest that formaldehyde-induced DNA damage was followed by DNA repair.

DNA repair inhibition

Formaldehyde can inhibit DNA repair and induce cell transformation ([Speit et al., 2000](#); [Grafstrom et al., 1984](#); [Emri et al., 2004](#); [Boreiko and Ragan, 1983](#)) as shown in Table C-17. Studies have shown that formaldehyde causes DNA repair inhibition at a concentration range of 0.125 mM to 10 mM in human bronchial epithelial cells ([Grafstrom et al., 1984](#)) and skin fibroblasts or keratinocytes ([Emri et al., 2004](#)), DNA repair proficient or deficient cell lines (e.g., XP), or cell lines hypersensitive to DNA-DNA crosslinks (e.g., FA) ([Speit et al., 2000](#)). In a study using human keratinocytes and fibroblasts, [Emri et al. \(2004\)](#) tested the formation of DNA SSBs induced by ultraviolet (UV) irradiation by UVB or UVC with or without prior treatment with 10 μ M formaldehyde. The authors reported that SSB induced by UV irradiation alone were repaired within 3–6 hours of exposure, while cells with UV irradiation followed by formaldehyde exposure had higher SSBs at the same time points due to increased chromosomal damage, suggesting that formaldehyde exposure altered the repair kinetics in these cells.

Aneuploidy

Studies on aneuploidy in various in vitro cell systems have provided mixed results as shown in Table C-17. For example, an increase in aneuploidy was observed in hamster CHO cells ([Kumari et al., 2012](#)) and human erythropoietic stem cells ([Ji et al., 2014](#)). However, no increase in

aneuploidy cells were observed in hamster V79 lung epithelial cells ([Speit et al., 2011a](#); [Kuehner et al., 2012](#)) or in human myeloid progenitor cells ([Kuehner et al., 2012](#)).

Table C-17. Summary of in vitro genotoxicity studies of formaldehyde in mammalian cells

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
p53 Mutations					
Rat Nasal tumor cell lines	NA	+	ND	cell lines derived from nasal tumors of rats from 2-year tumor study; rats exposed to 18.5 mg/m ³ HCHO, 6 hrs/d, 5 d/wk for 2 years	(Bermudez et al., 1994)
Deletion mutations					
Mouse Lymphoma L5178Y tk ^{+/-} cells	0.063 mM HCHO (commercial)	+	ND	2 hrs; mouse lymphoma assay; cytotoxic at 250 μM conc.	(Speit and Merk, 2002)
	0.8 mM 37% HCHO + 10% methanol	ND	+	3 hrs; MF at TK locus; 40–50% total growth at 0.8 mM dose	(Mackerer et al., 1996)
Hamster CHO cells/Hprt locus	0.3 mM HCHO (37% w/w)	+	ND	1 hr; 6-TG resistant mutants; dose-dependent ↓ in CFE and ↑ in MF	(Grafström et al., 1993)
	0.5 mM HCHO (commercial)	–	ND	4 hrs; HPRT assay; (T) by relative CE ≥ 0.125 mM	(Merk and Speit, 1998)
	1 mM HCHO (40% aq. Sol.)	+	ND	1 hr; 6-TG resistant colonies; base transversions at AT base pairs	(Graves et al., 1996)
Hamster V79 lung epithelial cells	0.5 mM HCHO (commercial)	–	ND	4 hrs; HPRT assay; (T) by relative CE ≥ 0.25 mM	(Merk and Speit, 1999)
Human Bronchial fibroblasts/epithelial cells (HPRT locus)	0.1 mM HCHO (commercial)	+	ND	5 hrs; 6-TG resistant mutants scored; MF nonlinear dose-dependent ↑; (T) > 0.1 mM by CFE	(Grafstrom et al., 1985)
Human Lymphoblasts/TK 6	0.03 mM 37% HCHO + 10-15% methanol	+	ND	2 hrs; MF at TK locus measured; single exposure (0–150 μm) nonlinear ↑ in MF; (T) at 0.125 mM	(Craft et al., 1987)
	0.13 mM 37% HCHO + 10-15% methanol	+	ND	2 hrs; MF at TK locus; cell survival was 15% at 0.15 mM; cells treated for 2 hrs with 0.07 mM methanol were not mutagenic, not cytotoxic	(Goldmacher and Thilly, 1983)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
	0.15 mM HCHO (commercial)	+	ND	8 exposures × 4 d, 2 hrs dosing; MF at HPRT locus; MF 12.4-fold higher over background; (T) 50% survival each treatment	(Crosby et al., 1988)
Point mutations					
Mouse Lymphoma cells/TK+/-	0.1 mM (-S9) and 0.5mM (+S9) 37% HCHO +10% methanol	+, -	+, -	NR; assay supplemented with FDH and NAD ⁺ ; MF at the TK locus; results indicate without and with FDH/NAD ⁺ , respectively; 50% (T) at 0.1 mM (-S9) and 0.5 mM (+S9) with FDH	(Blackburn et al., 1991)
	0.14 mM HCHO form not specified	+	ND	4 hrs; MF at TK locus; highly mutagenic but total growth is very low	(Wangenheim and Bolcsfoldi, 1988)
Hamster CHO cells/Hprt locus	1 mM HCHO (40% aq. Sol.)	+	ND	1 hr; 6-TG resistant colonies had base transversions at AT base pairs	(Graves et al., 1996)
Human Lymphoblast/TK6	0.15 mM HCHO (commercial)	+	ND	2 hrs (8 times); sequence analysis of HPRT mutants showed base substitutions at AT base pairs	(Liber et al., 1989)
DNA-protein crosslinks					
Mouse Hepatocytes	0.5 mM [¹⁴ C] HCHO (aq. Sol.)	+	ND	2 hrs; nonlinear dose-dependent ↑ in DPX.	(Casanova et al., 1997)
	0.5 mM [¹⁴ C] HCHO (aq. Sol.)	+	ND	2 hrs; HPLC analysis of DNA digest; Dose-dependent ↑ in DPX.	(Casanova and Heck, 1997)
Mouse L5178Y tk ^{+/-} Lymphoma cells	0.031 mM HCHO (commercial)	+	ND	2 hrs; DPX show dose-response; cytotoxic at 250 μM conc.	(Speit and Merk, 2002)
Mouse Leukemia L1210 cells	0.125 mM 37% HCHO	+	ND	1 hr; (T) at 0.3 μM conc.	(Ross et al., 1981)
	0.2 mM 37% HCHO	+	ND	2.5 hrs; (T) ≥ 0.175 mM	(Ross and Shipley, 1980)
Mouse Bone marrow mesenchymal cells	0.125 mM HCHO (37%)	+	ND	12 hrs; Alkaline comet assay; (T) from 0.175 mM to 0.2 mM	(She et al., 2013)
Rat C18 tracheal epithelial cell line	0.1 mM PFA in PBS	+	ND	1.5 hrs; DPX analyzed by alkaline elution; (T) at 0.4 mM	(Cosma and Marchok, 1988)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
Rat Aortic endothelial cells	0.5 mM HCHO (commercial)	+	ND	1.5 hrs; K+/SDS assay; dose- dependent ↑ in DPX ≥ 2 hrs; (T) by LDH release at 2 mM	(Lin et al., 2005)
Rat Primary tracheal epithelial cells	0.05 mM PFA in PBS	+	ND	1.5 hrs; DPX analyzed by alkaline elution; (T) > 0.2 mM	(Cosma and Marchok, 1988)
	3.34 mM HCHO/PBS	+	ND	3 hrs; dose-dependent ↑ in DPX	(Cosma and Marchok, 1988)
Rat Yoshida lymphosarcoma cells	0.25 mM HCHO (36% sol)	+	ND	4 hrs; alkaline elution assay; (T) ID ₅₀ 0.25 mM	(O'Connor and Fox, 1987)
Hamster CHO cells	0.125 mM HCHO (commercial)	+	ND	2 hrs; BrdU incorporation-FPG technique; conc.-related ↓ DNA migration inhibition	(Lorenti Garcia et al., 2009)
	0.2 mM HCHO (NS)	+	ND	1.5 hrs; dose-dependent ↑ in DPX up to 2 mM HCHO; values visually determined from graph	(Zhirkovich and Costa, 1992)
	0.25 mM HCHO (NS)	+	ND	1.5 hrs; dose-dependent ↑ in DPX formation up to 2 mM HCHO; values visually determined from graph	(Olin et al., 1996)
	0.5 mM HCHO (commercial)	+	ND	1.5 hrs; alkaline elution assay; DPX showed dose-dependent ↑ (0.5–4.5 mM); 82% viability at 4.5 mM HCHO	(Marinari et al., 1984)
Hamster V79 lung epithelial cells	0.01 mM 16% HCHO (ultrapure methanol free)	+	ND	1 hr; Comet assay; dose- dependent ↓ in DNA migration at HCHO ≥ 0.01 mM;	(Speit et al., 2007b)
	0.025 mM 16% HCHO (ultrapure methanol free);	+	ND	4 hrs; Comet assay; dose- dependent ↓ DNA migration; (T) at 0.2 mM by cell counts/proliferation index	(Speit et al., 2008a)
	0.0625 mM HCHO (commercial)	+	ND	4 hrs; Comet assay; dose- dependent ↑ migration inhibition (0.0625–0.5 mM); (T) by relative CE ≥ 0.25 mM	(Merk and Speit, 1999)
	0.125 mM HCHO (commercial)	+	ND	4 hrs; K-SDS assay; nonlinear dose-dependent ↑ in DPX (values visually determined from graph); HCHO (T) by relative CE assay ≥ 0.125	(Merk and Speit, 1998)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
Human Nasal epithelial cells	0.2 mM 16% HCHO (ultrapure methanol free)	+	ND	1 hr; Comet assay; dose-dependent ↑ DPX from 0.05–0.3 mM; (T) by CF ≥ 0.02 mM	(Speit et al., 2008b)
Human A549 lung epithelial cells	0.2 mM 16% HCHO (ultrapure Methanol free)	+	ND	1 hr & 4 hrs; Comet assay; dose-dependent ↑ migration inhibition from 0.1–0.3 mM; (T) by CF ≥ 0.02 mM	(Speit et al., 2008b)
	0.2 mM HCHO (stabilized with methanol)	+	ND	3 hrs; KCl/SDS method; DPX time-dependent ↑ up to 12 hrs; T ¹ / ₂ 12.5 hrs; (T) ≥ 0.2 mM by CF assay	(Quievryn and Zhitkovich, 2000)
	0.2 mM 16% HCHO (aqueous solution, methanol-free)	+	ND	1 or 3 × 24 hr intervals; comet assay	(NTP, 2010)
Human Lung/bronchial epithelial cells	0.1 mM HCHO (commercial)	+	ND	1 hr; alkaline elution technique; (T) 0.021 mM ID ₅₀ by growth inhibition	(Saladino et al., 1985)
	0.1 mM HCHO (commercial)	+	ND	1 hr; alkaline elution technique; (T) at 0.3 mM by CFE	(Grafstrom et al., 1986)
	0.2 mM 37% HCHO (w/w)	+	ND	1 hr; alkaline elution technique; (T) at 1 mM	(Grafstrom et al., 1984)
	2 mM HCHO (Not Specified)	+	ND	1 hr; Alkaline elusion technique;	(Grafstrom, 1990)
	0.39 mM HCHO	+	ND	4 hrs; KCl-SDS method	(Duan, 2011)
	0.8 mM 37% HCHO	+	ND	1 hr; alkaline elution	(Fornace et al., 1982)
Human Bronchial epithelial cells/fibroblasts	0.1 mM 37% HCHO	+	ND	1 hr; alkaline elution technique	(Grafstrom et al., 1983)
Human Fibroblasts (diploid)/HF/SV40	0.2 mM HCHO + Methanol)	+	ND	3 hrs; (T) ≥ 0.2 mM by CF assay; DPX half-life is 12.5 hrs	(Quievryn and Zhitkovich, 2000)
Human Fibroblast (Bronchial/Skin)	0.25 mM HCHO (NS)	+	ND	1.5 hrs; DPX dose-response not prominent; values visually determined from graph	(Olin et al., 1996)
Human Skin keratinocytes/fibroblasts	0.025 mM HCHO (NS)	+	ND	8 hrs with subsequent exposure to methyl methane sulfonate (0.25 mM)	(Emri et al., 2004)
Human XP fibroblasts	0.2 mM 37% HCHO (w/w)	+	ND	1 hr; alkaline elution technique; DPX T ¹ / ₂ 2–3 hrs	(Grafstrom et al., 1984)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
Human Normal, XPA and FA repair deficient fibroblasts	0.125 mM HCHO (commercial)	+	ND	2 hrs; Comet assay; dose-dependent DNA migration inhibition; No migration inhibition after 24 hrs	(Speit et al., 2000)
Human Fibroblasts/XP-F and XP-A	0.2 mM HCHO (stabilized with Methanol)	+	ND	3 hrs; DPX removal XP-A = XP-F cells; (T) ≥ 0.2 mM by CF assay	(Quievryn and Zhitkovich, 2000)
Human Lymphocytes	0.05 mM 10% formalin	+	ND	1 hr; comet assay; KCl/SDS assay; nonlinear dose-dependent ↑ ≥ 50 µM HCHO	(LICM, 2006)
	0.1 mM; 0.3 mM HCHO in water	+	-	3 hrs; (T) at 0.3 mM (+S9)	(Andersson et al., 2003)
	0.2 mM HCHO + Methanol)	+	ND	3 hrs; KCl/SDS method; DPX T ¹ / ₂ 18.1 hrs; (T) ≥ 0.2 mM by CF assay	(Quievryn and Zhitkovich, 2000)
Human White blood cells	0.001 mM HCHO (NS)	+	ND	1.5 hrs; Dose-dependent ↑ in DPX formation up to 2 mM HCHO; values visually determined from graph	(Shaham et al., 1996)
Human Whole blood cultures	0.025 mM 16% HCHO (ultrapure Methanol free)	+	ND	exposure duration not specified; Comet assay; dose-dependent migration inhibition; DPX ≥ 0.2 mM persist for 24 hrs	(Schmid and Speit, 2007)
Human Lymphoblast/TK6	0.05 mM 37% HCHO + 10-15% Methanol	+	ND	2 hrs; MF at TK locus measured; (T) at 0.125 mM	(Craft et al., 1987)
Human Lymphoblast/TK6	0.1 mM 16% HCHO (ultrapure MetOH free)	+	ND	2 hrs; Comet assay with g-irradiation; DPX formation dose-dependent; (T) at 0.1 mM 24 hrs by MTT assay	(Kuehner et al., 2013)
Human lymphoblasts (PD20 & PD20-D2)	0.125 mM 37% HCHO	+	ND	24 hrs; Dose-dependent ↑ in DPX from 0.05-0.15 mM; PD20>PD20-D2; (T) >0.15 mM	(Ren et al., 2013)
Human EBV-Burkitt's lymphoma cells	0.03% PFA in water	+	ND	18 hrs; Dose-dependent ↑ in DPX; (T) 0.01% PFA	(Costa et al., 1997)
Human T-leukemia (Jurkat E6-1) cells	1 mM HCHO (commercial)	+	ND	2 hrs; SDS-PAGE; (T) ≥ 1 mM by cell death assay	(Saito et al., 2005)
Human HeLa cells	0.05 mM 10% formalin	+	ND	1 hr; KCl/SDS precipitation method; (T) ≥ 100 mM by absorbance after 12 hrs; dose-	(LICM, 2006)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
				dependent ↑ in DPX; repaired within 18 hrs after HCHO removal	
Human Kidney cells/Ad293	0.2 mM HCHO + Methanol	+	ND	3 hrs; KCl/SDS method; DPX T ¹ / ₂ 12.5 hrs; (T) ≥ 0.2 mM by CF assay	(Quievryn and Zhitkovich, 2000)
Human Gastric mucosa cells	1 mM HCHO	+	ND	1 hr; (T) not reported	(Blasiak et al., 2000)
DNA adducts					
Hamster CHO cells	1 mM [³ H] 37% HCHO/10-15% Methanol	+	ND	2 hrs; (T) ≥ 2.5 mM	(Beland et al., 1984)
Human Nasal epithelial cells	0.33 mM 37% HCHO + 10% Methanol	+	ND	24 hrs; hmdA and hmdG adducts dose-dependent ↑. Viability showed dose-dependent from 10 500 mM;	(Zhong and Que Hee, 2004)
Human HeLa cells	0.5 mM [¹³ CD ₂]HCHO (20% in heavy water)	+	ND	3 hrs; No (T) information provided	(Lu et al., 2012a)
Chromosomal aberrations (CA)					
Hamster CHO cells (AA8) and their mutants (UV4, UV5, UV61)	0.15 mM HCHO (commercial)	+	ND	2 hrs; BrdU incorporation-FPG technique; dose-dependent ↑ in Cas	(Lorenti Garcia et al., 2009)
Hamster CHO cells	0.2 mM PFA in water	+	+	2 hrs; BrdU incorporation; dose-dependent ↑ in SCE +/- S9	(Natarajan et al., 1983)
Hamster CHO cells mutants (KO40)	0.2 mM HCHO (commercial)	+	ND	2 hrs; BrdU incorporation-FPG technique; dose-dependent ↑ in CAs	(Lorenti Garcia et al., 2009)
Hamster CHO cells	0.53 mM HCHO	(+)	(+)	8–12 hrs; Giemsa staining	(Galloway et al., 1985)
Hamster Lung fibroblasts	0.6 mM Formalin	+	ND	24 hrs; microscopic evaluation	(Ishidate et al., 1981)
Hamster/Syrian Embryo cells	0.033 mM 37% HCHO + 7–13% Methanol	+	ND	24 hrs; CA assay; 85% relative CFE at 0.099 mM	(Hikiba et al., 2005)
Human Fibroblasts	2 mM HCHO (NS)	+	ND	0.25 hr; Giemsa staining; dose-dependent ↑ in CA	(Levy et al., 1983)
Human Lymphocytes	0.125 mM HCHO (NS)	+	ND	1 hr; PCC technique; dose-dependent ↑ in CA	(Dresp and Bauchinger, 1988)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
Human lymphoblasts (PD20 & PD20-D2)	0.125 mM 37% HCHO	+	ND	24 hrs; Dose-dependent ↑ in CA from 0.05-0.15 mM; PD20=PD20-D2; (T) >0.15 mM	(Ren et al., 2013)
Human lymphocytes	0.25 mM, 0.5 M 37% HCHO + 10% Methanol	+	+	1 hr; conc. Respectively, for chromatid breaks and gaps; proliferation inhibition at 1 M (-S9) and 0.5 mM (+S9)	(Schmid et al., 1986)
Micronucleus (MN)					
Mouse erythropoietic cells	0.025 mM HCHO (37% + 10-15% methanol)	+	ND	1 hr; Dose-dependent in MN from 0.025-0.1 mM	(Ji et al., 2014)
Hamster V79 lung epithelial cells	0.075 mM 16% HCHO (ultrapure Methanol free);	+	ND	2 hrs; MN test; MN ≥ 0.075 mM; dose-dependent ↑ in MN	(Speit et al., 2007b)
	0.1 mM 16% HCHO (ultrapure Methanol-free);	+	ND	4 hrs; MN test; dose-dependent in MN; (T) at 0.2 mM by cell counts/proliferation index	(Speit et al., 2007b)
	0.125 mM HCHO (commercial)	+	ND	4 hrs; MN assay with AO staining; nonlinear dose-dependent ↑ in MN (values visually determined from graph); (T) by relative CE ≥ 0.125 mM	(Merk and Speit, 1998)
Human A549 lung epithelial cells	0.15 mM 16% HCHO (ultrapure, methanol-free)	+	ND	2 hrs (0.3 mM) or 30 hrs (0.15 mM); CBMN assay; Mostly centromere -ve by FISH analysis	(Speit et al., 2011a)
Human Normal, XPA and FA repair deficient fibroblasts	0.125 mM HCHO (commercial)	+	ND	2 hrs; MN test; MN ≥ 0.075 mM; dose-dependent ↑ in MN; normal<XPA<FA	(Speit et al., 2000)
Human lymphoblasts (PD20 & PD20-D2)	0.125 mM 37% HCHO	+	ND	24 hrs; Dose-dependent ↑ in MN from 0.05-0.15 mM; PD20>PD20-D2; (T) >0.15 mM	(Ren et al., 2013)
Human Whole blood cultures	0.3 mM 16% HCHO (ultrapure, methanol-free)	+	ND	27 hrs; CBMN assay; mostly centromere negative by FISH analysis	(Speit et al., 2011a)
Human Whole blood cultures	0.3 mM 16% HCHO (ultrapure Methanol free);	+	ND	24 hrs; HCHO dosed 44 hrs after culture; MN test; dose-dependent ↑ in MN (0.1–0.4 mM); (T) ≥ 0.3 mM by NDI	(Schmid and Speit, 2007)
Single strand breaks (SSB)					
Mouse	0.125 mM	-	ND	1 hr; (T) at 0.3 mM	(Ross et al., 1981)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
Leukemia L1210 cells	37% HCHO				
	0.2 mM 37% HCHO	(+)	ND	2.5 hrs; (T) ≥ 0.175 mM	(Ross and Shipley, 1980)
Rat Hepatocytes	1 mM HCHO (NS)	+	ND	4 hrs; HCHO cytotoxic ≥1.5 mM; dose-dependent ↑ in SSB, enhanced by GSH depletion	(Demkowicz-Dobrzanski and Castonguay, 1992)
Rat -tracheal epithelial cell line	0.2 mM PFA in PBS	+	ND	1.5 hrs; SSB analyzed by alkaline elution; HCHO toxic at 0.4 mM	(Cosma and Marchok, 1988)
Rat Yoshida lymphosarcoma cells	0.25 mM HCHO (36% sol)	+	ND	4 hrs; alkaline elution assay; (T) ID ₅₀ 0.25 mM	(O'Connor and Fox, 1987)
Hamster CHO cells	4.5 mM HCHO (commercial)	–	ND	1.5 hrs; 82% viability at 4.5 mM HCHO	(Marinari et al., 1984)
Hamster V79 lung epithelial cells	0.2 mM 16% HCHO (ultrapure Methanol free)	–	ND	1 hr; Comet assay	(Speit et al., 2007b)
Human Bronchial epithelial cell	0.1 mM 37% HCHO	+	ND	1 hr; alkaline elution technique; (T) at 0.3 mM	(Grafstrom et al., 1983)
	0.3 mM 37% HCHO (w/w)	+	ND	1 hr; SSB dose-dependent ↑; SSB 3 times higher than XP cells	(Grafstrom et al., 1984)
Human Lung/bronchial epithelial cells	0.1 mM HCHO (commercial)	+	ND	1 hr; alkaline elution technique; (T) 0.021 mM ID ₅₀ by growth inhibition	(Saladino et al., 1985)
	0.1 mM HCHO (commercial)	+	ND	1 hr; alkaline elution technique; (T) at 0.3 mM by CFE	(Grafstrom et al., 1986)
	0.8 mM 37% HCHO	+	ND	1 hr; alkaline elution	(Fornace, 1982)
Human Lung/bronchial epithelial (A549) cells	1.0 mM HCHO (commercial)	+	ND	8–72 hrs; Dose-dependent in ↑ DSB formation; DSB formed when viability, determined by MTT assay, was >60%	(Vock et al., 1999)
Human Skin keratinocytes/fibroblasts	0.1 mM HCHO (NS)	–	ND	20 hrs	(Emri et al., 2004)
Human XP fibroblasts	0.3 mM 37% HCHO (w/w)	+	ND	1 hr; SSB dose-dependent ↑	(Grafstrom et al., 1984)
Human	0.1 mM 37% HCHO + 10% Methanol	+	ND	0.5 hr; nick translation assay; low doses induce SSB	(Snyder and van Houten, 1986)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
Foreskin fibroblasts	0.25 mM 37% HCHO + 10% Methanol	–	ND	0.5 hr; alkaline sucrose sedimentation analysis; high doses don't induce SSB	(Snyder and van Houten, 1986)
Human HeLa cells	0.005 mM 10% formalin	+	ND	1 hr; Comet assay; (T) ≥ 100 μM after 12 hrs; SSB repaired within 90 min	(LICM, 2006)
Human Lymphocytes, peripheral blood	0.005 mM 10% formalin	+	ND	1 hr; comet assay; KCl/SDS assay; nonlinear dose-dependent ↑ ≥ 50 μM HCHO	(LICM, 2006)
Sister chromatid exchanges (SCE)					
Hamster CHO cells	0.03 mM 37% HCHO with 10% methanol	+	ND	24 hrs; BrdU incorporation; SCE dose-dependent ↑	(Obe and Beek, 1979)
	0.04 mM HCHO (commercial)	(+)	(+)	26 hrs; BrdU incorporation-FPG technique	(Galloway et al., 1985)
	0.2 mM PFA in water	+	+	2 hrs; BrdU incorporation; dose-dependent ↑ in SCE +/- S9	(Natarajan et al., 1983)
Hamster CHO cells (AA8) and their mutants (UV4, UV5, UV61, KO40)	0.15 mM HCHO (commercial)	+	ND	2 hrs; BrdU incorporation-FPG technique; dose-dependent ↑ in CAs	(Lorenti Garcia et al., 2009)
Hamster Embryo cells	0.01 mM 37% HCHO/7–13% Methanol;	+	ND	24 hrs; BrdU incorporation; dose-dependent ↑ in SCE; (T) by relative CE 68% at 0.033 mM	(Miyachi and Tsutsui, 2005)
Hamster V79 lung epithelial cells	0.05 mM 16% HCHO (ultrapure, methanol-free)	+	ND	24 or 28 hrs exposure to HCHO and BrdU; Aneuploidy and Toxicity measured by SCE and PI, respectively	(Speit et al., 2011a)
	0.06 mM 37% HCHO with 10% methanol	+	–	28 hrs; formalin + activation with primary rat hepatocytes; (T) at 0.54 mM (+S9) and 0.2 mM (–S9)	(Basler et al., 1985)
	0.1 mM 16% HCHO (ultrapure Methanol free);	+	ND	2 hrs; BrdU labeling; SCE ≥ 0.1 mM; genotoxicity paralleled cytotoxicity; (T) ≥ 0.1 mM by PI	(Speit et al., 2007b)
	0.1 mM 16% HCHO (ultrapure Methanol free);	+	ND	1 hr; BrdU labeling; SCE dose-dependent ↑ (0.1-0.2 mM)	(Neuss and Speit, 2008)
	0.1 mM 16% HCHO (ultrapure Methanol free);	+	ND	4 hrs; BrdU labeling; dose-dependent in SCE; (T) at 0.2 mM by cell counts/proliferation index	(Speit et al., 2008a)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
	0.125 mM HCHO (commercial)	+	ND	4 hrs; BrdU incorporation; dose-dependent ↑ in SCE; (T) by relative CE ≥ 0.125 mM	(Merk and Speit, 1998)
	0.125 mM HCHO (commercial)	+	ND	4 hrs; BrdU incorporation; dose-dependent ↑ in SCE; (T) by relative CE ≥ 0.25 mM	(Merk and Speit, 1999)
	0.13 mM 37% HCHO with 10% methanol	+	ND	2 hrs; (T) at 0.54 mM	(Basler et al., 1985)
	0.13 mM; 0.20 mM 37% HCHO with 10% methanol	+	–	3 hrs; (T) at 0.4 mM (–S9)	(Basler et al., 1985)
Human A549 lung epithelial cells	0.1 mM 16% HCHO (ultrapure Methanol free);	+	ND	1 hr; BrdU labeling; SCE dose-dependent ↑ (0.1–0.3 mM)	(Neuss and Speit, 2008)
Human A549 + V79 cells (co-cultivated)	0.05 mM 16% HCHO (ultrapure Methanol free);	+	ND	1 hr; BrdU labeling; SCE dose-dependent ↑ (0.05–0.2 mM); treated A549 cells not washed before adding V79 cells	(Neuss and Speit, 2008)
Human A549 + V79 cells (co-cultivated)	0.3 mM 16% HCHO (ultrapure Methanol free);	–	ND	1 hr; BrdU labeling; treated A549 cells washed before adding V79 cells	(Neuss and Speit, 2008)
Human Lymphocytes	0.125 mM 37% HCHO + 10% Methanol	+	+	1 hr; BrdU labeling; proliferation inhibition at 1 M (–S9) and 0.5 mM (+S9)	(Schmid et al., 1986)
	0.167 mM 37% HCHO + 10% Methanol	+	ND	24 hrs; BrdU incorporation; dose-dependent ↑ in SCE	(Obe and Beek, 1979)
	0.167 mM formalin or PFA	+	ND	72 hrs; BrdU incorporation with fluorescence + Giemsa method; (T) ≥ 0.33 mM and similar for formalin and PFA; dose-dependent ↑ for formalin reported	(Krieger et al., 1983)
Human Whole blood cultures	0.2 mM 16% HCHO (ultrapure Methanol free)	+	ND	72 hrs; BrdU labeling; no dose-response; (T) at 0.2 mM by PI	(Schmid and Speit, 2007)
Unscheduled DNA synthesis (UDS)					
Rat Hepatocytes	400 mM HCHO (NS)	+	ND	18–20 hrs; [³ H]dThd incorporation and autoradiography	(Williams et al., 1989a)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
Human Bronchial epithelial cells	0.1 mM 37% HCHO (reagent grade sol.)	–	ND	22 hrs; [³ H]dThd incorporation and autoradiography; (T) ≥ 1 mM	(Doolittle et al., 1985)
Human Foreskin fibroblasts	0.5 mM 37% HCHO + 10% Methanol	–	ND	0.5 hr; UDS	(Snyder and van Houten, 1986)
Human Bronchial fibroblasts	1 mM 37% HCHO	–	ND	1 hr; [³ H-Thymidine] incorporation.	(Grafstrom et al., 1983)
Human Embryo cells	0.1 mM HCHO (37% sol)	+	ND	1 hr; [³ H]dThd incorporation; dose-dependent ↑ in UDS (0.1-1 mM)	(Hamaguchi and Tsutui, 2000)
Human HeLa cells	0.001 mM HCHO (commercial)	+	ND	2.5 hrs; [³ H]dThd incorporation	(Martin et al., 1978)
DNA repair inhibition					
Human Skin keratinocytes/fibr oblasts	0.01 mM HCHO (NS)	+	ND	0.5 hr after exposure to UVB	(Emri et al., 2004)
Human Normal, XPA and FA repair deficient fibroblasts	0.125 mM HCHO (commercial)	+	ND	2 hrs	(Speit et al., 2000)
Cell transformation					
Mouse Embryo fibroblast/C3H10T ¹ / ₂ cells	0.003 mM HCHO (37%)	+	ND	24 hrs; HCHO treatment followed by TPA treatment, transformation +ve and dose-dependent; (T) ≥ 0.017 mM	(Boreiko and Ragan, 1983)
	0.017 mM HCHO (37% w/w) exposure	+	ND	24 hrs HCHO, 6 wks to medium ± TPA. HCHO +TPA +ve, dose- dependent ↑ (0.017–0.34 mM); HCHO alone –ve (0.083 mM); methano + TPA or formic acid + TPA –ve. HCHO cytotoxic at 0.033 mM	(Ragan and Boreiko, 1981)
Mouse Embryo fibroblast/C3H10T ¹ / ₂ cells	0.033 mM HCHO (37% w/w) exposure;	[+]	ND	4 hrs initiation with 0.5 µg/mL MNNG, promotion on days 5, 8, 15, 22, 29, 36 with HCHO with change of medium	(Frazelle et al., 1983)
Hamster	0.03 mM HCHO 37% aq.sol.	+	+	3 hrs; Style's cell transformation assay; transformation dose-	(Plesner and Hansen, 1983)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Dose/ Concentration ^a	Results ^b		Comments (duration; endpoint method; toxicity)	Reference
		-S9	+S9		
Kidney cell/BHK-21/cl.13				dependent ↑ (0.03–0.67 mM); (T) ≥ 0.67 mM	
Aneuploidy					
Hamster CHO cells (WT & XPF-deficient)	0.3 mM HCHO (Not Specified)	+	ND	4 hrs; Wright's stain and G-banding; +ve for tetraploidies and polyploidies	(Kumari et al., 2012)
Hamster V79 lung epithelial cells	0.05 mM HCHO, 16% ultra-pure, methanol-free	–	ND	7 d exposure; FISH analysis; (T) at 0.05 mM by CFA	(Kuehner et al., 2012)
Hamster V79 lung epithelial cells	0.1 mM HCHO, 16% ultra-pure, methanol-free	–	ND	24 or 28 hrs exposure to HCHO and BrdU; Aneuploidy and Toxicity measured by SCE and PI, respectively	(Speit et al., 2011a)
Human A549 lung epithelial cells	0.05 mM HCHO, 16% ultra-pure, methanol-free	–	ND	14 d exposure; FISH analysis; (T) at 0.02 mM by CFA	(Kuehner et al., 2012)
Human myeloid progenitor cells	0.05 mM HCHO, 16% ultra-pure, methanol-free	–	ND	9 d exposure; Aneuploidy in chromosomes 6, 7, and 8 tested by FISH analysis; (T) at 0.1 mM by CFA	(Kuehner et al., 2012)
Human erythropoietic stem cells	0.05 mM HCHO (37% +10–15% methanol)	+	ND	5 d; FISH analysis; Combined analysis of monosomies or trisomies of 7 and 8 are positive	(Ji et al., 2014)

^aLowest effective concentration (LEC) for positive results or highest ineffective concentration tested (HIC) for negative or equivocal results.

^b+ = positive; – = negative; (+), equivocal.

6-TG, 6-thioguanine; CF, colony formation; FA, Fanconi anemia; FDH, formaldehyde dehydrogenase; FPG, fluorescence plus Giemsa technique; HCHO, formaldehyde; hmdA, hydroxymethyl-deoxyadenosine; hmdG, hydroxymethyl-deoxyguanosine; hmDNA, hydroxymethyl-DNA; HPRT, hypoxanthine phosphoribosyl transferase; ID₅₀, HCHO concentration causing 50% growth inhibition compared to control cells; MF, mutation frequency; MN, micronucleus; NAD, nicotinamide adenine dinucleotide; ND, not done; NDI, nuclear division index; NR, not reported; NS, not specified; PFA, paraformaldehyde; PCC, premature chromosome condensation; PI, proliferation index; SCC, squamous cell carcinoma; SCE, sister chromatid exchange; (T), toxicity or cytotoxicity; TK, thymidine kinase; XP, xeroderma pigmentosum; AA8, parental CHO cells; CHO cell mutants deficient in nucleotide excision repair (UV4 & UV5), or transcription-coupled repair (UV61) or crosslink repair-deficient (KO40).

Summary of in vitro genotoxicity of formaldehyde

In vitro genotoxicity of formaldehyde has been reported in several mammalian cell culture systems (see Table C-17). Formaldehyde is mutagenic in several mouse lymphoma cells, Chinese hamster ovary (CHO) and hamster lung epithelial (V79) cells, a human lung epithelial carcinoma (A549) cell line, fibroblasts, gastric mucosa cells, and human peripheral blood lymphocytes (PBLs) and lymphoblasts. As shown in Table C-17, several genotoxicity endpoints, such as DNA-protein crosslinks, hydroxymethyl-DNA adducts, single strand breaks, cytogenetic markers, such as

micronucleus, chromosomal aberrations, and sister chromatid exchanges, and other genotoxic endpoints, such as unscheduled DNA synthesis, DNA repair inhibition, and cell transformation have been demonstrated in animal and human cell systems.

Cell lines derived from formaldehyde-induced rat nasal squamous cell carcinomas showed *p53* mutations and the mutant cells were tumorigenic when injected in nude mice, suggesting the mutagenicity and carcinogenicity of formaldehyde. Further, formaldehyde induced deletions and point mutations at the thymidine kinase (*tk*) locus in cultured mouse lymphoma cells and human lymphoblasts or at the hypoxanthine phosphoribosyl transferase (*hprt*) locus in CHO and V79 cells, and the mutations showed a dose-dependent increase. Further, these mutations contained base substitutions at the AT base pairs at both these loci.

Evidence of formaldehyde-induced genotoxicity was observed in rodent and human cells wherein a dose-dependent increase in DPX formation was reported over a range of formaldehyde concentrations (0.01–0.0625 mM) (see Table C-17). DPX are formed within an hour of exposure and removed within 24 hours after formaldehyde removal in cultured human cells. The average half-life ($t_{1/2}$) of DPX is 2–3 hours in xeroderma pigmentosum (XP) fibroblasts, 12.5 hours in Ad293 kidney cells and A549 cells, and 18.1 hours (range 1–60 hours) in PBLs. The higher removal time in PBLs is either due to low levels of glutathione in lymphocytes or inefficient repair. Thus, the existing data suggest that repair of DPX depends on the cell type. The removal of DPX is carried out either by spontaneous hydrolysis or other DNA repair processes; however, no difference in DPX removal has been observed between normal human fibroblasts and fibroblasts from XP or Fanconi anemia cell lines, suggesting a lack of involvement of nucleotide excision repair in the repair process. In proliferating cells, unrepaired DPX can arrest DNA replication and lead to the induction of other genotoxic effects such as SCEs. Further evidence of DNA reactivity was observed in CHO cells, HeLa cells, and human nasal epithelial cells wherein formaldehyde induced hm-DNA adducts.

Among the other types of genotoxicity, formaldehyde induced SSBs in several mammalian cell systems, including mouse leukemia cells; rat primary hepatocytes, tracheal epithelial cells, and lymphosarcoma cells; and human lung/bronchial epithelial cells, A549 and HeLa cells, skin fibroblasts, and PBLs, within an hour of exposure (see Table C-17). It has been shown that SSBs can be formed directly in lung/bronchial epithelial cells with formaldehyde exposure, independent of DNA repair.

Several studies have demonstrated formaldehyde-induced cytogenetic markers (CAs, MN and SCEs) in different rodent and human primary cells and cell lines (see Table C-17). For example, CAs are induced in CHO cells (normal and DNA repair deficient), V79 cells, and hamster embryo cells, with a dose-dependent increase in human fibroblasts and lymphocytes. Further evidence exists for formaldehyde-induced clastogenic effect as observed by MN induction in V79 cells and a dose-dependent increase in MN induction in both human whole blood cultures and normal and repair deficient fibroblast cells. Furthermore, formaldehyde induced SCEs in CHO cells (normal and repair-deficient) and V79 cells at various concentrations (0.01–0.5 mM). The dose-dependent

increase in SCE was higher in mutant CHO cells compared to the normal counterparts, suggesting the importance of DNA repair in SCE removal. Exposure of A549 cells for 1 hour with formaldehyde or co-culturing the exposed A549 cells with unexposed V79 cells beyond 1 hour induces SCE in both cell types, suggesting that formaldehyde is active in the medium for a longer time and continues to induce genotoxicity despite the high reactivity of formaldehyde with macromolecules.

In addition, formaldehyde induces DNA repair inhibition in normal as well as repair-deficient fibroblasts derived from XP and Fanconi anemia patients. In mouse embryo fibroblasts, formaldehyde acts as a potential initiator with a dose-dependent increase in cell transformation but acts as a weak promoter in hamster kidney cells. Overall, there is significant evidence that formaldehyde is genotoxic and mutagenic in several human and rodent cell culture systems.

C.3.5. Genotoxicity of Formaldehyde in Experimental Animals

In experimental animals, formaldehyde has been shown to induce DNA adducts, DPXs, DDXs, SSBs, cytogenetic alterations, such as, MN, SCEs, CAs, and mutations, as summarized in Tables C-18 and C-19.

DNA reactivity and DNA damage

Formaldehyde is highly DNA reactive. Based on numerous experimental animal studies across several species, exposure has been shown to cause damage at the site of contact and/or portal of entry (POE), including the formation of DNA adducts, DPXs, DDXs, SSBs and other cytogenetic effects (see Tables C-18 and C-19). In addition, some animal studies have reported evidence of effects on DNA at sites distal to the POE; however, these observations were not highly consistent across the available studies (acknowledging that the primary focus of most studies was the POE), and interpretations are complicated by the frequent use of test articles presumed to introduce methanol co-exposure (see Tables C-18 and C-19). This limitation is of significant concern for changes observed outside of the POE.

DNA adducts

[Beland et al. \(1984\)](#) demonstrated the formation of hmDNA mono adducts (e.g., N⁶-hmdA) from the in vitro reaction of formaldehyde with calf thymus DNA (see Appendix C.3.4). The hmDNA adducts are labile in nature and hence they were detected as methylDNA (me-DNA) adducts after chemically reducing them with NaBH₃CN followed by LC/MS analysis ([Wang et al., 2007b](#); [Wang et al., 2009a](#); [Moeller et al., 2011](#); [Lu et al., 2010a](#); [Lu et al., 2011](#)). Using [¹³CD₂]-formaldehyde inhalation exposures or orally administered [¹³CD₄]-methanol, one research group has reported the development of an LC/MS method that distinguishes formaldehyde-induced hmDNA mono adducts and DNA-DNA crosslinks originating from endogenous and exogenous exposures in different tissues of rats ([Lu et al., 2010a](#); [Lu et al., 2011](#); [Lu et al., 2012b](#)) and monkeys ([Moeller et al., 2011](#)). [Lu et al. \(2010a\)](#) exposed F344 rats to a single dose of 12.3 mg/m³ ¹³CD₂-formaldehyde by inhalation for 1 and 5 days. The authors detected three forms of endogenous DNA damage, i.e., the

N²-hmdG and N⁶-hmdA mono adducts and dG-CH₂-dG crosslinks, in all tested tissues (nose, lung, liver, spleen, bone marrow, thymus, and blood). The exogenous N²-hmdG adduct and dG-CH₂-dG crosslinks were detectable only in nasal tissue and their levels increased from 1 day to 5 days of exposure. However, the exogenous N⁶-hmdA adducts were not detectable in any of the tissues analyzed ([Lu et al., 2010a](#)).

The same group of investigators also exposed F344 rats to inhaled [¹³CD₂]-formaldehyde (0.9 to 18.7 mg/m³) for 6 hours and measured N²-hmdG adducts in the nasal epithelium ([Lu et al., 2011](#)). While both the endogenous and exogenous hmDNA adducts were analyzed in exposed rats, this study did not report the use of unexposed controls. Compared to the ¹³C-labeled exogenous mono adducts formed by exposures up to 11.2 mg/m³, endogenous N²-hmdG adducts formed at levels between 1.7 and over 90-fold higher, showing considerable variation in adduct levels across doses. Although the exogenous N²-hmdG adducts exhibited a nonlinear increase over the range of concentrations tested, their levels appeared to be above endogenous levels only at the highest formaldehyde concentration tested.

Further, the same group of investigators studied the distribution of hmDNA adducts in Cynomolgus monkeys that were exposed by inhalation to 2.34 or 7.5 mg/m³ of ¹³CD₂-formaldehyde (6 hours/day for 2 days) ([Moeller et al., 2011](#)). Endogenous N²-hmdG mono adducts were detected in the nasal maxilloturbinates and bone marrow, but exogenous DNA adducts were only detectable in the maxilloturbinates. The endogenous tissue levels of hmDNA adducts were 5–10 fold higher than corresponding exogenous adduct levels.

Recently, another study from the same research group examined endogenous and exogenous hm-DNA adducts in rats exposed to low levels of [¹³CD₂]-formaldehyde (1, 30, and 300 ppb) by nose-only inhalation for 28 days ([Leng et al., 2019](#)). The authors reported detectable levels of endogenous, but not exogenous hm-DNA adducts in several tissues including those in lower or upper respiratory tract (nasal epithelium, trachea and lung), blood and bone marrow, and in tissues other than respiratory tract, bone marrow and blood cells. Thus, any exogenous formaldehyde-induced hm-DNA adducts are below the limit of detection for exposure concentrations up to 300 ppb ([Leng et al., 2019](#)).

In addition to inhalation exposures, hmDNA adducts have been measured after exposure to chemicals (i.e., nitrosamines, methanol) that are metabolized to formaldehyde ([Wang et al., 2007b](#); [Lu et al., 2012b](#)). [Wang et al. \(2007b\)](#) have detected the N⁶-hmdA adduct in the liver and lung of rats injected subcutaneously with the tobacco-specific nitrosamines, N-nitrosodimethylamine (NDMA), or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) at 0, 0.025, and 0.01 mmol/kg b.w. doses. The N⁶-hmdA adduct showed a dose-response formation with both nitrosamines and was also detected endogenously in saline controls, albeit at low levels. Compared to saline controls, N⁶-hmdA levels in exposed rats were 4.5- to 15-fold higher in the liver, and 2.2- to 3.8-fold higher in the lung. Following gavage exposure with 500 and 2,000 mg/kg [¹³CD₄]-labeled methanol, hmDNA adducts were detectable in several tissues of Sprague-Dawley rats, including bone marrow ([Lu et](#)

[al., 2012b](#)). In this study, the authors also analyzed an unexposed control group. A dose-dependent increase in exogenous N²-hmdG adducts was reported in several tissues including bone marrow, suggesting that exogenous methanol is transported to bone marrow where it is converted to formaldehyde and results in the formation of exogenous hmDNA adducts that are identical to endogenous formaldehyde mono adducts. Interestingly however, the levels of endogenous N²-hmdG adducts, but not N⁶-hmdA adducts, in methanol-exposed animals were significantly increased in several tissues compared to endogenous N²-hmdG adduct levels in the corresponding tissues of unexposed controls. This observation suggests that exposure to exogenous methanol affects the formation and/or persistence of the endogenous N²-hmdG, but not N⁶-hmdA adducts, which may have also occurred in an earlier rat study that did not report the use of unexposed controls ([Lu et al., 2011](#)). From these studies, it appears that hmDNA adducts are likely to be formed in distal tissues when formaldehyde is produced as a metabolite of chemicals such as methanol ([Lu et al., 2012b](#)) or from NNK and NDMA ([Wang et al., 2007b](#)). Thus, oral exposure to methanol, but not inhaled formaldehyde, seems to produce formaldehyde-specific adducts in distal tissues of experimental animals.

DNA-protein crosslinks

Several in vivo studies involving rodents and monkeys have demonstrated DPX formation following inhalation exposure to formaldehyde (see Table C-18). In rats, several short- and long-term inhalation exposures of formaldehyde have been shown to induce DPX formation in nasal passages. For example, inhalation exposure to formaldehyde induced DPX in nasal mucosa with a single 3-hour ([Heck and Casanova, 1987](#); [Casanova and Heck, 1987](#)) or 6-hour exposure ([Lam et al., 1985](#); [Casanova et al., 1989](#)) or 6 hours daily exposure for 2 days ([Casanova-Schmitz and Heck, 1983](#); [Casanova-Schmitz et al., 1984b](#)).

DPX levels have been measured from the nasal lateral meatus, medial meatus, and posterior meatus ([Casanova et al., 1994](#)) or the entire nasal cavity showing a nonlinear dose-response effect at and above 0.37 mg/m³ dose ([Casanova et al., 1989](#)) after inhalation of ¹⁴C-formaldehyde. These sites have been shown to be associated with a high tumor incidence ([Morgan et al., 1986b](#)) or cellular proliferation ([Monticello et al., 1989](#); [Monticello et al., 1991](#)) in chronic formaldehyde exposure studies in rats.

Casanova-Schmitz and Heck ([1983](#)) have reported a significant increase in DPXs in respiratory, but not olfactory mucosa, at ≥ 7.37 mg/m³ of formaldehyde exposure of rats with a linear increase in the exposure range of 2.46–36.8 mg/m³. The inability of this study to detect DPXs at lower levels of formaldehyde exposure is likely due to the protective mechanism of GSH, which catalyzes the oxidative metabolism of formaldehyde to formate. [Lam et al. \(1985\)](#) have shown that co-exposure of rats with 4.6 mg/m³ acrolein and 7.4 mg/m³ formaldehyde for 6 hours resulted in higher DPX levels in the nasal mucosa of rats compared to rats given formaldehyde alone, suggesting that GSH depletion by acrolein enhanced the macromolecule binding of formaldehyde.

The same group in a different study did not detect DPX formation in the olfactory mucosa and bone marrow even at the high exposure concentration of 18.42 mg/m³ ([Casanova-Schmitz et al., 1984b](#)).

[Casanova and Heck \(1987\)](#) reported that GSH depletion caused an increase in DPX formation in the IF-DNA of the nasal mucosa of F344 rats when a dual-isotope (³H/¹⁴C) method was used. The dual isotope method distinguished between metabolic incorporation and covalent binding of formaldehyde. Formaldehyde is oxidized to formate, losing one hydrogen atom (indicated by a decrease in the ³H/¹⁴C ratio), and becomes metabolically incorporated into macromolecules. However, when GSH is not available (depleted), it leaves residual (unoxidized) formaldehyde to covalently bind to DNA, forming DPX. However, the residual formaldehyde may form adducts by reacting with deoxyribonucleosides in the DNA hydrolysates, which could also lead to an overestimation of the amount of DNA-bound formaldehyde. [Casanova et al. \(1989\)](#) used an improved method which is based on the determination of the total ¹⁴C-formaldehyde bound to DNA. This study showed that formaldehyde was exclusively bound to IF DNA, indicating the formation of DPXs. Hydrolysis of DPXs in different samples resulted in quantifiable amounts of formaldehyde to be released. DPX formation was detectable at all concentrations (0.37–12.3 mg/m³ for 6 hours) of formaldehyde exposure. Overall, these studies show that formaldehyde induces DPXs in nasal epithelial cells of rodents. However, there are no published rodent studies that assess DPXs beyond the nasal passages of the upper respiratory tract. [Neuss et al. \(2010b\)](#) did not detect a significant increase in DPX formation, as determined by the Comet assay in the bronchoalveolar lavage (BAL) cells of F344 rats exposed to up to 18.45 mg/m³ formaldehyde by whole-body inhalation compared to controls.

DPXs were also found in the nasal mucosa and extranasal tissues of rhesus monkeys exposed to 0.86, 2.45, or 7.36 mg/m³ formaldehyde 6 hours/day for 3 days ([Casanova et al., 1991](#)). These data were used as a basis for cross-species prediction of formaldehyde-induced DPXs in humans. The presence of DPXs in rhesus monkeys confirms formaldehyde's DNA reactivity as a general effect. Additionally, DPXs were detected in the larynx/trachea/carina (pooled sample) and in intrapulmonary airways of monkeys exposed to 2.5 or 7.4 mg/m³ formaldehyde. These data demonstrate direct effects of formaldehyde on DNA of tissues that correspond to observed tumor sites (e.g., nasal and nasopharynx) in humans.

Recent studies by ([Lai et al., 2016](#)) have shown that DPXs formed by endogenous formaldehyde were detectable in tissues at the portal of entry (nose) as well as at distal tissues (e.g., blood cells, and bone marrow) in rats or monkeys. However, when either species was exposed to [¹³CD₂]-labeled formaldehyde, exogenous DPXs were detectable only in the respiratory tissues. In rats, exogenous DPXs accumulated over a 28-day period of exposure and remained up to one week after removal of exposure (see Table C-18), suggesting a much slower repair of DPXs than indicated by *in vitro* data from earlier studies.

Recently, another study from the same research group examined endogenous and exogenous DPX adducts in rats exposed to low levels of [¹³CD₂]-formaldehyde (1, 30, and 300 ppb)

by nose-only inhalation for 28 days ([Leng et al., 2019](#)). The authors reported detectable levels of endogenous, but not exogenous DPXs in several tissues including those in lower or upper respiratory tract (nasal epithelium, trachea, and lung), blood and bone marrow, and in tissues other than respiratory tract, bone marrow and blood cells. Thus, any exogenous formaldehyde-induced DPX adducts are below the limit of detection for exposure concentrations up to 300 ppb ([Leng et al., 2019](#)).

DNA-DNA crosslinks

There is limited evidence showing the formation of DNA-DNA crosslinks (DDX) induced by inhalation exposure to formaldehyde. [Lu et al. \(2010a\)](#) reported dG-CH₂-dG crosslinks in the nasal epithelium of F344 rats exposed to 12.3 mg/m³ formaldehyde for 1 or 5 days (6 hours/day). However, roughly 65% of the dG-CH₂-dG crosslinks were considered artifacts formed during sample workup and storage. [Wang et al. \(2007b\)](#) reported very low levels of dA-CH₂-dA crosslinks of formaldehyde in rats exposed to NDMA and NNK but cautioned that these crosslinks may be generated artifactually upon DNA storage. Thus, DDX may not be a useful biomarker of formaldehyde exposure.

DNA SSBs by alkaline elution

Formaldehyde has been shown to induce DNA SSBs in a few studies involving mice ([Wang and Liu, 2006](#)) and rats ([Sul et al., 2007](#); [Im et al., 2006](#)), as summarized in Tables C-18 and C-19.

[Im et al. \(2006\)](#) reported a dose-dependent increase in DNA damage as analyzed by the comet assay in both PBLs and livers of Sprague-Dawley rats exposed by inhalation to 6.14 and 12.3 mg/m³ formaldehyde. In the same strain of rats, [Sul et al. \(2007\)](#) also observed a dose-dependent increase in SSBs in lung epithelial cells following inhalation exposure to 0, 6.15, and 12.3 mg/m³ formaldehyde for 2 weeks (6 hours/day, 5 days/week). In a developmental toxicity study, pregnant mice injected i.p. with formaldehyde from gestational days 6 to 19 exhibited DNA damage in maternal as well as fetal liver at 0.2 and 1 mg/kg, respectively ([Wang and Liu, 2006](#)).

Cytogenetic markers of genotoxicity

Micronucleus

Few studies examined the effect of formaldehyde exposure on MN induction in rodents by exposing the animals by inhalation, i.p. injection, or gavage as summarized in Tables C-18 and C-19. Inhalation exposure studies in rats were negative, while studies that used formalin by gavage in mice ([Ward et al., 1983](#)) and rats ([Migliore et al., 1989](#)) were positive for MN formation. Speit and coworkers did not observe MN formation in peripheral blood cells ([Speit et al., 2009](#)) and BAL cells ([Neuss et al., 2010b](#)) of F344 rats exposed to 0, 62, 1.23, 7.38, 12.3, and 18.45 mg/m³ formaldehyde. However, the Neuss et al. ([2010b](#)) study did not report the use of a positive control for MN induction, while in the other two studies, the use of cyclophosphamide as a positive control did not

appear to induce a high MN count and showed results within the range of control values ([Speit et al., 2009](#); [Speit et al., 2011b](#)). ([Ward et al., 1983](#)) observed aneuploidy and structural chromosomal aberrations (e.g., breaks, exchanges, aberrant chromosomes with and without gaps) in femoral bone marrow cells of mice dosed with formalin (100 mg/kg) or methanol (1,000 mg/kg). The cytogenetic effects seen in bone marrow suggest that the formalin or methanol given by gavage was able to reach bone marrow and induce genotoxicity. Similarly, ([Migliore et al., 1989](#)) observed MN formation in the gastric epithelial cells of Sprague-Dawley rats exposed to a single dose of formalin (200 mg/kg). Lastly, [Liu et al. \(2017\)](#) have shown that inhalation exposure to formaldehyde in ICR mice for 20 weeks caused a significant increase in the ratio of polychromatic erythrocytes/normochromatic erythrocytes, but not micronuclei induction in bone marrow ([Liu et al., 2017](#)).

Sister chromatid exchanges

Few studies examined the effect of formaldehyde exposure on SCEs in mice and rats (see Tables C-18 and C-19). Two of the three studies in rats were negative for SCEs in blood cells ([Speit et al., 2009](#); [Kligerman et al., 1984](#)), both of these studies used inhalation exposure to 18.45 mg/m³ formaldehyde for 6 hours/day, 5 days/week for 4 weeks.

In an inhalation study, ([Brusick, 1983](#)) exposed CD-1 mice to target concentrations of 0, 7.38, 14.76, or 30.75 mg/m³ formaldehyde vapors for 6 hours/day for 4–5 days. Significantly higher levels of SCEs/cell were reported in the bone marrow of female mice both at the mid and high concentrations, while the low-concentration group had levels that were not significantly different from the control group. Thus, formaldehyde exposure has provided equivocal results on the SCEs in rodents.

Chromosomal aberrations

Few studies reported the effect of formaldehyde inhalation on CA induction in rodents and these results were mixed (see Table C-18).

[Kligerman et al. \(1984\)](#) found no difference in the incidence of SCEs or CAs and mitotic index in the PBLs of male and female F344 rats exposed to formaldehyde for 5 days up to a 18.45 mg/m³ dose. Also, Dallas et al. ([1992](#)) reported no clastogenic effects in bone marrow of Sprague-Dawley rats exposed at the same concentration of formaldehyde for 8 weeks. However, the authors observed a modest, but statistically significant increase (1.7- to 1.8-fold) in CAs in pulmonary lavage cells at the high dose (18.45 mg/m³) compared to controls, but not at lower doses [0.61 and 3.7 mg/m³ ([Dallas et al., 1992](#))].

[Speit et al. \(2009\)](#) investigated the genotoxicity of formaldehyde in peripheral blood samples of Fischer-344 rats exposed to 0 to 18.45 mg/m³ formaldehyde for 4 weeks (6 hours/day, 5 days/week). Compared to controls, the authors found no significant increase in genotoxicity assays such as the comet assay (with or without γ -irradiation of blood samples), the SCEs assay, and micronucleus test. Earlier studies by ([Casanova-Schmitz et al., 1984b](#)) showed that formaldehyde

does not cause toxicity to bone marrow. Following formaldehyde exposure by i.p. injection in mice, data were negative for CAs in spermatocytes ([Fontignie-Houbrechts, 1981](#); [Fontignie-Houbrechts et al., 1982](#)) and polychromatic erythrocytes ([Natarajan et al., 1983](#)), while [Gomaa et al. \(2012\)](#) demonstrated an increase in chromosomal aberrations in bone marrow cells of adult male albino rats exposed to formaldehyde at 0.2 mg/kg-day i.p injection for 4 weeks. Oral administration of formaldehyde to rats showed positive results for CAs in the gastric epithelial cells ([Migliore et al., 1989](#)).

Since many leukemogens initiate leukemogenesis by directly damaging the hematopoietic stem cells/hematopoietic progenitor cells (HSP/HPC), [Zhao et al. \(2020\)](#) examined the effect of formaldehyde exposure either in vivo or ex vivo. They exposed either BALB/c mice to 3 mg/m³ formaldehyde by inhalation for 2 weeks or by ex vivo to cells from bone marrow, lung, nose, and spleen with 0, 50, 100, and 400 µM formaldehyde for 1 hour. Using a myeloid progenitor colony formation (MPCF) assay, they showed that formaldehyde exposure caused a decrease in burst-forming unit-erythroid (BFU-E) and colony-forming unit-granulocyte, macrophage (CFU-GM) colonies in all of the four tissues from both in vivo and ex vivo (up to 400 µM) exposure to formaldehyde. The authors concluded that their study confirms the presence of HSP/HPC in mouse lung and nose and hypothesize that following formaldehyde-induced DNA damage at the point of entry these damaged stem cells possibly migrate to bone marrow and induce leukemia ([Zhao et al., 2020](#)). However, the formaldehyde used in this study was generated from 10% formalin which contains methanol added as a stabilizer; it is likely that methanol could also contribute to the outcome, preventing attribution of the results to formaldehyde alone.

Overall, inhalation exposure to formaldehyde has produced mixed and equivocal results in rodents for cytogenetic markers of genotoxicity. Formaldehyde did not induce MN in bone marrow cells of male Sprague-Dawley rats ([Dallas et al., 1992](#)) and caused no increase in the frequency of SCEs or CAs and mitotic index in blood lymphocytes of F344 rats of either sex ([Kligerman et al., 1984](#)). However, a modest, but statistically significant, increase (1.7- to 1.8-fold) in CAs has been observed in pulmonary lavage cells of Sprague-Dawley rats after exposure to 18.45 mg/m³ ([Dallas et al., 1992](#)) and a significant increase in CAs in bone marrow cells of female Wistar rats exposed to 1.5 mg/m³ formaldehyde ([Kitaeva et al., 1990](#)); however, the latter finding involved methanol co-exposure, reducing confidence in these results. Also, formaldehyde exposure by inhalation in CD-1 mice induced SCEs in bone marrow cells at ≈15 mg/m³ ([Brusick, 1983](#)). Thus, some studies show that inhaled formaldehyde may be able to induce cytogenetic effects in distal tissues with repeated exposures, possibly only at very high formaldehyde concentrations.

Mutations

Formaldehyde exposure has been shown to induce mixed results for mutations in several test systems as summarized in Tables C-18 and C-19. The dominant lethal mutation test has been performed using mice and rats, where males were exposed to formaldehyde or formalin vapors by

inhalation or i.p. injection, mated with females, and where mutations were then scored in the offspring. In two of these studies, formaldehyde injected i.p. to CD-1 mice was negative for dominant lethal mutations ([Epstein and Shafner, 1968](#); [Epstein et al., 1972](#)), while another study which used a higher dose (50 mg/kg) of formaldehyde showed weakly positive results ([Fontignie-Houbrechts, 1981](#)). Specific pathogen-free ICR mice exposed to inhaled formaldehyde were positive for dominant lethal mutations ([Liu et al., 2009b](#)). In this study, mutation rates were dose dependent and mainly inherited from the paternal germ line.

Recio et al. ([1992](#)) demonstrated point mutations in the GC base pairs of the p53 tumor suppressor gene in 45% (5 out of 11) of the primary nasal squamous cell carcinomas (SCCs) from F344 rats that were chronically (2 years) exposed to 18.45 mg/m³ formaldehyde. Samples from this study were further analyzed by Wolf et al. ([1995](#)) who demonstrated the presence of the p53 tumor suppressor protein which correlated with the proliferating cell nuclear antigen (PCNA) but not TGF- α in the nasal SCCs. However, Meng et al. ([2010](#)) failed to detect p53 mutations in the nasal mucosa of rats exposed to 0.86 to 18.42 mg/m³ formaldehyde for 13 weeks. It is likely that the duration of exposure is important for the mutations to occur in these studies. In summary, formaldehyde produced mixed results in the DLM test. Short-term (13-week) exposure of rats to formaldehyde did not produce detectable mutations in the p53 tumor suppressor gene or Ha-ras oncogene; however, a chronic 2-year study resulted in SCC formation and mutations in the GC base pairs of the p53 gene in rats.

Table C-18. Summary of *in vivo* genotoxicity studies of formaldehyde inhalation exposure in experimental animals

Test system	Concentration ^a	Results ^b	Comments	Reference
Mutation				
<i>Evaluations specific to genotoxicity in the upper or lower respiratory tract</i>				
Rats/F344, nasal SCCs	18.45 mg/m ³ ; HCHO from PFA ^c	+	Inhalation, 6 hrs/d, 5 d/wk, 2 years	(Recio et al., 1992)
Rats/F344, nasal SCCs	18.45 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs/d, 5 d/wk, 2 years	(Wolf et al., 1995)
Rats/F344, nasal mucosa	18.45 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, 5 d/wk, 13 wks; Cell proliferation showed a conc.-dependent $\uparrow$; significant at 12.3 and 18.45 mg/m ³ exposures	(Meng et al., 2010)
<i>Evaluations specific to genotoxicity to systems other than the respiratory tract, bone marrow, or blood cells</i>				
Rats/Strain not specified - dominant lethal test	1.47 mg/m ³ ; HCHO (not specified)	(+)	Inhalation, 4 hrs/d for 4 wks	(Kitaeva et al., 1990)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Concentration ^a	Results ^b	Comments	Reference
Mice/ICR, specific pathogen-free dominant lethal test	200 mg/m ³ ; Formalin (37% HCHO w/w aq.sol.)	+	Whole-body inhalation exposure of ♂ mice for 2 hrs; 6 wks postexposure ♂ mated to ♀ at 1:1;	(Liu et al., 2009b)
DNA-protein crosslinks				
Evaluations specific to genotoxicity in the upper or lower respiratory tract				
Monkey/Rhesus nasal turbinates	0.86 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs; the LEC ↑ with the ↑ in distance from the portal of entry; DPX levels show conc.-dependent ↑ from 0.86–7.4 mg/m ³ , in the order of middle turbinates > lateral wall/septum, nasopharynx > larynx/trachea/carina.	(Casanova et al., 1991)
Monkey/Rhesus nasal, larynx, trachea, & carina	2.5 mg/m ³ ; HCHO from PFA	+		(Casanova et al., 1991)
Monkey/Rhesus maxillary sinuses, lungs	7.4 mg/m ³ ; HCHO from PFA	+		(Casanova et al., 1991)
Monkeys/Cynomolgus nose	7.4 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs/d, for 2 d	(Lai et al., 2016)
Rats/F344 nasal mucosa	0.37 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs; nonlinear conc.-dependent ↑ in DPX level between 0.37 to 12.1 mg/m ³	(Casanova et al., 1989)
Rats/F344 nasal mucosa	0.86 mg/m ³ ; HCHO from PFA	+	Inhalation 6 hrs/d, 5 d/wk, 11 wk + 4 d + 3 hrs (preexposed); or 3 hrs only (naïve); ↑cell proliferation ≥ 7.48 mg/m ³	(Casanova et al., 1994)
Rats/F344 nasal mucosa	2.5 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs/d, for 2 d; cytotoxicity ≥ 12.3 mg/m ³	(Casanova-Schmitz et al., 1984a)
Rats/F344 nasal mucosa	2.5 mg/m ³ ; HCHO from PFA	+	Inhalation, 3 hrs/d, for 2 d	(Casanova and Heck, 1987)
Rats/F344 nasal mucosa	2.5 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs/d; for 7 or 28 d	(Lai et al., 2016)
Rats/F344 nasal mucosa	7.4 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs/day, for 2 d	(Casanova-Schmitz and Heck, 1983) ^b

Supplemental Information—Formaldehyde (Inhalation)

Test system	Concentration ^a	Results ^b	Comments	Reference
Rats/F344 nasal mucosa	7.4 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs; co-exposure to 2 ppm acrolein caused a significant ↑ in toxicity and DPX formation	(Lam et al., 1985)
Rats/F344 nasal mucosa	18.45 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs/d; for 1, 2, and 4 d	(Lai et al., 2016)
Rats/F344 olfactory mucosa	18.45 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, for 2 d	(Casanova-Schmitz et al., 1984a)
Rats/F344 nasal mucosa	36.9 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, for 2 d	(Casanova-Schmitz and Heck, 1983) ^b
Rats/F344, nasal epithelium, trachea, lung	0.0012, 0.0369, 0.369 mg/m ³ [¹³ CD ₂]-HCHO	–	Inhalation, nose-only, 6 hrs/d, 28 d	(Leng et al., 2019)
Rats/F344 BAL cells	18.45 mg/m ³ ; HCHO from formalin vapors	–	Inhalation, 6 hrs/d, 5 d/wk, for 4 wks	(Neuss et al., 2010a)
Mice/BalbC lung	3.0 mg/m ³ ; HCHO vapor from 10% formalin	–	Inhalation, nose-only; 8 hrs/d for 7 d;	(Ye et al., 2013)
Evaluations specific to genotoxicity in cells of the blood and bone marrow				
Monkeys/Cynomolgus bone marrow, PBMC	7.4 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, for 2 d	(Lai et al., 2016)
Rats/F344 bone marrow	12.43 mg/m ³ ; HCHO from PFA	–	Inhalation, 3 hrs/d, for 2 d	(Casanova and Heck, 1987)
Rats/F344 bone marrow	18.45 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, for 2 d	(Casanova-Schmitz et al., 1984a)
Rats/F344 bone marrow, PBMC	18.45 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d; for 1, 2, and 4 d	(Lai et al., 2016)
Rats/F344, bone marrow, PB MC	0.0012, 0.0369, 0.369 mg/m ³ [¹³ CD ₂]-HCHO	–	Inhalation, nose-only, 6 hrs/d, 28 d	(Leng et al., 2019)
Rats/F344 peripheral blood	18.45 mg/m ³ ; HCHO from formalin vapors	–	Inhalation, 6 hrs/d, 5 d/wk, for 4 wks	(Speit et al., 2009)
Mice/BalbC bone marrow	1.0 mg/m ³ ; HCHO vapor from 10% formalin	+	Inhalation, nose-only; 8 hrs/d for 7 d; dose-dependent ↑ in DPX levels	(Ye et al., 2013)
Mice/BalbC PBM cells	3.0 mg/m ³ ; HCHO vapor from 10% formalin	+	Inhalation, nose-only; 8 hrs/d for 7 d; dose-dependent ↑ in DPX levels	(Ye et al., 2013)
Evaluations specific to genotoxicity in systems other than the respiratory tract, bone marrow or blood cells				
Monkeys/Cynomolgus liver	7.4 smg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/, for 2 d	(Lai et al., 2016)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Concentration ^a	Results ^b	Comments	Reference
Rats/F344, olfactory bulbs, liver, hippo campus, cerebellum	0.0012, 0.0369, 0.369 mg/m ³ [¹³ CD ₂]-HCHO	-	Inhalation, nose-only, 6 hrs/d, 28 d	(Leng et al., 2019)
Mice/Kunming kidney and testes	0.5 mg/m ³ ; HCHO vapor from 10% formalin	+	Inhalation, 72 hrs continuous exposure	(Peng et al., 2006)
Mice/Kunming liver	1.0 mg/m ³ ; HCHO vapor from 10% formalin	+	Inhalation, 72 hrs continuous exposure	(Zhao et al., 2009 ; Peng et al., 2006)
Mice/BalbC spleen, testes	1.0 mg/m ³ ; HCHO vapor from 10% formalin	+	Inhalation, nose-only; 8 hrs/d for 7 d; dose-dependent ↑ in DPX levels	(Ye et al., 2013)
DNA adducts				
Evaluations specific to genotoxicity in the upper or lower respiratory tract				
Monkey/Cynomolgus maxilloturninate	2.33 mg/m ³ ; HCHO (not specified)	+	Inhalation, 6 hrs/d, for 2 d; conc.-dependent ↑ in exogenous adducts	(Moeller et al., 2011)
Monkeys/Cynomolgus - nasal dorsal mucosa, nasopharynx, nasal septum, nasal posterior maxillary	7.5 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs/d, for 2 d	(Yu et al., 2015b)
Monkeys/Cynomolgus - trachea carina, trachea proximal	7.5 mg/m ³ ; HCHO from PFA	-	Inhalation, 6 hrs/d, for 2 d	(Yu et al., 2015b)
Rats/F344 nasal epithelium	0.86 mg/m ³ ; HCHO from PFA	+	Inhalation, for 6 hrs; conc.-dependent ↑ in exogenous adducts	(Lu et al., 2011)
Rats/F344 nasal epithelium	2.46 mg/m ³ ; HCHO from PFA	+	Inhalation, 6 hrs/d, for 7, 14, 21, or 28 d; recovery for 6, 24, 72, or 168 hrs; exposure-dependent ↑ in hmdG mono adducts	(Yu et al., 2015b)
Rats/F344 -nasal epithelium	12.3 mg/m ³ ; 20% HCHO in water	+	Inhalation, 1 and 5 d; exposure-dependent ↑ in exogenous hmdG adduct and dG-dG crosslinks	(Lu et al., 2010a)
Rats/F344 lung	12.3 mg/m ³ ; HCHO from PFA	-	Inhalation, 1 and 5 d	(Lu et al., 2010a)
Rats/F344, nasal epithelium, trachea, lung	0.0012, 0.0369, 0.369 mg/m ³ [¹³ CD ₂]-HCHO	-	Inhalation, nose-only, 6 hrs/d, 28 d	(Leng et al., 2019)
Evaluations specific to genotoxicity in cells of the blood and bone marrow				

Supplemental Information—Formaldehyde (Inhalation)

Test system	Concentration ^a	Results ^b	Comments	Reference
Monkey/Cynomologus bone marrow	2.33 mg/m ³ ; HCHO (not specified)	–	Inhalation, 6 hrs/d, for 2 d	(Moeller et al., 2011)
Monkeys/Cynomolgus bone marrow, white blood cells	7.5 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, for 2 d	(Yu et al., 2015b)
Rats/F344 white blood cells and bone marrow cells	12.3 mg/m ³ ; HCHO from PFA	–	Inhalation, 1 and 5 d	(Lu et al., 2010a)
Rats/F344, bone marrow, PB MC	0.0012, 0.0369, 0.369 mg/m ³ [¹³ CD ₂]-HCHO	-	Inhalation, nose-only, 6 hrs/d, 28 d	(Leng et al., 2019)
<i>Evaluations specific to genotoxicity in systems other than the respiratory tract, bone marrow or blood cells</i>				
Rats/F344 thymus, lymph nodes, trachea, lung, spleen, kidney, liver, brain	2.46 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, for 28 d	(Yu et al., 2015b)
Rats/F344 liver, spleen, thymus	12.3 mg/m ³ ; HCHO from PFA	–	Inhalation, 1 and 5 d	(Lu et al., 2010a)
Rats/F344, olfactory bulbs, liver, hippo campus, cerebellum	0.0012, 0.0369, 0.369 mg/m ³ [¹³ CD ₂]-HCHO	-	Inhalation, nose-only, 6 h/d, 28 d	(Leng et al., 2019)
Chromosomal aberrations				
<i>Evaluations specific to genotoxicity in the upper or lower respiratory tract</i>				
Rats/SD Pulmonary lavage cells	18.45 mg/m ³ ; HCHO from PFA	+	Inhalation, whole body; 6 hrs/d, 1 or 8 wks	(Dallas et al., 1992)
<i>Evaluations specific to genotoxicity in cells of the blood and bone marrow</i>				
Rats/Wistar Bone marrow	0.49 mg/m ³ ; HCHO (not specified)	+	Inhalation, 4 hrs/d, 4 mos	(Kitaeva et al., 1990)
Rats/SD Bone marrow	18.45 mg/m ³ ; HCHO from PFA	–	Inhalation, whole body; 6 hrs/d, 1 or 8 wks	(Dallas et al., 1992)
Rats/F344 Peripheral blood cells	18.45 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, 5 d/wk, for 4 wks	(Speit et al., 2009)
Rats/F344 Lymphocytes	18.45 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, 5 d; no significant dose-related effect on mitotic activity	(Kligerman et al., 1984)
Mice/CD-1, male & female, Bone marrow cells	30.75 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, 4–5 d	(Brusick, 1983)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Concentration ^a	Results ^b	Comments	Reference
Mice/BALB/c, bone marrow – hematopoietic stem and progenitor cells	3 mg/m ³ , HCHO from 10% formalin	+	Inhalation, 8 hrs/d, 5 d/wk, 2 wks	(Zhao et al., 2020)
Micronucleus				
<i>Evaluations specific to genotoxicity in the upper or lower respiratory tract</i>				
Rats/F344 BAL cells	18.45 mg/m ³ ; HCHO from formalin vapors	–	Inhalation, 6 hrs/d, 5 d/wk, for 4 wks; positive control was not used for the assay	(Neuss et al., 2010a)
<i>Evaluations specific to genotoxicity in cells of the blood and bone marrow</i>				
Rats/Outbred white polychromatophytic erythrocytes (bone marrow)	12.8 mg/m ³ , commercial formaldehyde	+	Inhalation; whole-body exposure; 4 hrs/d, 5 d/wk	(Katsnelson et al., 2013)
Rats/F344 -peripheral blood	18.45 mg/m ³ ; HCHO from formalin vapors	–	Inhalation, 6 hrs/day, 5 days/wk, for 4 wks	(Speit et al., 2009)
Mice/male ICR bone marrow cells	20 mg/m ³ 36.5%–38% HCHO in water (formalin)	+	Inhalation, 2 hrs/d for 15 d	(Yu et al., 2014a)
Mice/ICR, bone marrow cells	1, 10 mg/m ³ , HCHO source not reported	–	Inhalation, 2 hrs/d, 20 wks; micronucleus	(Liu et al., 2017)
Single strand breaks				
<i>Evaluations specific to genotoxicity in the upper or lower respiratory tract</i>				
Rats/SD lung epithelial cells	6.14 mg/m ³ ; HCHO (commercial)	+	Inhalation, 6 hrs/d, 5 d/wk for 2 wks; ↑ cytotoxicity (lipid peroxidation & protein carbonyl oxidation) observed at 18.42 mg/m ³	(Sul et al., 2007)
<i>Evaluations specific to genotoxicity in blood cells</i>				
Rats/SD, PBLs	6.14 mg/m ³ ; HCHO (commercial)	+	Inhalation, 5 d/wk for 2 wks	(Im et al., 2006)
<i>Evaluations specific to genotoxicity in systems other than the respiratory tract, bone marrow or blood cells</i>				
Rats/SD, liver	6.14 mg/m ³ ; HCHO (commercial)	+	Inhalation, 5 d/wk for 2 wks	(Im et al., 2006)
Sister chromatid exchanges				
<i>Evaluations specific to genotoxicity in cells of the blood and bone marrow</i>				

Supplemental Information—Formaldehyde (Inhalation)

Test system	Concentration ^a	Results ^b	Comments	Reference
Rats/F344 Lymphocyte	18.45 mg/m ³ ; HCHO from PFA	–	Inhalation, 6 hrs/d, 5 d; no significant dose-related effect on mitotic activity	(Kligerman et al., 1984)
Rats/F344 Peripheral blood cells	18.45 mg/m ³ ; Formalin vapors	–	Inhalation, 6 hrs/d 5 d/wk, for 4 wks	(Speit et al., 2009)
Mice/CD-1, male & female Bone marrow cells	14.76 mg/m ³ ; HCHO from PFA	–, +	Inhalation, 6 hrs/d, 5 d; ♂ mice: –ve; ♀ mice: +ve; conc.-dependent ↑ in SCEs	(Brusick, 1983)

Gray shading indicates experiments examining tissues or cells outside of the upper respiratory tract that are assumed to have included co-exposure to methanol, and are thus may be less reliable.

^aLowest effective concentration (LEC) for positive results or highest ineffective concentration tested (HIC) for negative or equivocal results.

^b+ = positive; – = negative; (+), equivocal.

^cThermal depolymerization of paraformaldehyde (PFA) or freshly prepared formalin (no methanol) are the preferred test article methods. Generation of formaldehyde from formalin, uncharacterized aqueous solutions (noted as **not specified**), or an unspecified source (also noted as **not specified**) is assumed to involve co-exposure to methanol, and the evidence is less reliable.

HCHO, formaldehyde; PFA, paraformaldehyde; hmdNA, hydroxymethylDNA; SCE, sister chromatid exchange; SCC, squamous cell carcinoma; hmdA, hydroxymethyl deoxyadenosine; hmdG, hydroxymethyl deoxyguanosine; MN, micronucleus.

Part of the data adapted from [NTP \(2010\)](#).

Table C-19. Summary of in vivo genotoxicity studies of formaldehyde exposure by intraperitoneal and oral routes of exposure in experimental animals

Test system	Concentration ^a	Results ^b	Comments	Reference
Mutation				
Rats/Albino Spermatocyte; DLM	0.125 mg/kg; test article: <u>37% HCHO (+ 10% methanol)</u>	+	i.p., ♂ given 5 daily doses and mated to ♀; dose-dependent ↑ in DLM index; effects greater with shorter time gap postexposure	(Odeigah, 1997)
Mice/CD-1 DLM test	20 mg/kg HCHO; test article: <u>Not Specified</u>	–	i.p. injection to ♂; mated to ♀ and autopsied 13 d past mid-wk of mating	(Epstein and Shafner, 1968)
DNA-protein crosslinks				
Rats/F344 tracheal implants	0.01% HCHO in PBS; test article: <u>Not Specified</u>	+	instillation, twice weekly for 2, 4, or 8 wks	(Cosma et al., 1988)
Mice/NS liver (Fetal) [Chinese lang-English Abstract]	0.2 mg/kg; test article: <u>HCHO (not specified)</u>	+	i.p. injection to pregnant mice from GD 6 to 19	(Wang and Liu, 2006)

Supplemental Information—Formaldehyde (Inhalation)

Test system	Concentration ^a	Results ^b	Comments	Reference
Mice/NS Liver (maternal) [Chinese lang-English Abstract]	20 mg/kg; test article: <u>HCHO (not specified)</u>	–	i.p. injection to pregnant mice from GD 6 to 19	(Wang and Liu, 2006)
Chromosomal aberrations				
Mice/CBA femoral polychromatic erythrocytes	25 mg/kg; test article: HCHO (PFA in water)	–	i.p. injections (2) within 24 hr interval; cells sampled 16 and 40 hrs post 2nd injection	(Natarajan et al., 1983)
Mice/Q strain Spermatocytes	50 mg/kg; test article: <u>HCHO (35% sol.)</u>	–	i.p. injection, single	(Fontignie- Houbrechts, 1981)
Mice/Q strain Spermatogonia	30 mg/kg; test article: <u>HCHO (commercial)</u>	–	i.p. injection, 35% HCHO solution + 90 mg/kg H ₂ O ₂	(Fontignie- Houbrechts et al., 1982)
Rats/SD gastric epithelial cells (stomach, duodenum, ileum, colon)	200 mg/kg; test article: HCHO (in water)	+	p.o. gavage, 16, 24, or 30 hrs; time-dependent ↑ in CA in all tissues; toxic at 30 hrs; no significant change in mitotic index	(Migliore et al., 1989)
Mice/B6C3F1-bone marrow	100 mg/kg; test article: <u>formalin</u> ; or 1,000 mg/kg methanol	+	Gavage, single exposure; HCHO and methanol showed 21- and 15-fold increase compared to controls, respectively	(Ward et al., 1983)
Rats (male albino), bone marrow cells	0.2 mg/kg-day; test article: HCHO (source not specified)	+	i.p injection, single injection for 4 wks	(Gomaa et al., 2012)
Micronucleus				
Mice/CBA femoral polychromatic erythrocyte and spleen cell	25 mg/kg; test article: HCHO (PFA in water)	–	i.p. injections (two) of HCHO solution within 24 hr interval; cells sampled 16 and 40 hrs post 2nd injection	(Natarajan et al., 1983)
Mice/NMRI bone marrow	30 mg/kg; test article: <u>HCHO (commercial)</u>	–	i.p. injection, single	(Gocke et al., 1981)
Mice/CD-1 reticulocytes	30 mg/kg; test article: <u>HCHO (35%)</u>	–	i.v. 2 injections; sampled 24, 48, or 72 hrs after exposure	(Morita et al., 1997)
Mice/CD-1 bone marrow or peripheral blood	200 mg/kg; test article: <u>35% HCHO</u>	–	Gavage twice (bone marrow) or once (peripheral blood); all mice killed at 300 mg/kg dose	(Morita et al., 1997)
Rats/SD gastric epithelial cells (stomach, duodenum, ileum, colon)	200 mg/kg; test article: HCHO (in water)	+	p.o. gavage, 16, 24, or 30 hrs; time-dependent ↑ in MN in all tissues; toxic at 30 hrs; no significant change in mitotic index	(Migliore et al., 1989)

^aLowest effective concentration (LEC) for positive results or highest ineffective concentration (HIC) tested for negative or equivocal results.

^b+ = positive; – = negative; (+), equivocal.

^cThermal depolymerization of paraformaldehyde (PFA) or freshly prepared formalin (no methanol) are the preferred test article methods. Generation of formaldehyde from formalin, uncharacterized aqueous solutions (noted as **not specified**), or an unspecified source (also noted as **not specified**) is assumed to involve co-exposure to methanol, and the evidence is less reliable.

HCHO, formaldehyde; PFA, paraformaldehyde; DLM, dominant lethal mutation; i.p., intra peritoneal; i.v., intra venous; GD, gestation day; MN, micronucleus.

Part of the data adapted from [NTP \(2010\)](#).

Summary of in vivo genotoxicity studies of formaldehyde by routes of exposure in experimental animals

Formaldehyde reacts with cellular macromolecules at the portal of entry causing genotoxicity. Genotoxicity of inhaled formaldehyde involves direct interaction with DNA inducing DNA-protein crosslinks and/or hydroxymethylDNA adducts or DNA mono adducts, single strand breaks, micronuclei, and chromosomal aberrations in nasal passages of experimental animals. DPX are formed predominantly by crosslinking of the epsilon-amino groups of lysine and the exocyclic amino groups of DNA, especially at the N-terminus of histone. Due to the differences in the anatomy of nasal passages and breathing patterns of rats and monkeys, the location of DPX formation differs. Over a range of 0.86 to 7.37 mg/m³, formaldehyde-induced DPX levels showed a concentration-dependent increase in the monkey respiratory tract in the order of middle turbinates > anterior lateral wall/septum > maxillary sinuses and lungs. Thus, the lowest effective concentration (LEC) was higher with increased anatomical distance from the portal of entry. Furthermore, these anatomical sites are known to be associated with formaldehyde-induced proliferative response in monkeys. In rats, DPX formation showed concentration dependence between 0.37–12.1 mg/m³ formaldehyde, which was nonlinear with a sharp increase above 4.9 mg/m³. With exposures up to 28 days, DPXs were shown to accumulate and persisted for an additional 7 days at a concentration of 2.5 mg/m³. In addition, DPX formation was six-fold higher in the lateral meatus compared to the medial and posterior meatus, corresponding, respectively, to high and low tumor incidence sites in rats. DPXs were not detected in olfactory mucosa, bronchoalveolar lavage (BAL) cells of rats or in lungs of mice exposed to formaldehyde. DPXs (from exogenous formaldehyde) also were not detected in bone marrow and peripheral blood monocyte cells (rats and monkeys) and liver (monkeys) following inhalation exposure. Since DPXs are likely to induce replication errors, they have been considered to be a marker of mutagenicity. The repair of DPX in eukaryotes appears to depend on the dose and duration of formaldehyde exposure. The overall evidence indicates that the DPXs are markers of exposure as well as genotoxic endpoints.

HydroxymethylDNA adducts in experimental animals can result from DNA reacting with endogenously-produced or exogenous formaldehyde. Mono adducts formed from endogenous formaldehyde (produced during normal cellular metabolism) are distinguished from those formed by exogenous exposure using stable isotope (¹³C)-labeled formaldehyde coupled with sensitive MS

techniques. Inhaled formaldehyde induces N2-hmdG adducts in the nasal epithelium of F344 rats, but not in distal tissues, and the adduct levels are associated with concentration and duration of exposure. In rhesus monkeys, formaldehyde induces N2-hmdG adducts in the maxilloturbinates, and the mono adduct levels are associated with the exposure concentration of formaldehyde. Endogenous N2-hmdG mono adducts and dG-dG crosslinks are also detected in rats and monkeys, but in all experimental animals exposed exogenously to formaldehyde by inhalation, N2-hmdG adducts were only elevated in nasal passages, not in tissues beyond the portal of entry. However, formaldehyde-specific hmDNA adducts have been detected in rodent tissues distal to the portal of entry when the animals were exposed to methanol or nitrosamines, which are known to release formaldehyde as a metabolic intermediate in vivo. These studies suggest a lack of transport of formaldehyde beyond the portal of entry when given by inhalation in animals. Although the hmDNA adducts are considered to be genotoxic endpoints of formaldehyde exposure, their mutagenicity has not been established.

There is limited evidence about mutagenicity of formaldehyde in experimental animals. Formaldehyde did not induce mutations in the nasal mucosa of rats with inhalation exposure to 18.5 mg/m³ for 13 weeks, but there are no available studies involving longer periods of exposure (see Table C-18). However, formaldehyde inhalation exposure caused other genotoxic endpoints, including chromosomal aberrations and single strand breaks but not micronuclei in cells of the respiratory system (Table C-18).

Twelve out of 17 that analyzed formaldehyde-induced genotoxic endpoints in bone marrow or blood cells were negative. Conflicting results have been obtained in terms of the source of formaldehyde. Formaldehyde derived from paraformaldehyde or commercial formalin was negative for DPX formation in bone marrow and peripheral blood cells, although one recent study, which used 10% formalin as a source of formaldehyde, induced DPX formation in bone marrow and peripheral blood mononuclear cells. Formaldehyde did not induce hmDNA adducts in the bone marrow of monkeys and rats, suggesting that inhaled exogenous formaldehyde may not be transported to the tissues distal to the portal of entry. Formaldehyde failed to induce CAs in 4/5 studies in the bone marrow or peripheral blood cells of rats and mice (see Tables C-18 and C-19), although one study detected CAs in bone marrow of rats. Limited available evidence shows that inhaled formaldehyde did not induce micronuclei in the peripheral blood cells of rats, but was positive for inducing SSBs in peripheral blood and bone marrow cells and produced mixed results on SCE formation. The above studies clearly indicate the complexity of data analyses with contradicting results even when using the same assay system, type of exposure, and/or methodology.

Formaldehyde produced mixed results in tissues other than the respiratory and hematopoietic systems (see Tables C-18 and C-19). Three studies demonstrated DPX formation in mouse kidney, testes, liver and spleen when 10% formalin was used as a source of formaldehyde.

Inhaled formaldehyde did not induce hmDNA adducts in the liver, spleen, and thymus of rats, but SSBs were detectable in the liver of rats following inhalation exposure.

Several studies evaluated the genotoxicity and mutagenicity of formaldehyde by routes other than inhalation exposure and reported mixed results (see Table C-19), suggesting that formaldehyde induced genotoxicity might depend on the route of exposure and formulation of formaldehyde administered.

C.3.6. Genotoxic Endpoints in Humans

A large set of research studies in several countries, involving different exposure settings, found that exposure to formaldehyde is associated with damage or changes to human DNA that inform mechanisms of carcinogenesis. These studies have observed increased levels of DNA damage, DNA-protein crosslinks, and chromosomal breaks in buccal and nasal epithelial cells, and peripheral blood lymphocytes (PBLs). Chromosomal damage, manifested as an increased frequency of different types of chromosomal aberrations, has been reported. It has been shown that an increased frequency of chromosomal aberrations and micronuclei are associated with increased cancer mortality, and these endpoints are considered by EPA to be highly relevant to the assessment of genotoxicity in humans ([U.S. EPA, 2005](#); [Bonassi et al., 2004b](#); [Bonassi et al., 2007](#); [Bonassi et al., 2008](#); [Bonassi et al., 2011](#)). Single strand breaks in DNA, indicating genetic instability also are considered by EPA to be highly relevant to the assessment of genotoxicity for humans. However, an increased level of sister chromatid exchange in peripheral lymphocytes has not been found to be associated with cancer mortality in a large collaborative evaluation ([Bonassi et al., 2004a](#)). Although sister chromatid exchange is an indication of genotoxicity, this endpoint is considered to be less relevant as a predictor of cancer risk. The studies that reported SCE results were evaluated and are summarized in tables but are not synthesized because of the large amount of evidence for other genotoxicity endpoints.

EPA evaluated the studies, focusing on study design, comparison groups, assessment of exposure and cytogenetic endpoints, and analytic methods. As discussed in this synthesis, although the entire set of studies contributed to the assessment, those with the stronger study designs and methods, and which provided adequate details, were given more weight. Most of the studies reporting on measures of genotoxicity did not describe the details of population selection, recruitment, and participation, which makes it difficult to evaluate potential selection bias. However, most did report details on the population, and since knowledge of a person's status regarding these endpoints would not be a factor in his or her decision to participate, the population selection reporting deficiency is likely not a serious limitation.

Chromosomal Aberrations in Peripheral Blood Lymphocytes

A total of 16 studies were available that evaluated chromosomal aberrations in peripheral blood lymphocytes (PBLs) or less differentiated subsets among individuals in a variety of exposure settings, including students in anatomy and embalming courses, workers in industrial settings, and

workers in pathology laboratories (Table C-20). Average formaldehyde concentrations in these occupational settings generally were above 0.1 mg/m³, although two studies evaluated chromosomal aberrations among groups exposed to lower average concentrations ([Santovito et al., 2011](#); [Pala et al., 2008](#)). Study results were heterogeneous, and the studies were variable in their study designs and reporting detail. Several did not state whether sample analysis was blinded with respect to exposure status, did not provide demographic information on exposed and referent groups to support assertions of similarity, had extremely small sample sizes ($N < 15$), or incubated cells for longer than 48–50 hours (thus not restricting to M₁ metaphases, and/ or did not describe their approach to data analysis: ([Vasudeva and Anand, 1996](#); [Vargová et al., 1992](#); [Thomson et al., 1984](#); [Suskov and Sazonova, 1982](#); [Lazutka et al., 1999](#); [Kitaeva et al., 1996](#); [He et al., 1998](#); [Gomaa et al., 2012](#); [Fleig et al., 1982](#)). Nine publications for 8 occupational groups provided detailed descriptions of study methods and important attributes of the exposed and referent groups ([Zhang et al., 2010](#); [Santovito et al., 2011](#); [Santovito et al., 2014](#); [Pala et al., 2008](#); [Musak et al., 2013](#); [Lan et al., 2015](#); [Jakab et al., 2010](#); [Costa et al., 2015](#); [Bauchinger and Schmid, 1985](#)).

Formaldehyde was associated with a higher prevalence of chromosomal aberrations among workers in pathology laboratories ([Santovito et al., 2011](#); [Musak et al., 2013](#); [Jakab et al., 2010](#); [Costa et al., 2015](#)); these effects included chromatid-type aberrations ([Jakab et al., 2010](#); [Costa et al., 2015](#)), chromosome-type aberrations ([Musak et al., 2013](#); [Costa et al., 2015](#)), chromosomal exchange ([Musak et al., 2013](#)), and premature centromere division ([Jakab et al., 2010](#)). Costa et al. (2015) also reported an increase in aneuploidies and in the number of aberrant and multiaberrant cells. In one study of paper makers, formaldehyde exposure was associated with dicentrics and centric rings ([Bauchinger and Schmid, 1985](#)). Average 8-hour TWA formaldehyde concentrations of 0.32, 0.47, and 0.9 mg/m³ were associated with a 1.7–1.9-fold increase in total chromosomal aberrations among exposed groups ([Musak et al., 2013](#); [Jakab et al., 2010](#); [Costa et al., 2015](#)). An increased mean number of chromosomal aberrations per cell was significantly associated with an 8-hour TWA concentration of 0.07 mg/m³ among pathologists compared to referent hospital workers exposed to 0.04 mg/m³ by Santovito et al. (2011). One well-conducted study did not observe associations ([Pala et al., 2008](#)), possibly because the group of laboratory workers was exposed to very low formaldehyde concentrations (75% of workers at < 0.026 mg/m³). Another study in nurses found no differences with their referent group, although this group likely experienced a wide variation in the intensity of their formaldehyde exposure, and no formaldehyde measurements were conducted ([Santovito et al., 2014](#)). An increased frequency of chromosomal aberrations or aberrant cells was also found in a few studies that incubated cell cultures for a longer period (72 hours) ([Lazutka et al., 1999](#); [Kitaeva et al., 1996](#); [Gomaa et al., 2012](#)), but not by all ([Vasudeva and Anand, 1996](#); [Fleig et al., 1982](#)). Incubation times longer than required to achieve first generation metaphase would be expected to result in greater heterogeneity in the aberration frequencies detected.

Zhang et al. (2010), using fluorescence in situ hybridization techniques, observed an increased level of chromosome aneuploidy (monosomy 7 and trisomy 8) in cultured CFU-GM colony cells in a small group of highly exposed formaldehyde-melamine production workers ($n = 10$) compared to a referent group matched by age and gender ($n = 12$). Although only a small number of workers were evaluated, this report provided complete details on study design, participation, population characteristics, exposure measurements, cytogenetic analyses, and data analysis and results. Subsequently, a larger group of the same cohort ($n = 29$ exposed, $n = 23$ referent) were included in a chromosome-wide evaluation of aneuploidy, again using cultured CFU-GM colony cells (Lan et al., 2015). An elevated risk ratio for monosomy, trisomy, and tetrasomy was found in several chromosomes, including chromosomes 5 and 7, a finding that was predicted a priori. In addition, investigators reported an increased frequency of structural chromosome aberrations in chromosome 5 (IRR 4.15, 95% CI 1.20–14.35). Gentry et al. (2013) reported on analyses using data on the cohort studied by Zhang et al. (2010) and noted that few of the DNA analyses scored 150 or more cells per individual as specified by the study protocol. Although the pilot study methods were criticized for not adhering to the assay protocol (Gentry et al., 2013), a clarification of the assay protocol was provided by the investigators with a description of how the study adhered to it (Rothman et al., 2017). The criticism by Gentry et al. (2013) applied to both the exposed and unexposed groups; thus, no bias should have occurred. Analyzing fewer cells per individual may have increased the variability in the prevalence estimates of aneuploidy, which may have attenuated the measures of association. Although the chromosome anomalies may have arisen either in vivo or during the in vitro cell culture period (Gentry et al., 2013), there was a significant increase in the exposed workers compared to the referent group, indicating a formaldehyde-associated tendency toward aneuploidy or other chromosomal aberrations. Median formaldehyde concentrations measured in the exposed and referent groups were 1.7 mg/m³ and 0.032 mg/m³, respectively. Personal exposure monitoring was conducted for several other chemical exposures, including chloroform, methylene chloride, tetrachloroethylene, trichloroethylene, benzene, or other hydrocarbons, which were not detected. Statistical models were adjusted for potential confounders including age, gender, recent infection, body mass index, and current tobacco, alcohol, and medication use.

The differences in lymphocyte subset levels between exposed and unexposed workers reported by Zhang et al. (2010) were challenged by Mundt et al. (2017) in a reanalysis that did not find evidence of an exposure-response trend within the exposed group, although the difference between unexposed and exposed subjects was reconfirmed. Rothman et al. (2017) also responded to the critique by Mundt et al. (2017) explaining that the exposure levels in the exposed group were relatively homogenous and the study was not designed to provide a range of exposures wide enough to evaluate exposure-response relationships given the expected effect size and sample size in the study. Overall, the evidence from the set of studies in which there is higher confidence is

consistent with the finding that formaldehyde exposure is associated with chromosomal aberrations in PBLs.

Micronuclei

An increase in micronuclei in buccal mucosa, nasal mucosal cells and peripheral blood lymphocytes (PBLs) was associated with formaldehyde exposure in a large number of studies (see Table C-20). Micronuclei were reported in a diverse set of exposed populations including plywood production workers, formaldehyde production and other chemical workers, pathologists and other laboratory workers, and anatomy and mortuary lab students, and were observed at average concentrations of 0.1 mg/m³ ([Wang et al., 2019](#); [Ballarin et al., 1992](#)), 0.2 mg/m³ ([Ladeira et al., 2011](#); [Costa et al., 2019](#)), and 0.5 mg/m³ ([Ying et al., 1997](#); [Costa et al., 2008](#); [Costa et al., 2011](#); [Costa et al., 2013](#)). Micronuclei in peripheral lymphocytes and exfoliated cells are considered biomarkers of genotoxic events and chromosomal instability, including errors in DNA repair mechanisms, dysfunction or lack of telomeres, and other failures during DNA replication and repair processes ([Bonassi et al., 2011](#)). Micronuclei in PBL is a validated predictor of cancer risk in epidemiology studies ([Bonassi et al., 2007](#)). Studies of exposure to formaldehyde over a short duration found no changes in micronucleus frequency in nasal mucosal cells ([Zeller et al., 2011](#)), buccal mucosal cells after a 4-hour exposure for 10 days ([Speit et al., 2007a](#)) or PBLs with an 8-hour cross-shift change ([Lin et al., 2013](#)).

Measurements in exfoliated buccal cells (EBC) revealed a consistently increased frequency of micronuclei or binucleated cells among exposed individuals ([Ying et al., 1997](#); [Viegas et al., 2010](#); [Titenko-Holland et al., 1996](#); [Suruda et al., 1993](#); [Peteffi et al., 2015](#); [Ladeira et al., 2011](#); [Costa et al., 2019](#); [Burgaz et al., 2002](#); [Aglan and Mansour, 2018](#)). Differences were reported using various study designs, including changes in anatomy and embalming students before and after lab courses and prevalence studies comparing exposed workers and referent groups. Generally, differences were observed at formaldehyde exposure levels averaging 0.2 mg/m³ and above. Micronuclei frequencies were greater by 1.5 to 6-fold in exposed workers with mean formaldehyde concentrations of 0.2 to 0.5 mg/m³ compared to referent groups ([Viegas et al., 2010](#); [Ladeira et al., 2011](#); [Costa et al., 2019](#)). Most of the studies of micronuclei frequency in buccal cells provided detailed discussions of design, methods, and results; potential confounders and other exposures that could pose a risk of genotoxicity were considered and excluded either in the design or data analysis. Associations with exposure duration also were observed by some researchers. Aglan ([2018](#)) analyzed micronuclei frequency in EBC from hair stylists who routinely conducted hair straightening treatments and compared them to a group of hair stylists who did not conduct these treatments. Formaldehyde concentrations can be high when hair straightening treatments are used, and 15-minute TWA concentrations greater than 1.9 mg/m³ were measured in this group. An increase in MN frequency was observed between the referent group and exposed groups stratified by exposure duration (below or above 5 years). However, there is more uncertainty in these results because reporting deficiencies prevented analysis of the potential for selection bias. While Costa

(2019) reported a nonsignificant increase across tertiles of formaldehyde concentration above 0.2 ppm among anatomy/ pathology workers, the authors did not observe a trend in the frequency of nuclear buds across exposure duration from less than 8 years to over 14 years. Other studies of workers with mean exposure duration over 5 years also reported associations with exposure duration ([Viegas et al., 2010](#); [Ladeira et al., 2011](#)).

Fewer studies are available that assessed micronuclei in nasal cells, but results were generally consistent. Significant differences in nasal micronuclei frequency were observed among anatomy students after an 8-week course ([Ying et al., 1997](#)), pathology workers compared to unexposed workers at the same institutions ([Burgaz et al., 2001](#)), and between formaldehyde production workers ([Ye et al., 2005](#)) or plywood production workers ([Ballarin et al., 1992](#)) compared to their referent groups. Formaldehyde concentrations among exposed groups averaged 0.1–>1.0 mg/m³. One study did not observe formaldehyde-related changes in nasal cells of embalming students ([Suruda et al., 1993](#)), but did report an increase in micronuclei with acentric fragments (centromere negative micronuclei) using fluorescence in situ hybridization (FISH) ([Titenko-Holland et al., 1996](#)). These results suggest that the predominant damage in these cells consisted of DNA and/or chromosomal breaks.

Most of a large set of studies that measured micronuclei in PBLs reported increased levels among exposed participants working in diverse exposure settings and in several countries ([Ye et al., 2005](#); [Wang et al., 2019](#); [Viegas et al., 2010](#); [Suruda et al., 1993](#); [Souza and Devi, 2014](#); [Orsiere et al., 2006](#); [Ladeira et al., 2011](#); [Jiang et al., 2010](#); [He et al., 1998](#); [Costa et al., 2008](#); [Costa et al., 2011](#); [Costa et al., 2013](#); [Costa et al., 2019](#); [Bouraoui et al., 2013](#); [Aglan and Mansour, 2018](#)). Several of these studies included a large sample size, and all provided detailed discussions of design, methods, and results, including how potential confounders and other exposures that could pose a risk of genotoxicity were considered and excluded, either in the design or data analysis. Costa et al. (2019) reported that the frequency of micronuclei in PBL and EBC were correlated in their study population. A clear concentration-related response in micronucleus frequency measured in PBLs was reported among plywood production workers in two studies that evaluated effects across multiple exposure categories ([Ye et al., 2005](#); [Jiang et al., 2010](#)). Micronuclei frequency (and centromeric micronuclei) increased with cumulative exposure ([Wang et al., 2019](#); [Suruda et al., 1993](#)) and the duration of exposure ([Viegas et al., 2010](#); [Souza and Devi, 2014](#); [Lin et al., 2013](#); [Ladeira et al., 2011](#); [Jiang et al., 2010](#); [Bouraoui et al., 2013](#); [Aglan and Mansour, 2018](#)). Observed effects were independent of confounding by age, gender, or smoking status.

A study of anatomy students did not observe changes in micronuclei in PBLs after an 8-week course, although increased levels were observed in buccal and nasal cells, suggesting that changes in lymphocytes may occur after a longer duration of formaldehyde exposure ([Ying et al., 1997](#)). Lin et al. (2013) did not observe an increase in micronucleus frequency across formaldehyde exposure categories among plywood workers in China. However, the referent group was exposed to

mean concentrations of 0.13 mg/m³, a level associated with increased micronucleus frequency in another study of plywood workers ([Jiang et al., 2010](#)).

The sensitivity of the micronucleus assay can be enhanced by probing cells with pancentromeric DNA probes. A micronucleus that has a single centromere (C1 + MN) suggests chromosome migration impairment, and the presence of two or more centromeres (Cx + MN) indicates centromere amplification, with both conditions indicating aneuploidy ([Iarmarcovai et al., 2006](#)). Orsiere et al. ([2006](#)) and Bouraoui et al. ([2013](#)) evaluated micronuclei in lymphocytes using FISH and a pancentromeric probe and found increased levels of centromeric micronuclei, including monocentromeric micronuclei (C1 + MN) and multicentromeric micronuclei (Cx + MN) among exposed pathology and anatomy lab workers. The enhanced chromosome loss is consistent with the increase in aneuploidy in lymphocytes reported by Zhang et al. ([2010](#)).

DNA Damage

Most studies of DNA single-strand breaks, DNA crosslinks, apurinic or apyrimidinic sites, and sites with incomplete DNA repair using the Comet assay observed associations in PBLs with occupational formaldehyde exposure involving workers in plywood or furniture manufacturing, use of melamine resins, and in pathology laboratories ([Zendehdel et al., 2017](#); [Peteffi et al., 2015](#); [Lin et al., 2013](#); [Jiang et al., 2010](#); [Gomaa et al., 2012](#); [Costa et al., 2008](#); [Costa et al., 2011](#); [Costa et al., 2015](#)) (see Table C-20). A 1.5- to 3-fold difference was observed comparing exposed groups to their referent groups at average concentrations as low as 0.09 mg/m³ ([Zendehdel et al., 2017](#)), 0.14 mg/m³ ([Jiang et al., 2010](#)) or 0.04–0.11 mg/m³ ([Peteffi et al., 2015](#)). A clear concentration-related response was observed in plywood plant workers ([Lin et al., 2013](#); [Jiang et al., 2010](#)). In addition to the cross-sectional comparisons, an increased level of damage to DNA, indicated by increased tail moment levels in the Comet assay, was associated with formaldehyde exposure over an 8-hour work shift ([Lin et al., 2013](#)) and after an exposure for 4 hours/day for 5 days during a controlled human exposure study ([Zeller et al., 2011](#)). One study of workers in medium density fiberboard manufacture did not observe increases in Comet assay measures in the exposed group at a mean 8-hour TWA 0.25 ± 0.07 mg/m³ ([Aydin et al., 2013](#)). The range of exposure levels (0.12–0.41 mg/m³) was lower than most of the studies that evaluated DNA damage using the Comet assay, and almost half of the exposed workers in this study reported using personal protective equipment.

An increased level of DPXs was associated with formaldehyde exposure in a few studies, both across an 8-hour work shift ([Lin et al., 2013](#)), and in comparisons of formaldehyde-exposed workers and their referent groups ([Shaham et al., 1997](#); [Shaham et al., 2003](#)). Lin et al. ([2013](#)) also compared DPX rates between formaldehyde-exposed plywood workers and a referent group but did not observe differences by exposure group. There was no trend across levels of exposure or duration of employment, possibly because the comparison group had significant exposure to formaldehyde (0.019–0.252 mg/m³) and workers had been employed only for a mean of 2.5 years. Shaham et al. ([2003](#)) found higher DPX levels in peripheral lymphocytes among a group of pathologists with a mean duration of exposure of 16 years compared to administrative workers

from the same hospitals. While DPX levels in the exposed group were comparable to the exposed groups studied by Lin et al. (2013), DPX levels in the administrative workers were 60% lower than those measured in the referent group of woodworkers, perhaps reflecting their lower formaldehyde exposure. Analyses ruled out potential confounding by age, gender, smoking, education, and country of origin. Shaham et al. (2003) also observed higher levels of pantropic p53 protein (mutant plus wild-type protein) in serum in the exposed group compared to serum in the unexposed group, with a particularly strong association in males (pantropic p53 >150 pg/mL, adjusted OR = 2.0 (95% CI 0.9–4.4)). Increased serum pantropic p53 levels (p53 >150 pg/mL) was associated with mutant p53 content, and also with elevated DPX levels (OR = 2.5, 95% CI 1.2–5.4), suggesting a link between increases in DPX levels and overexpression of mutant p53 protein, an indication of loss of tumor suppressor gene capability.

Malondialdehyde-deoxyguanosine (M₁dG) adducts in DNA extracted from whole blood were elevated in pathologists who spent time conducting tissue fixation (mean formaldehyde 0.212 ± 0.047 mg/m³) compared to workers and students in other science labs (Bono et al., 2010). The prevalence of M₁dG DNA adducts was increased in the entire group of pathologists compared to the referent group among whom average formaldehyde concentrations were 0.028 mg/m³. Increased levels also were observed among a subgroup exposed to 0.07 mg/m³ formaldehyde and higher. This finding suggests the presence of formaldehyde-associated DNA damage concurrent with the induction of oxidative stress. An increase in oxidative stress, indicated by elevated plasma levels of malondialdehyde (MDA), was observed among employees at a cosmetic manufacturing company, who also had higher plasma levels of p53 compared to a group of employees in a hospital administrative department with no formaldehyde exposure (Attia et al., 2014). Although no air monitoring was conducted, the cosmetics workers had higher urinary formate levels compared to the referent group. Both plasma MDA and plasma p53 levels were related to urinary formate levels and also to each other. Regression analyses were adjusted for age and gender. Together, these two studies suggest that formaldehyde may increase systemic oxidative stress, which may be related to observed increases in peripheral white blood cell genotoxicity.

DNA Repair Protein Activity

O⁶-alkylguanine DNA alkyl-transferase activity in PBLs of students after 9 weeks or 3-months exposure to formaldehyde in embalming or anatomy labs was compared to enzyme activity prior to the beginning of the courses. Although an association with decreased activity was indicated in one study of embalming students (Hayes et al., 1997), this finding was not confirmed by a subsequent study of anatomy students (Schlink et al., 1999).

Susceptibility: Gene-Environment Interaction

A few studies of genotoxicity among formaldehyde-exposed groups also evaluated differences in subgroups defined by polymorphic variants in genes coding for proteins involved in the detoxification of xenobiotic toxic substances, including glutathione-S-transferases (GSTM1,

GSTT1, GSTP1), CYP2E1, and specifically, formaldehyde (alcohol dehydrogenase (ADH5) (see Table C-20). Polymorphisms in DNA repair proteins also were studied including the X-ray repair cross-complementing genes (XRCC1, XRCC2, XRCC3), RAD51, PARP1, and MUTYH. This included genes of the Fanconi anemia pathway (FANCA, BRIP1). The frequency of chromosomal aberrations in lymphocytes was higher in a formaldehyde-exposed group but did not vary by GSTT or GSTM polymorphism ([Santovito et al., 2011](#)). However, the GSTM1 null variant and the GSTP1 codon 105 Val allele were associated with an increased olive tail moment and MN frequency, respectively, among exposed individuals, but not in the referent group ([Jiang et al., 2010](#)). Costa et al. ([2015](#)) and Costa et al. ([2019](#)) also reported an increase in MN frequency in exfoliated buccal cells among exposed individuals with the Val variant in the GSTP1 rs1695 polymorphism, whereas chromosomal aberrations (CSAs) were more prevalent among the exposed group homozygous for the Ile allele. This research group also reported an increase in nuclear buds in buccal cells among exposed individuals with the A variant in the CYP2E1 rs6413432 polymorphism while exposed individuals homozygous for the wildtype T allele had a higher % tDNA measured in the comet assay. These associations were not observed in the referent group. In addition, the variant allele for the ADH5 Val309Ile polymorphism was associated with an increased frequency of micronuclei in lymphocytes among exposed individuals, but not in the referent group ([Ladeira et al., 2013](#)). The frequency of nuclear buds was associated with formaldehyde exposure and among carriers of the XRCC3 *Met* variant allele in both exposed and referent individuals, but effect modification was not apparent ([Ladeira et al., 2013](#)). Costa et al. ([2019](#)) did not observe associations with the XRCC gene polymorphisms and micronuclei frequency in EBC or PBL among formaldehyde exposed workers. However, micronuclei frequency was increased in PBL among exposed individuals with the Ala variant in the FANCA rs719823 variant. Therefore, genetic differences may alter susceptibility to the cytogenetic effects of formaldehyde, but more definitive research is needed.

Table C-20. Summary of genotoxicity of formaldehyde in human studies

Reference and study design	Exposure	Results																														
Chromosomal Damage and Induction of DNA repair																																
Prevalence Studies																																
Costa et al. (2015) Portugal Prevalence study Population: 84 anatomy pathology workers from 9 hospital laboratories, exposed to formaldehyde for at least 1 year, compared to 87 unexposed employees from administrative offices in same geographic area. Exclusions: cancer history, radiation therapy or chemotherapy, surgery with anesthesia or blood transfusion in last year. Exposed and referent similar for mean age 39 years, 77% females, 25% smokers. Outcome: Peripheral blood samples, coded, analyses blinded to exposure status. Chromosomal aberrations (CAs) structural and numerical), duplicates cultured 51 hrs (cited Roma-Torres et al., 2006), 4% Giemsa stain; scored 100 metaphases per person, CTAs & CSAs according to Savage et al. (1976); gaps not included. Exposed compared to unexposed using Mann-Whitney U-test for CA measures; negative binomial regression for untransformed total-CAs, CSAs, CTAs, gaps, aneuploidies, & aberrant cells; Poisson regression for untransformed multiaberrant cells.	Exposure assessed via air sampling and deriving an 8-hr TWA for each subject. Exposure concentration: Mean: 0.38 ppm (0.47 mg/m³) Range: 0.28–0.85 ppm (0.34–1.05 mg/m³) Exposure duration 12.0 ± 8.2 years	Comparison of exposed (N = 84) and referent (N = 87), frequencies of chromosomal aberrations (CA), structural and numerical <table> <tr> <th>Aberration</th><th>MR^a</th><th>95% CI</th></tr> <tr> <td>Total CA</td><td>1.91</td><td>1.44–2.53</td></tr> <tr> <td>CSAs</td><td>2.07</td><td>1.27–3.38</td></tr> <tr> <td>CTAs</td><td>1.86</td><td>1.39–2.48</td></tr> <tr> <td>Gaps</td><td>1.65</td><td>1.34–2.03</td></tr> <tr> <td>Aneuploidies</td><td>1.64</td><td>1.36–1.98</td></tr> <tr> <td>Aberrant cells</td><td>1.66</td><td>1.28–2.17</td></tr> <tr> <td>Multi-aberrant cells</td><td>3.96</td><td>2.09–7.48</td></tr> </table> ^a MR – mean ratio; all models adjusted for age, gender and smoking habit, multi-aberrant cells MR also adjusted for fruit consumption (# pieces eaten per day) No associations observed for models of formaldehyde exposure as continuous variable, exposure duration or professional activity on genotoxicity endpoints (data not provided by authors) Mean SCE per cell in peripheral lymphocytes: ratio of exposed to referent <table> <tr> <th></th><th>Ratio</th><th>95% CI</th></tr> <tr> <td>SCE/cell</td><td>1.27</td><td>1.10 –1.46</td></tr> </table> Poisson regression adjusted for gender, smoking, and age.	Aberration	MR ^a	95% CI	Total CA	1.91	1.44–2.53	CSAs	2.07	1.27–3.38	CTAs	1.86	1.39–2.48	Gaps	1.65	1.34–2.03	Aneuploidies	1.64	1.36–1.98	Aberrant cells	1.66	1.28–2.17	Multi-aberrant cells	3.96	2.09–7.48		Ratio	95% CI	SCE/cell	1.27	1.10 –1.46
Aberration	MR ^a	95% CI																														
Total CA	1.91	1.44–2.53																														
CSAs	2.07	1.27–3.38																														
CTAs	1.86	1.39–2.48																														
Gaps	1.65	1.34–2.03																														
Aneuploidies	1.64	1.36–1.98																														
Aberrant cells	1.66	1.28–2.17																														
Multi-aberrant cells	3.96	2.09–7.48																														
	Ratio	95% CI																														
SCE/cell	1.27	1.10 –1.46																														
Lan et al. (2015) China Prevalence study Population: 43 formaldehyde-melamine workers (95% employed for >1 year) compared to 51 workers from other	Personal monitors for 3 d over entire shift within a 3-wk period.	Among all 24 chromosomes analyzed, elevated IRR for monosomy found for chromosomes 1, 5, 7, 4, 19, 10, 16, 21, 2, 8, 18, 12, 20, 13, 6, and 14 ($p < 0.05$, Table 2 in Lan et al.); elevated IRR for trisomy found for chromosomes 5, 19, 21, 1, 20, and 16; elevated IRR for																														

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																																																																				
regional factories no formaldehyde exposure frequency-matched by age and gender; participation rates exposed 92%, referent 95%; selected subset with scorable metaphases, high formaldehyde levels among exposed, comparable referents with scorable metaphases (29 exposed and 23 referent). Outcome: Chromosome-wide aneuploidy in CFU-GM colony cells cultured for 14 d using OctoChrome FISH; scored minimum 150 cells/ subject; analysis blinded to exposure. Analyzed using negative binomial regression controlling for age and gender; incidence rate ratio (IRR). Also evaluated potential confounding from current smoking and alcohol use, recent infections, current medication use, and body mass index (Supplemental tables in the paper) Related reference: Zhang et al. (2010)	Formaldehyde concentration: 8 hr TWA Exposed Median: 1.38 ppm (1.7 mg/m³) 10th & 90th percentile: 0.78, 2.61 ppm (0.96, 3.2 mg/m³) Referent 0.026 ppm (0.032 mg/m³) 10th & 90th percentile: 0.015, 0.026 ppm (0.019, 0.032 mg/m³) Formaldehyde LOD: 0.012 ppm Personal sampling for organic compounds on 2 or more occasions. No chloroform, methylene chloride, tetra-chloroethylene, trichloro-ethylene, benzene, or hydrocarbons were detected; urinary benzene at background levels and similar between groups	tetrasomy found for chromosomes 4, 15, 17, 14, 3, 18, 8, 12, 2, 10, and 6. Selected Comparison of Chromosomal Aberration Rates* <table><tr><th>Chromosome</th><th>IRR</th><th>95% CI</th><th>p-Value</th></tr><tr><td colspan="4"><i>Monosomy</i></td></tr><tr><td>1</td><td>2.31</td><td>1.61–3.31</td><td>6.02E-06</td></tr><tr><td>5</td><td>2.24</td><td>1.57–3.20</td><td>9.01E-06</td></tr><tr><td>7</td><td>2.17</td><td>1.53–3.08</td><td>1.57E-05</td></tr><tr><td>4</td><td>2.02</td><td>1.40–2.90</td><td>0.00015</td></tr><tr><td>19</td><td>1.74</td><td>1.29–2.34</td><td>0.00026</td></tr><tr><td>10</td><td>1.86</td><td>1.30–2.65</td><td>0.00064</td></tr><tr><td>16</td><td>1.54</td><td>1.12–2.12</td><td>0.0075</td></tr><tr><td colspan="4"><i>Trisomy</i></td></tr><tr><td>5</td><td>3.40</td><td>1.94–5.97</td><td>1.98E-05</td></tr><tr><td>19</td><td>2.07</td><td>1.24–3.46</td><td>0.0055</td></tr><tr><td>21</td><td>2.09</td><td>1.22–3.57</td><td>0.0071</td></tr><tr><td colspan="4"><i>Tetrasomy</i></td></tr><tr><td>4</td><td>1.64</td><td>1.21–2.21</td><td>0.0012</td></tr><tr><td>15</td><td>3.10</td><td>1.53–6.28</td><td>0.0017</td></tr><tr><td>17</td><td>2.40</td><td>1.33–4.32</td><td>0.0036</td></tr></table> * Chromosomes with IRR with p-values < 0.001. Increased frequency of structural chromosome aberrations in chromosome 5, IRR 4.15, 95% CI 1.20–14.35 (p = 0.024).	Chromosome	IRR	95% CI	p-Value	<i>Monosomy</i>				1	2.31	1.61–3.31	6.02E-06	5	2.24	1.57–3.20	9.01E-06	7	2.17	1.53–3.08	1.57E-05	4	2.02	1.40–2.90	0.00015	19	1.74	1.29–2.34	0.00026	10	1.86	1.30–2.65	0.00064	16	1.54	1.12–2.12	0.0075	<i>Trisomy</i>				5	3.40	1.94–5.97	1.98E-05	19	2.07	1.24–3.46	0.0055	21	2.09	1.22–3.57	0.0071	<i>Tetrasomy</i>				4	1.64	1.21–2.21	0.0012	15	3.10	1.53–6.28	0.0017	17	2.40	1.33–4.32	0.0036
Chromosome	IRR	95% CI	p-Value																																																																			
<i>Monosomy</i>																																																																						
1	2.31	1.61–3.31	6.02E-06																																																																			
5	2.24	1.57–3.20	9.01E-06																																																																			
7	2.17	1.53–3.08	1.57E-05																																																																			
4	2.02	1.40–2.90	0.00015																																																																			
19	1.74	1.29–2.34	0.00026																																																																			
10	1.86	1.30–2.65	0.00064																																																																			
16	1.54	1.12–2.12	0.0075																																																																			
<i>Trisomy</i>																																																																						
5	3.40	1.94–5.97	1.98E-05																																																																			
19	2.07	1.24–3.46	0.0055																																																																			
21	2.09	1.22–3.57	0.0071																																																																			
<i>Tetrasomy</i>																																																																						
4	1.64	1.21–2.21	0.0012																																																																			
15	3.10	1.53–6.28	0.0017																																																																			
17	2.40	1.33–4.32	0.0036																																																																			
Santovito et al. (2014) Italy Prevalence study Population: 20 female nurses from 2 analogous departments in 2 hospitals (mean age 37 year) ; 20 unexposed from administrative departments of same	All exposed used protective equipment; no formaldehyde measurements; nurses also exposed to antibiotics, cytostatic drugs, anesthetics and sterilants	Frequency of Chromosomal Aberrations and SCEs among nurses and referent (mean ± SE) <table><tr><th></th><th>#</th><th>Nurses</th><th>Referent</th></tr><tr><td>CA/ NSM</td><td>20</td><td>0.025 ± 0.003</td><td>0.02 ± 0.003</td></tr><tr><td>Cells with aberrations / NSM</td><td>20</td><td>0.025 ± 0.003</td><td>0.02 ± 0.003</td></tr></table>		#	Nurses	Referent	CA/ NSM	20	0.025 ± 0.003	0.02 ± 0.003	Cells with aberrations / NSM	20	0.025 ± 0.003	0.02 ± 0.003																																																								
	#	Nurses	Referent																																																																			
CA/ NSM	20	0.025 ± 0.003	0.02 ± 0.003																																																																			
Cells with aberrations / NSM	20	0.025 ± 0.003	0.02 ± 0.003																																																																			

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results															
hospital (mean age 39.6 year); all nonsmokers and did not consume alcohol Outcome: Peripheral blood samples, coded. Cultures incubated for 48 hr for CA and 72 hr for SCE; CA slides stained with 5% Giemsa, scored 200 metaphases per subject, SCE 50 metaphases scored per subject; Mean frequencies compared, Wilcoxon test	Employment duration: Exposed 11.8 year, range 1–28 year; Referent 11.2 year, range 7–20 year	<table><tr><td>SCEs/ NSM</td><td>20</td><td>6.55 ± 0.033*</td><td>4.10 ± 0.37</td></tr></table> NSM: number of scored metaphases * <i>p</i> <0.001 No association CAs or SCEs with age or duration	SCEs/ NSM	20	6.55 ± 0.033*	4.10 ± 0.37											
SCEs/ NSM	20	6.55 ± 0.033*	4.10 ± 0.37														
Costa et al. (2013) Portugal Prevalence study Population: 35 pathology workers from 4 hospital laboratories, exposed to formaldehyde for at least 1 year (88.6% female, mean age 41.2 year, 20% smokers), compared to 35 unexposed employees from same work area (80% female, mean age 39.8 year, 20% smokers). Outcome: SCE, coding and analysis blinded; stain fluorescence plus Giemsa, scored 50 M ₂ metaphases/ subject by 1 reader Related references: Costa et al. (2008) ; Costa et al. (2011)	Exposure assessed via air sampling and deriving an 8-hr TWA for each subject. Exposure concentration: Mean: 0.44 mg/m ³ Range: (0.28–0.85) mg/m ³ Exposure duration 12.5 (1–30) years	Mean SCE per cell 1.3-fold higher in exposed workers compared to controls (<i>p</i> <0.05, Student’s t-test). Univariate analyses presented in Figure 1 of Costa et al. (2013) Mean SCE per cell in peripheral lymphocytes: ratio of exposed to referent <table><tr><td></td><td>Ratio</td><td>95% CI</td></tr><tr><td>SCE/cell</td><td>1.245</td><td>0.594 –1.897</td></tr></table> Multivariate analysis adjusted for gender, smoking, and age		Ratio	95% CI	SCE/cell	1.245	0.594 –1.897									
	Ratio	95% CI															
SCE/cell	1.245	0.594 –1.897															
Musak et al. (2013) Slovakia Prevalence study Population: 105 technicians and pathologists at hospital labs (79% female, mean age 41.7 years, 27.6% smokers) compared to 250 other medical staff (89% female, mean age 36.2 years, 19.2% smokers), all healthy. Outcome: Differences in frequency of chromosomal aberration in peripheral blood lymphocytes, blinded analysis, 100 mitoses scored/ subject, 2 scorers	Air monitoring once per year (no details provided). Exposure conc.: Mean: 0.32 mg/m ³ Range: 0.14–0.66 mg/m ³ Exposure duration: Mean: 14.7 ± 10.4 years Range: NR	Chromosomal aberrations in peripheral lymphocytes <table><tr><td>Aberration</td><td>OR</td><td>95% CI</td></tr><tr><td>CA</td><td>1.70</td><td>1.6–2.72</td></tr><tr><td>CTA</td><td>1.37</td><td>0.85–2.19</td></tr><tr><td>CSA</td><td>1.57</td><td>0.98–2.53</td></tr><tr><td>Chromosomal exchange</td><td>2.6</td><td>1.1–5.9</td></tr></table> Binary logistic regression controlling for age, gender, job type, and smoking	Aberration	OR	95% CI	CA	1.70	1.6–2.72	CTA	1.37	0.85–2.19	CSA	1.57	0.98–2.53	Chromosomal exchange	2.6	1.1–5.9
Aberration	OR	95% CI															
CA	1.70	1.6–2.72															
CTA	1.37	0.85–2.19															
CSA	1.57	0.98–2.53															
Chromosomal exchange	2.6	1.1–5.9															
Gomaa et al. (2012) Egypt Prevalence study Population: 30 workers in pathology, histology and anatomy laboratories at a university (30% female, mean age 42.5	No formaldehyde measurements; exposure defined by job type	Chromosomal aberrations in peripheral lymphocytes <table><tr><td>Structural</td><td>Referent</td><td>Exposed</td></tr><tr><td>Chromatid gap & break</td><td>1.9 ± 0.36</td><td>6.5 ± 0.65*</td></tr></table>	Structural	Referent	Exposed	Chromatid gap & break	1.9 ± 0.36	6.5 ± 0.65*									
Structural	Referent	Exposed															
Chromatid gap & break	1.9 ± 0.36	6.5 ± 0.65*															

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results		
year) compared to 15 referents (46.7% female, mean age 39.3 year). Source of referent was not described. Outcome: Chromosome aberrations in peripheral blood lymphocytes, cultured 72 hr, blinding not described; mean # per 100 metaphases; Difference between exposed and referent, Student's <i>t</i> -test	Mean employment duration 14.3 year	Chromatid deletion	8.7 ± 0.55	15.5 ± 0.47*
		Ring chromosome	5.5 ± 0.33	16.4 ± 0.29*
		Dicentric chromosome	0.9 ± 0.41	9.0 ± 0.54*
		Total	20.0 ± 0.27	46.4 ± 0.35
		Numerical		
		Aneuploidy	0.2 ± 0.12	0.7 ± 0.10
		Polyploidy	0.6 ± 0.14	0.9 ± 0.09
		* Student's <i>t</i> -test, <i>p</i> <0.05; mean per 100 metaphases ± SE		
		No association with age or gender, ANOVA		
Santovito et al. (2011) Italy Prevalence study Population: 20 pathology workers (70% female, mean age 45.7 year) compared to 16 workers from the same hospital (43.8% female, mean age 42.1 year). All subjects were non-smokers and had not consumed alcohol in 1 year. Outcome: Frequency of chromosome aberrations per cell and mean % cells with aberrations; Venous blood sample collected at end of shift on same day as formaldehyde measurements, samples coded and processed within 4 hrs of collection, cells harvested 48 hr, 5% Giemsa stain, scored 100 metaphases/subject	Exposure conc: Personal air sampling, 8-hr duration. Referent: Mean: 0.036 ± 0.002 mg/m ³ Pathologists: Mean: 0.073 ± 0.013 mg/m ³ LOD 0.05 mg/mL Exposure duration: Mean: 13 years Range: 2–27 years	Chromosomal aberrations in peripheral lymphocytes		
			Referent	Exposed
		Mean CA/cell	0.011 ± 0.004	0.03 ± 0.004*
		% of cells with aberrations	1.00 ± 0.342	2.50 ± 0.286
		* <i>p</i> <0.001, Mann-Whitney U test		
		Effects of exposure on chromosomal aberrations and cells with aberrations (coefficient (SE))		
			Exposure	<i>p</i> - Value
		# CA	0.960 (0.275)	0.001
		# cell with aberrations	0.838 (0.287)	0.004
		Generalized linear models with Poisson error distribution, adjusted for age		
Jakab et al. (2010) Hungary Prevalence study Population: 37 female workers in 3 hospitals & 1 university pathology department (21 exposed to formaldehyde alone	Exposure assessed via records on area air samples, measured within	Cytogenetic analysis in cultured peripheral lymphocytes (mean ± SD)		
			Unexposed	Exposed
		Total CA	1.62 ± 0.26	3.05 ± 0.62*

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																					
(mean age 43.3 year, 23.8% smokers), compared to 37 healthy female unexposed health-service staff (mean age 41.8 year, 16.2% smokers). Outcome: Peripheral lymphocytes; CA, SCE, premature centromere division (PCD), mitoses with >3 chromosomes with PCD (centromere separation general (CSG)), CA stain 5% Giemsa, cells harvested 50 hr, scored 100 metaphases/subject. SCE fluorescence plus Giemsa; scored 50 cells/subject; analyses blinded	1–3 years of data collection. Exposure Concentration: 8-hr TWA: 0.9 mg/m³ Range: 0.23–1.21 mg/m³ Exposure duration: Mean: 17.7 years Range: 4–34 years	<table> <tr> <td>Chromatid-type aberrations</td><td>1.00 ± 0.20</td><td>2.35 ± 0.46*</td></tr> <tr> <td>Chromosome-type aberrations</td><td>0.62 ± 0.18</td><td>0.70 ± 0.26</td></tr> <tr> <td>Aneuploidy</td><td>8.89 ± 0.66</td><td>5.4 ± 0.61*</td></tr> <tr> <td>SCE (%/cell)</td><td>6.16 ± 0.16</td><td>6.36 ± 0.26</td></tr> <tr> <td>High frequency SCE</td><td>3.76 ± 1.14</td><td>7.05 ± 2.19</td></tr> <tr> <td>PCD (%)</td><td>7.6 ± 0.84</td><td>13.65 ± 1.59*</td></tr> <tr> <td>PCD (CSG)</td><td>5.57 ± 0.66</td><td>8.8 ± 1.07*</td></tr> </table> *<i>p</i> < 0.05, Student's <i>t</i>-test, compared to controls SCE % and mean HF/SCE higher in referent and exposed smokers; mean SCE % associated with older age	Chromatid-type aberrations	1.00 ± 0.20	2.35 ± 0.46*	Chromosome-type aberrations	0.62 ± 0.18	0.70 ± 0.26	Aneuploidy	8.89 ± 0.66	5.4 ± 0.61*	SCE (%/cell)	6.16 ± 0.16	6.36 ± 0.26	High frequency SCE	3.76 ± 1.14	7.05 ± 2.19	PCD (%)	7.6 ± 0.84	13.65 ± 1.59*	PCD (CSG)	5.57 ± 0.66	8.8 ± 1.07*
Chromatid-type aberrations	1.00 ± 0.20	2.35 ± 0.46*																					
Chromosome-type aberrations	0.62 ± 0.18	0.70 ± 0.26																					
Aneuploidy	8.89 ± 0.66	5.4 ± 0.61*																					
SCE (%/cell)	6.16 ± 0.16	6.36 ± 0.26																					
High frequency SCE	3.76 ± 1.14	7.05 ± 2.19																					
PCD (%)	7.6 ± 0.84	13.65 ± 1.59*																					
PCD (CSG)	5.57 ± 0.66	8.8 ± 1.07*																					
Zhang et al. (2010) China Prevalence study Population: 43 formaldehyde-melamine workers (95% employed for >1 year) compared to 51 workers from other regional factories frequency-matched by age and gender; participation rates exposed 92%, referent 95%; Analyzed subset of exposed (<i>n</i> = 10, 9 male, 1 female, mean age 31 year) and referent (<i>n</i> = 12, 11 male, 1 female, mean age 32 year) Outcome: Chromosome aberration in peripheral blood cells, blinded to exposure. Chromosome aneuploidy in cultured CFU-GM colony cells using FISH; monosomy 7 and Trisomy 8; scored minimum 150 cells/ subject. Related references: Mundt et al. (2017); Lan et al. (2015); Gentry et al. (2013)	Personal monitors for 3 d within a 3-wk period. Formaldehyde concentration: 8 h TWA Exposed Median: 1.57 mg/m³ 10th & 90th percentile: 0.74, 3.08 mg/m³ Referent 0.039 mg/m³ 10th & 90th percentile: 0.022, 0.039	Leukemia-specific chromosome changes: Significant increase chromosome aneuploidy in cultured CFU-GM colony cells among subset of high exposed (<i>n</i> = 10) compared to matched controls (<i>n</i> = 12) <i>Data provided in Figure 4 of Zhang et al. (2010).</i> Analyzed using negative binomial regression (exposed compared to unexposed) controlling for age, gender, and smoking Mundt et al. presented individual data in graphs for chromosome 7 and chromosome 8 (<i>n</i> = 10 exposed and <i>n</i> = 12 controls), noting smoking status and whether 150 or more cells were evaluated. No patterns apparent.																					
Costa et al. (2008) Portugal Prevalence study	Exposure assessed via air sampling at breathing zone	Mean SCE per cell in peripheral lymphocytes <table> <tr> <td></td><td>Controls</td><td>Exposed</td></tr> <tr> <td>SCE/ cell</td><td>4.49 ± 0.16</td><td>6.13 ± 0.29*</td></tr> </table>		Controls	Exposed	SCE/ cell	4.49 ± 0.16	6.13 ± 0.29*															
	Controls	Exposed																					
SCE/ cell	4.49 ± 0.16	6.13 ± 0.29*																					

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results															
Population: 30 pathology lab workers (4 hospitals), (70% female, mean age 38 year, 27% smokers) compared to 30 administrative employees matched by age, gender, lifestyle, smoking habits and work area (63.3% female, mean age 37 years, 23% smokers). Outcome: Peripheral lymphocytes; blood samples collected 10–11 am; processed immediately; stain fluorescence plus 5% Giemsa, SCE/ cell 50 s division metaphases scored by one observer, Scored blind to exposure status. Effect of smoking and gender also analyzed	and deriving an 8-hr TWA for each subject Concentration: Mean: 0.54 mg/m³ Range: (0.05–1.94) mg/m³ Duration: 11 years Range: (0.5–27) years	<i>*p</i> <0.05, Student’s <i>t</i>-test No association of SCE with gender or age. Smoking increased SCE among referent group (smoking prevalence 23% in referent, 27% in exposed). No association of SCE with duration of exposure															
Pala et al. (2008) Italy Prevalence study Population: 36 lab workers (66.7% female, mean age 40.1 year, 16.7% smokers) Outcome: CA and SCE, in peripheral lymphocytes (blood sampled at end of 8-hr) Blinded analyses, CA: cells harvested at 48 hr, 100 metaphases/ subject, SCE: harvest at 72 hr, 30 2nd division cells/ subject.	Personal air monitoring (8-hr sample) High exposure group: ≥ 0.026 mg/m³, 75th percentile (range 0.005–0.269 mg/m³) and low-exposure group: <0.026 mg/m³ Concentration: Low (<i>n</i> = 27): 0.015 (0.005–0.0254) mg/m³ High (<i>n</i> = 9): 0.056 (0.026–0.269) mg/m³	<table><tr><th colspan="3">Frequency chromosomal aberrations in peripheral lymphocytes</th></tr><tr><th></th><th>CA</th><th>SCE</th></tr><tr><td>< 0.026 mg/m³</td><td>2.95 ± 1.79 (<i>n</i>=19)</td><td>6.57 ± 1.38 (<i>n</i>=17)</td></tr><tr><td>≥ 0.026 mg/m³</td><td>2.22 ± 1.27 (<i>n</i>=5)</td><td>5.06 ± 0.76 (<i>n</i>=2)</td></tr><tr><td>Means ratio (95% CI)</td><td>0.83 (0.42–1.64)</td><td>0.81 (0.56–1.18)</td></tr></table> Multivariate regression models adjusting for gender, age and smoking; Poisson model for CA, SCE log-normal random effects model Authors did not use a referent group	Frequency chromosomal aberrations in peripheral lymphocytes				CA	SCE	< 0.026 mg/m ³	2.95 ± 1.79 (<i>n</i> =19)	6.57 ± 1.38 (<i>n</i> =17)	≥ 0.026 mg/m ³	2.22 ± 1.27 (<i>n</i> =5)	5.06 ± 0.76 (<i>n</i> =2)	Means ratio (95% CI)	0.83 (0.42–1.64)	0.81 (0.56–1.18)
Frequency chromosomal aberrations in peripheral lymphocytes																	
	CA	SCE															
< 0.026 mg/m ³	2.95 ± 1.79 (<i>n</i> =19)	6.57 ± 1.38 (<i>n</i> =17)															
≥ 0.026 mg/m ³	2.22 ± 1.27 (<i>n</i> =5)	5.06 ± 0.76 (<i>n</i> =2)															
Means ratio (95% CI)	0.83 (0.42–1.64)	0.81 (0.56–1.18)															
Ye et al. (2005) China Population: 18 workers at a formaldehyde plant at least 1 year (38.9% female, mean age 29 year, and 16 workers exposed to indoor air formaldehyde via building materials (75% female, mean age 22 year) compared to 23 students with no known source of formaldehyde exposure (dormitories) (48% female, mean age 19 year); all nonsmokers Outcome: SCE in peripheral lymphocytes, time of sample not stated; stain Giemsa solution, analysis blinded, 30 M₂ lymphocytes analyzed/ subject.	Area samples; Exposure duration: Workers 8.5 (1–15) years Waiters 12 wks TWA Concentration Controls 0.011 ± 0.0025 mg/m³ Max. 0.015 mg/m³ Wait staff 0.107 ± 0.067 mg/m³	<table><tr><th colspan="4">SCE frequency by exposure group</th></tr><tr><th></th><th>Referent</th><th>Wait Staff</th><th>Formaldehyde workers</th></tr><tr><td>Mean SCE</td><td>6.38 ± 0.41</td><td>6.25</td><td>8.24 ± 0.89*</td></tr></table> <i>*p</i> <0.05, ANOVA. Values estimated from graph in Figure 2 of Ye et al.	SCE frequency by exposure group					Referent	Wait Staff	Formaldehyde workers	Mean SCE	6.38 ± 0.41	6.25	8.24 ± 0.89*			
SCE frequency by exposure group																	
	Referent	Wait Staff	Formaldehyde workers														
Mean SCE	6.38 ± 0.41	6.25	8.24 ± 0.89*														

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																											
	Max. 0.30 mg/m ³ Workers 0.985 ± 0.286 mg/m ³ Max. 1.694 mg/m ³																												
(Shaham et al., 2002) Israel Prevalence study Population: 90 workers from 14 hospital pathology departments (65 females, 25 males; mean age 44.2 year, 34% smokers) compared to 52 administrative workers from the same hospitals (8 females, 44 males; mean age 41.7 year, 46.9% active smokers, 53.1% nonsmokers) Outcome: SCE in peripheral lymphocytes; Mean # SCEs per chromosome and proportion of high frequency cells compared between exposed and referent. High frequency cells defined as > 8 SCEs; blinding not described, stain fluorescence plus 5% Giemsa, scored 30–32 cells/ subject. Related references: Shaham et al. (1997)	Personal and area samples, sampling at different points in work day, sampling duration averaged 15 min Exposure concentration: Low level exposure: Mean: 0.49 mg/m³ Range: 0.05–0.86 mg/m³ High level exposure: Mean: 2.76 mg/m³ Range: 0.89–6.89 mg/m³ Exposure duration: Mean: 15.4 years Range: 1–39 years	SCE frequency in peripheral lymphocytes by exposure group and smoking status (mean ± SE) <table> <tr> <th></th><th>Mean number SCEs per chromosome</th><th>Mean proportion of high frequency cells</th></tr> <tr> <td>Unexposed</td><td>0.19 ± 0.004</td><td>0.44 ± 0.02</td></tr> <tr> <td>Exposed</td><td>0.27 ± 0.003*</td><td>0.88 ± 0.01*</td></tr> <tr> <td><i>No smoking</i></td><td></td><td></td></tr> <tr> <td>Low</td><td>0.28 ± 0.004</td><td>0.88 ± 0.015</td></tr> <tr> <td>High</td><td>0.26 ± 0.021</td><td>0.86 ± 0.016</td></tr> <tr> <td><i>Smoking</i></td><td></td><td></td></tr> <tr> <td>Low</td><td>0.27 ± 0.007</td><td>0.89 ± 0.018</td></tr> <tr> <td>High</td><td>0.28 ± 0.006</td><td>0.92 ± 0.021</td></tr> </table> *p <0.01, ANOVA adjusting for age, gender, smoking status, education years and origin (ethnicity) No association with exposure duration (≤15 years and >15 years) with adjustment for age, gender, smoking status, education years and origin (ethnicity)		Mean number SCEs per chromosome	Mean proportion of high frequency cells	Unexposed	0.19 ± 0.004	0.44 ± 0.02	Exposed	0.27 ± 0.003*	0.88 ± 0.01*	<i>No smoking</i>			Low	0.28 ± 0.004	0.88 ± 0.015	High	0.26 ± 0.021	0.86 ± 0.016	<i>Smoking</i>			Low	0.27 ± 0.007	0.89 ± 0.018	High	0.28 ± 0.006	0.92 ± 0.021
	Mean number SCEs per chromosome	Mean proportion of high frequency cells																											
Unexposed	0.19 ± 0.004	0.44 ± 0.02																											
Exposed	0.27 ± 0.003*	0.88 ± 0.01*																											
<i>No smoking</i>																													
Low	0.28 ± 0.004	0.88 ± 0.015																											
High	0.26 ± 0.021	0.86 ± 0.016																											
<i>Smoking</i>																													
Low	0.27 ± 0.007	0.89 ± 0.018																											
High	0.28 ± 0.006	0.92 ± 0.021																											
Lazutka et al. (1999) Lithuania Prevalence study Population: Carpet and plastic manufacturing; Carpet plant, exposed, 38 male, 41 female (age 22–65 year, 49% smokers); unexposed, 64 male, 26 female, 30% smokers; Plastic plant, exposed 34 male, 63 female (age 28–64 year, 37% smokers); unexposed 64 males, 26 females Outcome: CA in peripheral blood lymphocytes; fluorescence plus Giemsa stain, cells harvested 72 hr, scored 100 metaphases/ subject on coded slides.	Industrial hygiene area measurements reported by plants; carpet plant, formaldehyde 0.3–1.2 mg/m³, styrene 0.13–1.4 mg/m³, phenol 0.3 mg/m³; plasticware plant, formaldehyde 0.5–0.9 mg/m³, styrene 4.4–6.2	Frequency of chromosomal aberrations in peripheral blood lymphocytes by exposure (CA/ 100 cells ± SEM) <table> <tr> <th></th><th>#</th><th>CA Frequency</th></tr> <tr> <td><i>Carpet Workers</i></td><td></td><td></td></tr> <tr> <td>Exposed</td><td>79</td><td>3.79 ± 0.32*</td></tr> <tr> <td>Referent</td><td>90</td><td>1.68 ± 0.13</td></tr> <tr> <td><i>Plasticware workers</i></td><td></td><td></td></tr> <tr> <td>Exposed</td><td>97</td><td>4.17 ± 0.29*</td></tr> <tr> <td>Referent</td><td>90</td><td>1.68 ± 0.13</td></tr> </table>		#	CA Frequency	<i>Carpet Workers</i>			Exposed	79	3.79 ± 0.32*	Referent	90	1.68 ± 0.13	<i>Plasticware workers</i>			Exposed	97	4.17 ± 0.29*	Referent	90	1.68 ± 0.13						
	#	CA Frequency																											
<i>Carpet Workers</i>																													
Exposed	79	3.79 ± 0.32*																											
Referent	90	1.68 ± 0.13																											
<i>Plasticware workers</i>																													
Exposed	97	4.17 ± 0.29*																											
Referent	90	1.68 ± 0.13																											

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results						
	mg/m ³ , phenol 0.5–0.75 mg/m ³ Duration exposure, carpet plant: 2 mo–21 year; plastic plant: 2 mo–25 year	* <i>p</i> < 0.0001; ANOVA adjusted for age Predominant types of damage were chromatid and chromosome breaks Duration of exposure not associated with CA frequency; Age and smoking (data not shown) were not associated with CA frequency						
Shaham et al. (1997) Israel Prevalence study Population: 13 pathology workers (mean age 42 year, 23% smokers) compared to 20 referent workers matched by age (mean age 39 year, 30% smokers). Outcome: SCE in peripheral lymphocytes, Mean # per chromosome, stain fluorescence plus 5% Giemsa, blinding not described, mean of 30 cells/ individual, Related references: Shaham et al. (1996)	Field and personal air sampling, sample duration 15 min, multiple times during work-day (# not reported). Concentration: Mean: not reported Range: 1.7–1.97 mg/m ³ Personal samples: Range: 3.4–3.8 mg/m ³ Exposure duration mean 13 years (range 2–25 years)	SCE (mean # per chromosome) in peripheral lymphocytes <table><tr><td></td><td>Unexposed</td><td>Exposed</td></tr><tr><td>SCE</td><td>0.186 ± 0.035</td><td>0.22 ± 0.039*</td></tr></table> * <i>p</i> = 0.05, ANOVA adjusted for smoking status years of exposure linearly correlated with mean number of SCE per chromosome, adjusting for smoking		Unexposed	Exposed	SCE	0.186 ± 0.035	0.22 ± 0.039*
	Unexposed	Exposed						
SCE	0.186 ± 0.035	0.22 ± 0.039*						
Kitaeva et al. (1996) Russia (translated) Prevalence study Population: 15 formaldehyde production workers (5 females, 10 males, mean age 38 year), anatomy instructors (6 female, 2 male), mean age 41 year) compared to 6 unexposed (mean age 28.5 year) Outcome: Blood collection in 1988. CA: cells harvested at 72 hr; blinding not described. Unclear if statistical analyses were performed.	No quantitative exposure assessment Exposure duration: Formaldehyde production 9.7 years Anatomy instructors 17 years	CA (% aberrant metaphases) in peripheral lymphocytes <table><tr><td></td><td>Referent (n=6)</td><td>Exposed Workers (n=8)</td></tr><tr><td>% of metaphases at 72 hrs lymphocyte culture</td><td>1.8 ± 0.6 (547 metaphases examined)</td><td>5.4 ± 1.9 (148 metaphases examined)</td></tr></table> No metaphases observed at 72 hrs in lymphocyte cultures from anatomy instructors Authors reported that % CA was not dependent on age, gender and length of employment		Referent (n=6)	Exposed Workers (n=8)	% of metaphases at 72 hrs lymphocyte culture	1.8 ± 0.6 (547 metaphases examined)	5.4 ± 1.9 (148 metaphases examined)
	Referent (n=6)	Exposed Workers (n=8)						
% of metaphases at 72 hrs lymphocyte culture	1.8 ± 0.6 (547 metaphases examined)	5.4 ± 1.9 (148 metaphases examined)						

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																								
Vasudeva and Anand (1996) India Prevalence study Population: 30 female medical students exposed 15 mos, compared to 30 age-matched nonmedical students. All 17–19 years old Outcome: chromosomal aberrations in peripheral blood samples, mean frequency aberrant metaphases, cells harvested at 72 hr, 100 cells/ subject; blinding not reported.	Exposure not quantified Exposure conc.: < 1.23 mg/m³ Exposure duration: 15 mos	No significant difference in chromosomal aberrations between groups ($p > 0.5$). Mean frequency of aberrant metaphases Exposed: 1.2% Unexposed: 0.9% <i>No additional quantitative information available</i>																								
Vargová et al. (1992) Czechoslovakia Prevalence study Population: 20 wood workers with at least 5 years of exposure (10 females, 10 males, mean age 42.3 year), compared to 19 workers from the same plant with no known occupational contact with chemicals. Outcome: CA frequency, peripheral lymphocytes, Giemsa staining, cells harvested 48 hr, 100 cells/ subject. Blinding not described.	Task-based air sampling in breathing zone over 8 hours Exposure conc.: Range: 0.55–10.36 mg/m³ Exposure duration: 5–>16 years	Frequency of chromosomal aberrations in peripheral lymphocytes by exposure group <table> <tr> <th></th><th>Exposed</th><th>Unexposed^a</th></tr> <tr> <td>% aberrant cells</td><td>3.08</td><td>3.60</td></tr> <tr> <td># breaks per cell^a</td><td>0.045</td><td>0.030</td></tr> </table> ^aAccording to authors, both groups reported % aberrant cell levels above normal range (1.2–2%)		Exposed	Unexposed ^a	% aberrant cells	3.08	3.60	# breaks per cell ^a	0.045	0.030															
	Exposed	Unexposed ^a																								
% aberrant cells	3.08	3.60																								
# breaks per cell ^a	0.045	0.030																								
Bauchinger and Schmid (1985) Germany Prevalence study Population: 20 male paper makers exposed for at least 2 years (mean age 40.8 year, 30% smokers) compared to 20 unexposed male workers from the same factory Outcome: Peripheral lymphocytes, CA/ cell (scored 500 cells/ subject), cells harvested 48 hr, Giemsa staining; SCE/ cell (scored 50/ subject) analyzed using coded slides, SCE stratified by smoking status.	Exposure assessment based on air monitoring and job-function. Exposure concentration.: ≈ 1.47 mg/m³, plus 3.7 mg/m³ for 45 min (supervisors) or 90 min (operators) per 8 hrs Exposure duration Mean: 14.5 years Range: 2–30 years	Frequency of CA and SCE/cell (mean $\pm$ SE) in peripheral lymphocytes <table> <tr> <th></th><th>Referent</th><th>Exposed</th></tr> <tr> <td>% cell with CA</td><td>0.86 $\pm$ 0.10</td><td>0.87 $\pm$ 0.08</td></tr> <tr> <td>SCE/ cell</td><td>9.53 $\pm$.035</td><td>8.87 $\pm$ 0.24</td></tr> <tr> <td><i>Aberrations/ cell</i></td><td></td><td></td></tr> <tr> <td>Chromatid</td><td>0.0038 $\pm$ 0.0005</td><td>0.0042 $\pm$ 0.0005</td></tr> <tr> <td>Acentric fragments</td><td>0.0046 $\pm$ 0.0006</td><td>0.0034 $\pm$ 0.0005</td></tr> <tr> <td>Dicentrics</td><td>0.0005 $\pm$ 0.0002</td><td>0.0013 $\pm$ 0.0003*</td></tr> <tr> <td>Centric rings</td><td>0.0001 $\pm$ 0.0001</td><td>0.0003 $\pm$ 0.0001*</td></tr> </table> *$p < 0.05$, Mann-Whitney rank U test Frequency of SCE was not associated with exposure when stratified by smoking		Referent	Exposed	% cell with CA	0.86 $\pm$ 0.10	0.87 $\pm$ 0.08	SCE/ cell	9.53 $\pm$.035	8.87 $\pm$ 0.24	<i>Aberrations/ cell</i>			Chromatid	0.0038 $\pm$ 0.0005	0.0042 $\pm$ 0.0005	Acentric fragments	0.0046 $\pm$ 0.0006	0.0034 $\pm$ 0.0005	Dicentrics	0.0005 $\pm$ 0.0002	0.0013 $\pm$ 0.0003*	Centric rings	0.0001 $\pm$ 0.0001	0.0003 $\pm$ 0.0001*
	Referent	Exposed																								
% cell with CA	0.86 $\pm$ 0.10	0.87 $\pm$ 0.08																								
SCE/ cell	9.53 $\pm$.035	8.87 $\pm$ 0.24																								
<i>Aberrations/ cell</i>																										
Chromatid	0.0038 $\pm$ 0.0005	0.0042 $\pm$ 0.0005																								
Acentric fragments	0.0046 $\pm$ 0.0006	0.0034 $\pm$ 0.0005																								
Dicentrics	0.0005 $\pm$ 0.0002	0.0013 $\pm$ 0.0003*																								
Centric rings	0.0001 $\pm$ 0.0001	0.0003 $\pm$ 0.0001*																								

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results															
Thomson et al. (1984) Great Britain Prevalence study Population: 6 pathology workers (2 female, 4 male, mean age 33.5 year) compared to 5 referents (3 female, 2 male, mean age 27.8 year) (study details on referent not provided) Outcome: CA frequency, stain fluorescence plus Giemsa technique (Perry and Wolff, 1974), cells harvested 48 hr, slides coded and scored 100 1st division metaphases/ subject; SCE frequency, cells harvested 72 hr, 50 cells/ subject	Personal air monitoring over 1–3 mos before blood samples Exposure conc.: TWA Mean: 2.26 mg/m³ Range: 1.14–6.93 mg/m³ Exposure duration: 4–11 years, 2–4 hr/d, 2–3 d/wk	No significant difference in incidence of chromosome aberrations or SCE frequency found between groups. SCE frequency (mean per cell) Exposed (<i>N</i> = 6) 6.78 ± 0.31 Referent (<i>N</i> = 5) 6.44 ± 0.38 (individual data reported, analytic methods were not described)															
Fleig et al. (1982) Germany Prevalence study Population: 15 formaldehyde-manufacturing workers (mean age 50 year) compared to 15 age-and gender matched unexposed workers from same plant. Outcome: Chromosome aberrations in peripheral blood lymphocytes cells harvested 70–72 hrs, 10% Giemsa stain; slides coded; scored 100 metaphases/ subject.	Personal air sampling. 1946–1971: <6.15 mg/m³ (MAK) 1971–1982: <1.23 mg/m³ (MAK) Duration: Mean: 28 years Range: 23–35 years	Chromosomal aberrations in peripheral blood lymphocytes <table> <tr> <th></th><th>Unexposed</th><th>Exposed</th></tr> <tr> <td>Mean % aberrant cells including gaps</td><td>3.33</td><td>3.07</td></tr> <tr> <td>Mean % aberrant cells excluding gaps</td><td>1.07</td><td>1.67</td></tr> </table> <i>P</i> > 0.05, Fisher's exact text Smoking habit not associated with CA (data not reported)		Unexposed	Exposed	Mean % aberrant cells including gaps	3.33	3.07	Mean % aberrant cells excluding gaps	1.07	1.67						
	Unexposed	Exposed															
Mean % aberrant cells including gaps	3.33	3.07															
Mean % aberrant cells excluding gaps	1.07	1.67															
Suskov and Sazonova (1982) Russia Prevalence study Population: 31 phenol-formaldehyde workers (mean age 39.1 year) compared to 74 referents matched by gender, smoking, alcohol consumption, and medication Outcome: Chromosomal aberrations via mean frequency of aberrant metaphases, Buckton and Evans (1973) method; cells harvested at 50 hr	Workers exposed to both phenol and FA. Area samples Exposure conc.: Formaldehyde Mean: 0.5 mg/m³ Phenol mean: 0.3 mg/m³ Exposure duration: 4 mos to 30 years	Frequency of chromosomal aberrations by exposure group <table> <tr> <th></th><th>Referent</th><th>Exposed</th></tr> <tr> <td>Mean % aberrant cells</td><td></td><td></td></tr> <tr> <td>Aberrant metaphases</td><td>2.4 ± 0.22</td><td>5.0 ± 0.40*</td></tr> <tr> <td>Aberrant chromosomes per cell</td><td>0.024 ± 0.002</td><td>0.058 ± 0.006*</td></tr> <tr> <td>Chromosomal breaks per aberrant chromosome</td><td>1.26 ± 0.076</td><td>1.27 ± 0.044</td></tr> </table> *<i>p</i> < 0.001, chi-square		Referent	Exposed	Mean % aberrant cells			Aberrant metaphases	2.4 ± 0.22	5.0 ± 0.40*	Aberrant chromosomes per cell	0.024 ± 0.002	0.058 ± 0.006*	Chromosomal breaks per aberrant chromosome	1.26 ± 0.076	1.27 ± 0.044
	Referent	Exposed															
Mean % aberrant cells																	
Aberrant metaphases	2.4 ± 0.22	5.0 ± 0.40*															
Aberrant chromosomes per cell	0.024 ± 0.002	0.058 ± 0.006*															
Chromosomal breaks per aberrant chromosome	1.26 ± 0.076	1.27 ± 0.044															

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results									
Short-term Studies											
Ying et al. (1999) China Population: 23 nonsmoking anatomy students (11 males, 12 females, age not reported) exposed during 8-week course, 3-hr session, 3 times/ wk. Outcome: SCE in peripheral blood lymphocytes, assessed before the start of the course and at the end of 8-week period. Blinded analysis of slides, one observer with repeat by second; 30 M₂ lymphocytes per subject analyzed; Lymphocyte transformation rate (LTR)	Air sampling, estimated TWA and peak levels during class and in the dorms. Anatomy labs: Mean 3-hr TWA: 0.51 ± 0.299 mg/m³, range: 0.07–1.28 mg/m³ Dormitories: Mean TWA: 0.012 ± 0.003 mg/m³, range: 0.011–0.016 mg/m³ Duration: 8 wks	Frequency SCE and lymphocyte transformation rate (LTR) (%) (Mean±SEM), Change over 8 wks <table> <tr> <td></td><td>Before exposure</td><td>After exposure</td></tr> <tr> <td>SCE</td><td>6.383 ± 0.405</td><td>6.613 ± 0.786</td></tr> <tr> <td>LTR</td><td>59.07 ± 6.35</td><td>56.92 ± 8.64</td></tr> </table> *<i>p</i> <0.05, paired <i>t</i>-test Levels in males and females were similar		Before exposure	After exposure	SCE	6.383 ± 0.405	6.613 ± 0.786	LTR	59.07 ± 6.35	56.92 ± 8.64
	Before exposure	After exposure									
SCE	6.383 ± 0.405	6.613 ± 0.786									
LTR	59.07 ± 6.35	56.92 ± 8.64									
He et al. (1998) China Prevalence study Population: 13 anatomy students exposed during a 12-week course compared to 10 students. Age and gender similar between groups, all nonsmokers (data not shown). Outcome: CA and SCE in peripheral lymphocytes, CA: modified fluorescence plus Giemsa stain, cells harvested 48 hr, scored 100 metaphases/ subject. SCE: cells harvested 72 hr, 50 metaphases/ subject. Blinding not described	Breathing zone air samples in location of exposed students. Concentration in breathing zone: Mean 2.92 mg/m³ Duration: 12 wks (10 hrs/wk)	Frequency of SCE and chromosomal aberrations in peripheral lymphocytes <table> <tr> <td></td><td>Referent</td><td>Exposed</td></tr> <tr> <td>Mean SCE per cell</td><td>5.26 ± 0.51</td><td>5.91 ± 0.71*</td></tr> <tr> <td>Lymphocyte CA</td><td>3.40 ± 1.57</td><td>5.92 ± 2.40*</td></tr> </table> *<i>p</i> <0.05, analytic test not described		Referent	Exposed	Mean SCE per cell	5.26 ± 0.51	5.91 ± 0.71*	Lymphocyte CA	3.40 ± 1.57	5.92 ± 2.40*
	Referent	Exposed									
Mean SCE per cell	5.26 ± 0.51	5.91 ± 0.71*									
Lymphocyte CA	3.40 ± 1.57	5.92 ± 2.40*									
Suruda et al. (1993) USA Panel study Population: 29 students (with adequate samples) (24.1% female, mean age 23.6 year, 17.2% smokers) exposed to formaldehyde for 9 wks during embalming course, with baseline samples taken. Mean duration of embalming 125 min. Possible exposure prior to course. Outcome: SCE in peripheral lymphocytes, stain fluorescein plus Giemsa, 50 s division metaphases scored/ subject;	Personal sampling for 121 of 144 embalmings; Exposure concentration: Mean: 1.72 mg/m³ Range: (0.18–5.29) mg/m³ Duration: 9 wks (0.173 years)	Frequency of SCE before and after a 9-wk embalming course <table> <tr> <td></td><td>Before exposure</td><td>After exposure</td></tr> <tr> <td>SCE</td><td>7.72 ± 1.26</td><td>7.14 ± 0.89*</td></tr> </table> *<i>p</i> <0.01, difference in mean before and after exposure, matched Student's <i>t</i>-test		Before exposure	After exposure	SCE	7.72 ± 1.26	7.14 ± 0.89*			
	Before exposure	After exposure									
SCE	7.72 ± 1.26	7.14 ± 0.89*									

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results									
blood samples collected in morning before 1 st class and after 9 wks; analysis of slides blinded to exposure status											
Yager et al. (1986) USA Panel study Population: 8 anatomy students (1 male, 7 females, mean age 26 year, all nonsmokers) exposed to formaldehyde during a 10 wk course (2 sessions/ wk). No occupational or lab formaldehyde exposure during previous year. Outcome: Mean SCEs per cell in peripheral lymphocytes; before and after 10 wks, samples coded and randomized together for analysis	Ambient air and breathing zone monitoring. Breathing zone concentration: Mean:1.5 mg/m ³ Range: 0.9–2.4 mg/m ³ Exposure duration: 10 wks	Mean SCE per cell before and after 10-wk course (mean ± SEM) <table><tr><td></td><td>Before</td><td>After</td></tr><tr><td>Mean SCE per cell</td><td>6.39 ± 0.11</td><td>7.20 ± 0.33*</td></tr></table> * <i>p</i> = 0.02, paired <i>t</i> -test		Before	After	Mean SCE per cell	6.39 ± 0.11	7.20 ± 0.33*			
	Before	After									
Mean SCE per cell	6.39 ± 0.11	7.20 ± 0.33*									
Zeller et al. (2011) Germany Controlled human exposure study Subjects: 41 healthy volunteers exposed 4 hr/d for 5 d, all male, nonsmokers Outcome: SCE in peripheral lymphocytes: method according to Schmid and Speit (2007), scored 30 cells/ sample. Proliferation index (PI) calculated from 1 st , 2 nd , and 3 rd mitoses in 100 metaphases. Analyzed using Wilcoxon Sign Rank test	12 groups of 2 to 4 persons in a chamber, exposures randomly assigned. Formaldehyde concentrations: 0 (i.e., background level of 0.01 ppm); 0.3 ppm (0.37 mg/m ³) ^a with 4 peaks of 0.6 ppm (0.74 mg/m ³); 0.4 ppm (0.49 mg/m ³) with four peaks of 0.8 ppm (0.98 mg/m ³); 0.5 ppm (0.67 mg/m ³); and 0.7 ppm (0.86 mg/m ³) Peaks were 15 min each, Four 15-min exercise sessions during exposure.	Frequency of SCE/ metaphase and PI in lymphocytes before and after 4-hour exposure (N = 40) <table><tr><td></td><td>SCE/ metaphase</td><td>PI</td></tr><tr><td>Lymphocytes Before</td><td>6.1 ± 0.898^a</td><td>2.46 ± 0.114</td></tr><tr><td>After</td><td>6.1 ± 0.938</td><td>2.47 ± 0.145</td></tr></table> ^a <i>p</i> = 0.689		SCE/ metaphase	PI	Lymphocytes Before	6.1 ± 0.898 ^a	2.46 ± 0.114	After	6.1 ± 0.938	2.47 ± 0.145
	SCE/ metaphase	PI									
Lymphocytes Before	6.1 ± 0.898 ^a	2.46 ± 0.114									
After	6.1 ± 0.938	2.47 ± 0.145									
Chromosomal Breaks or Aneuploidy											
Prevalence Studies											
Aglan and Mansour (2018) Egypt Prevalence study, June 2015 - September 2016	Passive air sampling (Umex-100) at fixed position in breathing zone,	MN frequency (%) in PBL and buccal cells by duration of employment (< 5 and > 5 years) <table><tr><td>PBL</td><td>EBC</td></tr></table>	PBL	EBC							
PBL	EBC										

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results														
Population: 60 hair stylists who routinely conducted hair straightening compared to 60 stylists who did not conduct this treatment. Excluded subjects with chronic disease and /or regular medications, family history of cancer, recurrent abortions, smoking or pregnancy. Ages 20–36 years. Outcome: Blood collected at end of 8-hr shift. CB Micronucleus test in lymphocytes. Replicate cultures for each sample, incubated 72 hrs. 2,000 binucleated cells from coded slides (1,000 from each replicate culture), scored using criteria by Fenech et al. (2003). MN frequency % altered cells. MN in exfoliated buccal cells. Cheeks scraped with wooden spatula, fixed in 3:1 methanol/ acetic acid and dropped onto slides, stained with Feulgen/ Fast Green, examined at 400× according to Tolbert et al. (1991). Analyzed independently by 2 people, 1,500 cells scored per person using criteria by Sarto et al. (1987). % altered cells.	15-min samples during hair straightening process; 15-min TWA Group 1 (work duration < 5 years): 1.68 ± 0.27 ppm Group 2 (work duration > 5 years): 1.83 ± 0.16 ppm	<table><thead><tr><th></th><th>Mean ± SD</th><th>Mean ± SD</th></tr></thead><tbody><tr><td>Referent (n=60)</td><td>0.22 ± 0.42*</td><td>0.17 ± 0.38**</td></tr><tr><td>< 5 yrs (n=31)</td><td>0.61 ± 0.50</td><td>0.32 ± 0.48</td></tr><tr><td>> 5 yrs (n=29)</td><td>1.66 ± 0.48</td><td>0.94 ± 0.58</td></tr></tbody></table> *<i>p</i> < 0.01, ** <i>p</i> < 0.001, Kruskal Wallis test Between group differences statistically significant in PBL and for EBC except between referent and < 5 year exposure group (least significant difference test)		Mean ± SD	Mean ± SD	Referent (n=60)	0.22 ± 0.42*	0.17 ± 0.38**	< 5 yrs (n=31)	0.61 ± 0.50	0.32 ± 0.48	> 5 yrs (n=29)	1.66 ± 0.48	0.94 ± 0.58		
	Mean ± SD	Mean ± SD														
Referent (n=60)	0.22 ± 0.42*	0.17 ± 0.38**														
< 5 yrs (n=31)	0.61 ± 0.50	0.32 ± 0.48														
> 5 yrs (n=29)	1.66 ± 0.48	0.94 ± 0.58														
Costa et al. (2019) Portugal Prevalence study (extension of Costa et al. (2015)). Population: 85 anatomy pathology workers from 9 hospital laboratories, exposed to formaldehyde for at least 1 year, compared to 87 unexposed employees from administrative offices in same geographic area. Exclusions: cancer history, radiation therapy or chemotherapy, surgery with anesthesia or blood transfusion in last year. Exposed and referent similar for mean age 39 years, 77% females, 25% smokers. Outcome: Peripheral blood samples, coded, analyses blinded to exposure status. Exfoliated cells were collected for each cheek separately. Cytokinesis-blocked MN test, Costa et al. (2008); culture incubation 72 hr; stain 4% Giemsa; scored 1,000 binucleated cells/subject, criteria defined by Fenech (2007). Buccal MN cytome assay. 2,000 differentiated cells scored for frequency of MN, nuclear buds and nucleoplasmic	Exposure assessed via air sampling and deriving an 8-hr TWA for each subject. Exposure concentration: Mean: 0.38 ppm (0.47 mg/m³) Range: 0.28–1.39 ppm (0.34–1.72 mg/m³) Exposure duration 12.0 ± 8.2 years	MN frequency (%) in peripheral lymphocytes, exposed relative to referent group, Mean Ratio (MR) <table><thead><tr><th></th><th>Ratio</th><th>95% CI</th></tr></thead><tbody><tr><td>Exposure</td><td>1.55**</td><td>1.2–1.99</td></tr></tbody></table> Poisson regression models adjusted for age, gender, smoking habits **<i>p</i> < 0.01 MN frequency in exfoliated buccal cells, Mean Ratio (MR) <table><thead><tr><th></th><th>Exposed: Unexposed</th><th>MR</th><th>95% CI</th></tr></thead><tbody><tr><td>MNB</td><td>63:69</td><td>4.08** *</td><td>2.12 – 7.87</td></tr></tbody></table>		Ratio	95% CI	Exposure	1.55**	1.2–1.99		Exposed: Unexposed	MR	95% CI	MNB	63:69	4.08** *	2.12 – 7.87
	Ratio	95% CI														
Exposure	1.55**	1.2–1.99														
	Exposed: Unexposed	MR	95% CI													
MNB	63:69	4.08** *	2.12 – 7.87													

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																	
bridges according to Tolbert et al. (1991) and Thomas et al. (2009) . T-Cell Receptor mutation assay in mononuclear leukocytes, flow cytometry, minimum of 2.5 × 10 ⁵ lymphocyte-gated events were acquired, # events in mutation cell window (CD3-CD4+ cells) divided by total number of events for CD4+ cells		<table><tr><td>BNbud</td><td>63:69</td><td>2.8</td><td>1.76 – 4.71</td></tr><tr><td></td><td></td><td>8**</td><td></td></tr><tr><td></td><td></td><td>*</td><td></td></tr></table>	BNbud	63:69	2.8	1.76 – 4.71			8**				*						
		BNbud	63:69	2.8	1.76 – 4.71														
				8**															
				*															
		Poisson regression models adjusted for age, gender, smoking habits; *** <i>p</i> < 0.001																	
		Correlation between MNL and MNB: <i>r</i> = 0.359, <i>p</i> < 0.001																	
		MN frequency in PBL and exfoliated buccal cells by level and duration in exposed, Mean Ratio (MR)																	
		<table><tr><td></td><td colspan="3">MNL</td><td colspan="3">BNbud</td></tr><tr><td></td><td>N</td><td>MR</td><td>95% CI</td><td>N</td><td>MR</td><td>95% CI</td></tr></table>					MNL			BNbud				N	MR	95% CI	N	MR	95% CI
			MNL			BNbud													
			N	MR	95% CI	N	MR	95% CI											
Level (ppm)																			
0.08-0.22 27 1.0																			

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																								
Exposed and referent were comparable for mean age, smoking and alcohol consumption. Outcome: CBMN according to Fenech (1993, 2000) . Blinded analysis. Venous peripheral blood cultured for 44 hr, Cytochalasin-B added to cultures, cells harvested 28 hrs later, air dried slides stained with Giemsa, MN detected at 400× with confirmation at 1,000×. 1,000 binucleated cells scored/ subject	Cumulative dose (mg/m ³ -year) determined for each worker (C × T). C = geometric mean of concentration for a year at a sampling site, T = years. Exposed: 0.90 (0.60-1.78) Referent: 0.06 (0.02-0.10)	<table><tr><th colspan="4">Micronucleus frequency (per 1,000, frequency ratio (FR)) in PBL</th></tr><tr><th>CED (mg/m³-year)</th><th>N</th><th>Exposed</th><th>FR (95% CI)</th></tr><tr><td>0.01 – 0.06</td><td>45</td><td>1.36 ± 0.86</td><td>1</td></tr><tr><td>0.06 – 0.125</td><td>55</td><td>1.87 ± 0.92</td><td>1.38 (1.00-1.91)</td></tr><tr><td>0.125 – 0.9</td><td>46</td><td>2.50 ± 1.17</td><td>1.83 (1.34-2.52)</td></tr><tr><td>0.9 – 3.75</td><td>54</td><td>3.65 ± 1.40</td><td>2.67 (1.99-3.64)</td></tr></table> Poisson regression models with adjustment for age, smoking status and alcohol use	Micronucleus frequency (per 1,000, frequency ratio (FR)) in PBL				CED (mg/m ³ -year)	N	Exposed	FR (95% CI)	0.01 – 0.06	45	1.36 ± 0.86	1	0.06 – 0.125	55	1.87 ± 0.92	1.38 (1.00-1.91)	0.125 – 0.9	46	2.50 ± 1.17	1.83 (1.34-2.52)	0.9 – 3.75	54	3.65 ± 1.40	2.67 (1.99-3.64)
Micronucleus frequency (per 1,000, frequency ratio (FR)) in PBL																										
CED (mg/m ³ -year)	N	Exposed	FR (95% CI)																							
0.01 – 0.06	45	1.36 ± 0.86	1																							
0.06 – 0.125	55	1.87 ± 0.92	1.38 (1.00-1.91)																							
0.125 – 0.9	46	2.50 ± 1.17	1.83 (1.34-2.52)																							
0.9 – 3.75	54	3.65 ± 1.40	2.67 (1.99-3.64)																							
Petteffi et al. (2015) Brazil Prevalence study Population: 46 workers in furniture manufacturing facility (mean age 34.5 year, 56.5% male, 1 smoker) and unexposed group (n = 45) recruited from employees and students of local university with no history of occupational exposure to potentially genotoxic agents or substances metabolized to formic acid. (mean age 35.4 year, 33.3% male, 0 smokers) Outcome: Oral buccal epithelial cell samples (scraped with endocervical brush), micronucleus test, DNA-specific Feulgen staining and counterstaining with Fast Green according to Tolbert et al. (1992) ; analyzed 2,000 cells/ person by 2 independent observers (1,000 ea).	Monitoring in 7 sections in facility; referent monitoring in 5 areas of university; breathing zone 8 hr samples collected on same day as biological samples. Urine samples collected at end of work day on 5 th day of work; correlation of formaldehyde concentration in air with urinary formic acid concentration, r = 0.626, p <0.001 UV painting, lamination/press, packaging, edge lamination	<table><tr><th colspan="4">Comparisons of micronucleus frequency and other DNA damage in buccal cells, median (interquartile range)</th></tr><tr><th></th><th>Referent</th><th>Exposed</th><th>p-Value</th></tr><tr><td>Micronuclei</td><td>0</td><td>0</td><td>0.08</td></tr><tr><td>Nuclear buds</td><td>0 (0–0.50)</td><td>0.24 (0–0.63)</td><td>0.126</td></tr><tr><td>Binucleated cells</td><td>0.50 (0–1.38)</td><td>1.34 (0.64–2.38)</td><td>0.003</td></tr><tr><td>Karyorrhexis</td><td>1.0 (0.49–2.04)</td><td>1.31 (0.58–2.49)</td><td>0.372</td></tr></table> Nonparametric tests used because data were not normally distributed. Exposed and referent compared using Mann-Whitney test. No differences between men and women for measures of DNA damage in either exposed or referent.	Comparisons of micronucleus frequency and other DNA damage in buccal cells, median (interquartile range)					Referent	Exposed	p-Value	Micronuclei	0	0	0.08	Nuclear buds	0 (0–0.50)	0.24 (0–0.63)	0.126	Binucleated cells	0.50 (0–1.38)	1.34 (0.64–2.38)	0.003	Karyorrhexis	1.0 (0.49–2.04)	1.31 (0.58–2.49)	0.372
Comparisons of micronucleus frequency and other DNA damage in buccal cells, median (interquartile range)																										
	Referent	Exposed	p-Value																							
Micronuclei	0	0	0.08																							
Nuclear buds	0 (0–0.50)	0.24 (0–0.63)	0.126																							
Binucleated cells	0.50 (0–1.38)	1.34 (0.64–2.38)	0.003																							
Karyorrhexis	1.0 (0.49–2.04)	1.31 (0.58–2.49)	0.372																							

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results												
	0.03–0.04 ppm (0.037–0.05 mg/m³) Edge painting, machining and drilling center, board cutting 0.06–0.09 ppm (0.07–0.11 mg/m³) Referent mean (SD) 0.012 (0.008) ppm (0.015 (0.01) mg/m³) Formic acid median Exposed 20.47 mg/L Referent 4.57 mg/L Exposure duration 5.76 year	No correlation between urinary formic acid and measures of DNA damage.												
Souza and Devi (2014) India Prevalence study Population: 30 male workers in anatomy departments (embalming) in several medical colleges (mean age 39.9 year, 50% smokers); compared to 30 male clerical workers in same facilities (mean age 37.8 year, 30% smokers). Outcome: Total MN/ 1,000 cells in peripheral lymphocytes. Assays conducted blinded. Cytokinesis -blocked micronucleus assay Costa et al. (2008), 1,000 binucleated cells/ subject.	No measurements reported. Duration exposure mean 10.66 year, range 1–30 year	MN frequency in Lymphocytes by Exposure Group (mean (SD)) <table> <tr> <th></th><th>Mean ± SD</th><th>95% CI</th></tr> <tr> <td>Exposed (N = 30)</td><td>9.5 ± 3.23</td><td>8.29–10.7</td></tr> <tr> <td>Comparison group (N = 30)</td><td>3.73 ± 1.43</td><td>3.19–4.26</td></tr> <tr> <td>Difference in means^a</td><td>5.76</td><td>4.47–7.06^a</td></tr> </table> ^aNo difference = 0. Association of MN frequency with exposure and smoking evaluated using two-way ANOVA. Smoking was not associated with MN frequency. Pearson's correlation test showed a positive correlation ($r = 0.5$, $P = 0.02$) between the duration of exposure and the frequency of MN in lymphocytes.		Mean ± SD	95% CI	Exposed (N = 30)	9.5 ± 3.23	8.29–10.7	Comparison group (N = 30)	3.73 ± 1.43	3.19–4.26	Difference in means ^a	5.76	4.47–7.06 ^a
	Mean ± SD	95% CI												
Exposed (N = 30)	9.5 ± 3.23	8.29–10.7												
Comparison group (N = 30)	3.73 ± 1.43	3.19–4.26												
Difference in means ^a	5.76	4.47–7.06 ^a												

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																					
Bouraoui et al. (2013) Tunisia Prevalence study Population: 31 pathology workers (60% female, mean age 42, 9.6% smokers) compared to 31 unexposed administrative staff in same facility (60% female, mean age 43 year, 12.9% smokers). Outcome: MN peripheral lymphocytes: Cytokinesis-blocked MN assay in combination with FISH using all-chromosome centromeric probe Sari-Minodier et al. (2002); stain 5% Giemsa, 2,000 binucleated cells scored/ subject, Fenech (2000), blinding not described	Exposure assessed by job title and duration of employment. Atmospheric air sampling performed in area of potential exposure Concentration: Means of 3 samplings: 0.25 mg/m³ 2.21 mg/m³ 4.2 mg/m³ Duration: Mean 15.68 years (6.53 ± 0.7 hrs/d)	MN frequency in peripheral lymphocytes (Mean ± SD) <table> <tr> <th></th><th>Referent</th><th>Exposed</th></tr> <tr> <td>MN (%/1,000 binucleated cells)</td><td>7.08 ± 4.62</td><td>25.35 ± 6.28*</td></tr> <tr> <td>FISH MN (%/2,000 cells)</td><td>6.12 ± 4.24</td><td>23.25 ± 5.92*</td></tr> <tr> <td>C + MN</td><td>4.03 ± 3.64</td><td>18.38 ± 5.94*</td></tr> <tr> <td>C – MN</td><td>2.09 ± 0.74</td><td>4.87 ± 3.22</td></tr> <tr> <td>C1+ MN</td><td>2.93 ± 2.74</td><td>15.35 ± 6.03*</td></tr> <tr> <td>Cx + MN</td><td>1.1 ± 1.16</td><td>3.03 ± 2.7*</td></tr> </table> *p <0.05, Student's t-test Duration of exposure was associated with all of the cytogenetic alterations. Abbreviations: C +, C –, C1 + MN, Cx + MN		Referent	Exposed	MN (%/1,000 binucleated cells)	7.08 ± 4.62	25.35 ± 6.28*	FISH MN (%/2,000 cells)	6.12 ± 4.24	23.25 ± 5.92*	C + MN	4.03 ± 3.64	18.38 ± 5.94*	C – MN	2.09 ± 0.74	4.87 ± 3.22	C1+ MN	2.93 ± 2.74	15.35 ± 6.03*	Cx + MN	1.1 ± 1.16	3.03 ± 2.7*
	Referent	Exposed																					
MN (%/1,000 binucleated cells)	7.08 ± 4.62	25.35 ± 6.28*																					
FISH MN (%/2,000 cells)	6.12 ± 4.24	23.25 ± 5.92*																					
C + MN	4.03 ± 3.64	18.38 ± 5.94*																					
C – MN	2.09 ± 0.74	4.87 ± 3.22																					
C1+ MN	2.93 ± 2.74	15.35 ± 6.03*																					
Cx + MN	1.1 ± 1.16	3.03 ± 2.7*																					
Costa et al. (2013) Portugal Prevalence study Population: 35 pathology workers from 4 hospital laboratories, exposed to formaldehyde for at least 1 year (88.6% female, mean age 41.2 year, 20% smokers), compared to 35 unexposed employees from same work area (80% female, mean age 39.8 year, 20% smokers). Outcome: MN in peripheral lymphocytes, samples collected between 10 & 11 am. Cytokinesis-blocked MN test Teixeira et al. (2004). 1,000 cells analyzed/ subject, MN per 1,000 binucleated cells, scored blindly by one reader, criteria Fenech (2007) Related references: Costa et al. (2008); Costa et al. (2011)	Exposure assessed via air sampling and deriving an 8-hr TWA for each subject. Exposure conc.: Mean 0.44 mg/m³, range 0.28–0.85 mg/m³ Exposure duration 12.5 ± 8.1 years, range 1–30 year	Univariate analyses presented in Figure 1 of the paper. MN frequency was 2.5-fold higher in exposed group compared to referent group. MN frequency (%) in peripheral lymphocytes, exposed relative to referent group <table> <tr> <th></th><th>Ratio</th><th>95% CI</th></tr> <tr> <td>Exposure</td><td>2.1</td><td>1.025–3.174</td></tr> </table> Multivariate analysis, adjusted for gender, smoking and age		Ratio	95% CI	Exposure	2.1	1.025–3.174															
	Ratio	95% CI																					
Exposure	2.1	1.025–3.174																					
Lin et al. (2013) China Prevalence study	Personal air monitoring and job assignment.	MN Frequency in peripheral lymphocytes by formaldehyde exposure level and work years																					

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																								
Population: 96 plywood workers exposed to formaldehyde (13.5% female, mean age 33 year, 30.2% smokers) compared to referent group (N = 82) (4% female, mean age 31 year, 40% smokers). Outcome: MN assay in peripheral lymphocytes, analyzed 1,000 binucleated cells/ subject, scoring criteria Fenech (1993) , Fenech et al. (2003) , blinded analysis MN assessed by exposure group and # years worked.	Average concentration: High, N = 38 (making glue): 1.48 mg/m ³ , range 0.914–2.044 mg/m ³ Low, N = 58 (sanding boards, pressing wood scraps with glue at high temp): 0.68 mg/m ³ , range 0.455–0.792 mg/m ³ Referent group, N = 82 (grinding wood scraps): 0.13 mg/m ³ , range 0.019–0.252 mg/m ³ Exposure duration: 2.52 years	<table><tr><th colspan="4">By Exposure levels</th></tr><tr><th></th><th>Referent</th><th>Low</th><th>High</th></tr><tr><td>MN freq (%)</td><td>2.05 ± 1.72</td><td>2.02 ± 1.81</td><td>2.37 ± 1.79</td></tr></table> ANOVA p-value = 0.455; Poisson regression p-value = 0.288 <table><tr><th colspan="4">Number of Work Years</th></tr><tr><th></th><th><1 (N= 57)</th><th>1–3 (N= 64)</th><th>>3 (N= 57)</th></tr><tr><td>MN freq (%)</td><td>1.02 ± 1.10</td><td>2.25 ± 1.56*</td><td>2.90 ± 1.96*</td></tr></table> ANOVA p-value < 0.001; Poisson regression p-value < 0.001 ANOVA and Poisson regression adjusting for age, gender, smoking status, alcohol, duration of employment	By Exposure levels					Referent	Low	High	MN freq (%)	2.05 ± 1.72	2.02 ± 1.81	2.37 ± 1.79	Number of Work Years					<1 (N= 57)	1–3 (N= 64)	>3 (N= 57)	MN freq (%)	1.02 ± 1.10	2.25 ± 1.56*	2.90 ± 1.96*
By Exposure levels																										
	Referent	Low	High																							
MN freq (%)	2.05 ± 1.72	2.02 ± 1.81	2.37 ± 1.79																							
Number of Work Years																										
	<1 (N= 57)	1–3 (N= 64)	>3 (N= 57)																							
MN freq (%)	1.02 ± 1.10	2.25 ± 1.56*	2.90 ± 1.96*																							
Costa et al. (2011) Portugal Prevalence study Population: 48 pathology workers from 5 hospital laboratories, exposed for at least 1 year (28% female, mean age 40 year, 21% smokers), compared to 50 unexposed employees matched by age, gender, lifestyle, smoking habits and work area (25% female, mean age 37 year, 14% smokers). Outcome: MN in peripheral blood lymphocytes, (Teixeira et al., 2004); stain 4% Giemsa; scored 1,000 binucleated cells/ subject, scored blind by one reader, criteria Fenech (2007)	Exposure assessed via air sampling in breathing zone and deriving an 8-hr TWA for each subject. Concentration: Mean: 0.53 mg/m ³ , range 0.05–1.94 mg/m ³ Duration: Mean: 13.6 years, range: 1–31 years	<table><tr><th colspan="3">MN frequency (%) in peripheral lymphocytes</th></tr><tr><th></th><th>Referent</th><th>Exposed</th></tr><tr><td>MN</td><td>3.66 ± 0.51</td><td>6.19 ± 0.62*</td></tr></table> *p <0.05; Mann-Whitney U test and Kruskal-Wallis test	MN frequency (%) in peripheral lymphocytes				Referent	Exposed	MN	3.66 ± 0.51	6.19 ± 0.62*															
MN frequency (%) in peripheral lymphocytes																										
	Referent	Exposed																								
MN	3.66 ± 0.51	6.19 ± 0.62*																								
Ladeira et al. (2011) Portugal Prevalence study Population: 56 hospital workers in histopathology labs (66% female, mean age 39.5 years, 19.6% smokers) compared to 85 administrative staff (64% female, mean age 32.4 years, 29.4% smokers).	Personal air sampling, 6–8 hrs, estimated 8-hr TWA Exposure conc.: Mean TWA 8 hr 0.2 ± 0.14 mg/m ³	<table><tr><th colspan="3">MN frequency (Mean ± SD) by cell type</th></tr><tr><th></th><th>Lymphocytes</th><th>Buccal cells</th></tr><tr><td>Referent</td><td>0.81 ± 0.172</td><td>0.16 ± 0.058</td></tr><tr><td>Exposed</td><td>3.96 ± 0.525*</td><td>0.96 ± 0.277*</td></tr><tr><td>OR^a</td><td>9.67</td><td>3.99</td></tr><tr><td>95% CI</td><td>3.81–24.52</td><td>1.38–11.58</td></tr></table>	MN frequency (Mean ± SD) by cell type				Lymphocytes	Buccal cells	Referent	0.81 ± 0.172	0.16 ± 0.058	Exposed	3.96 ± 0.525*	0.96 ± 0.277*	OR ^a	9.67	3.99	95% CI	3.81–24.52	1.38–11.58						
MN frequency (Mean ± SD) by cell type																										
	Lymphocytes	Buccal cells																								
Referent	0.81 ± 0.172	0.16 ± 0.058																								
Exposed	3.96 ± 0.525*	0.96 ± 0.277*																								
OR ^a	9.67	3.99																								
95% CI	3.81–24.52	1.38–11.58																								

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																								
Outcome: MN in peripheral lymphocytes and buccal cells. Samples coded and analyzed blinded. Lymphocytes, cytokinesis-block micronucleus cytome assay, stain May-Grunwald-Giemsa, 1,000 binucleated cells scored/ subject by 2 readers; buccal mucosa cells, stain Feulgen, 2,000 cells scored/ subject, 2 readers Related references: Viegas et al. (2010); Speit et al. (2012)	Mean ceiling value: 1.4 ± 0.91 mg/m³, range 0.22–3.6 mg/m³ Exposure duration: 14.5 (1–33) years	*<i>p</i>≤0.002, Mann-Whitney test ^aOdds ratio for risk of presence of MN; binary logistic regression MN frequency (Mean ± SD) by years of exposure <table><tr><th>Years</th><th>N</th><th>Lymphocytes</th><th>Buccal cells</th></tr><tr><td><5</td><td>8</td><td>2.75 ± 0.940</td><td>0.63 ± 0.625</td></tr><tr><td>6–10</td><td>19</td><td>3.05 ± 0.775</td><td>0.63 ± 0.326</td></tr><tr><td>11–20</td><td>12</td><td>5.50 ± 1.317</td><td>0.83 ± 0.458</td></tr><tr><td>>21</td><td>15</td><td>5.00 ± 1.151</td><td>1.20 ± 0.8</td></tr></table> Evaluated potential confounding by age, gender, smoking and alcohol, no major evidence of confounding noted	Years	N	Lymphocytes	Buccal cells	<5	8	2.75 ± 0.940	0.63 ± 0.625	6–10	19	3.05 ± 0.775	0.63 ± 0.326	11–20	12	5.50 ± 1.317	0.83 ± 0.458	>21	15	5.00 ± 1.151	1.20 ± 0.8				
Years	N	Lymphocytes	Buccal cells																							
<5	8	2.75 ± 0.940	0.63 ± 0.625																							
6–10	19	3.05 ± 0.775	0.63 ± 0.326																							
11–20	12	5.50 ± 1.317	0.83 ± 0.458																							
>21	15	5.00 ± 1.151	1.20 ± 0.8																							
Jiang et al. (2010) China Prevalence Population: 151 male workers from 2 plywood plants (mean age 27.4 years, 52.3% smokers) compared to 112 unexposed workers at a machine manufacturer in same town (mean age 28.7 years, 42.9% smokers). Outcome: Cytokinesis-block micronucleus (CB-MN), Fenech (1993), scoring criteria Fenech et al. (2003), 1,000 binucleated lymphocytes/ subject, blinded analysis	Exposure assessed by job title and personal air monitoring. Exposure concentration ppm converted to mg/m³ by EPA. Exposed: 1.08 mg/m³, range 0.1–7.75 mg/m³ Referent: <0.01 mg/m³ (LOD) Duration: Mean 2.51 years Range: (0.5–25) years	Lymphocyte MN frequency by duration and formaldehyde concentration <table><tr><th>Duration (years)</th><th>MN^a</th><th>Conc. (mg/m³)</th><th>MN^b</th></tr><tr><td>0.6–1</td><td>4.33 ± 2.81</td><td>0.0123^c</td><td>2.67 ± 1.32</td></tr><tr><td>1–3</td><td>5.84 ± 3.63</td><td>0.1353</td><td>4.03 ± 2.40</td></tr><tr><td>3–25</td><td>5.84 ± 3.24*</td><td>0.3444</td><td>5.74 ± 3.13*</td></tr><tr><td></td><td></td><td>0.4797</td><td>6.76 ± 3.81*</td></tr><tr><td></td><td></td><td>3.1488</td><td>8.25 ± 3.53*</td></tr></table> ^aANOVA, Dunnett-Hsu test, <i>p</i> =0.04, adjusted for age, formaldehyde concentration, current smoking status, alcohol ^bANOVA, <i>p</i> <0.05; Trend <i>p</i> <0.001 ^cReferent group	Duration (years)	MN ^a	Conc. (mg/m ³)	MN ^b	0.6–1	4.33 ± 2.81	0.0123 ^c	2.67 ± 1.32	1–3	5.84 ± 3.63	0.1353	4.03 ± 2.40	3–25	5.84 ± 3.24*	0.3444	5.74 ± 3.13*			0.4797	6.76 ± 3.81*			3.1488	8.25 ± 3.53*
Duration (years)	MN ^a	Conc. (mg/m ³)	MN ^b																							
0.6–1	4.33 ± 2.81	0.0123 ^c	2.67 ± 1.32																							
1–3	5.84 ± 3.63	0.1353	4.03 ± 2.40																							
3–25	5.84 ± 3.24*	0.3444	5.74 ± 3.13*																							
		0.4797	6.76 ± 3.81*																							
		3.1488	8.25 ± 3.53*																							
Viegas et al. (2010) Portugal Prevalence study	Personal air sampling, (<i>N</i> = 2 in factory, <i>N</i> = 29 in labs)	MN Frequency by cell type (mean ± SD) <table><tr><th>Referent</th><th>Factory</th><th>Laboratory</th></tr></table>	Referent	Factory	Laboratory																					
Referent	Factory	Laboratory																								

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results									
Population: 30 formaldehyde factory workers and 50 pathology/anatomy lab workers exposed for >1 year (40% female, mean age 35.7 year, 31.3% smokers), compared to 85 unexposed individuals (63.5% female, mean age 33.9 year, 30.6% smokers) Outcome: MN assay, buccal mucosa cells and peripheral lymphocytes. Blinded coding and analysis, Buccal cells, Feulgen stain, 2,000 cells scored/ subject by 4 observers, scoring criteria Tolbert et al. (1992), peripheral lymphocytes, stain May-Grunwald-Giemsa, 1,000 binucleated cells scored/ subject Also discussed in Viegas et al. (2013)	6–8 hrs, estimated 8-hr TWA Exposure duration: Factory workers: 6.2 (1–27) year Lab workers: 14.5 (1–33) year 8-hr TWA Concentration in: Factory: 0.26 mg/m³, range 0.25–0.27 mg/m³ Lab: 0.34 mg/m³, range 0.06–0.63 mg/m³ Ceiling Concentrations Factory: 0.64 mg/m³, range 0.004–1.28 mg/m³ Lab: 3.1 mg/m³, range 0.03–6.18 mg/m³	<table><tr><td>Peripheral lymphocytes</td><td>1.17 ± 1.95</td><td>1.76 ± 2.07</td><td>3.7 ± 3.86*</td></tr><tr><td>Buccal cells</td><td>0.13 ± 0.48</td><td>1.27 ± 1.55*</td><td>0.64 ± 1.74*</td></tr></table> *<i>p</i> <0.01, Spearman’s correlation test Years of exposure correlated with MN in peripheral lymphocytes (<i>r</i> = 0.401, <i>p</i> <0.01), and MN in buccal cells (<i>r</i> = 0.209, <i>p</i> = 0.008); Spearman’s test No correlation between MN frequency and smoking or gender, small magnitude of correlation with age (<i>r</i> = +0.194; <i>p</i> <0.05 for blood lymphocytes, <i>r</i> = -0.168; <i>p</i> <0.05 for buccal cells).	Peripheral lymphocytes	1.17 ± 1.95	1.76 ± 2.07	3.7 ± 3.86*	Buccal cells	0.13 ± 0.48	1.27 ± 1.55*	0.64 ± 1.74*	
Peripheral lymphocytes	1.17 ± 1.95	1.76 ± 2.07	3.7 ± 3.86*								
Buccal cells	0.13 ± 0.48	1.27 ± 1.55*	0.64 ± 1.74*								
Costa et al. (2008) Portugal Prevalence study Population: 30 pathology lab workers (4 hospitals), (70% female, mean age 38 year, 27% smokers) compared to 30 administrative employees matched by age, gender, lifestyle, smoking habits, and work area (63.3% female, mean age 37 years, 23% smokers). Outcome: MN in peripheral lymphocytes (Teixeira et al., 2004), stain 4% Giemsa; scored 1,000 binucleated cells/ subject, scored blind by one reader, criteria Caria et al. (1995)	Air sampling in breathing zone, derived an 8-hr TWA for each subject Concentration: Mean: 0.54 mg/m³, range: 0.05–1.94 mg/m³ Duration: 11 years Range: (0.5–27) years	<table><tr><th colspan="3">MN frequency in peripheral lymphocytes</th></tr><tr><th></th><th>Referent</th><th>Exposed</th></tr><tr><td>Lymphocyte MN</td><td>3.27 ± 0.69</td><td>5.47 ± 0.76*</td></tr></table> <i>P</i>=0.003, Mann-Whitney U-test and Kruskal-Wallis test. Authors reported positive correlation between formaldehyde exposure levels and MN frequency (<i>r</i>=0.384, <i>p</i>=0.001)	MN frequency in peripheral lymphocytes				Referent	Exposed	Lymphocyte MN	3.27 ± 0.69	5.47 ± 0.76*
MN frequency in peripheral lymphocytes											
	Referent	Exposed									
Lymphocyte MN	3.27 ± 0.69	5.47 ± 0.76*									
Pala et al. (2008) Italy Prevalence study Population: 36 lab workers (66.7% female, mean age 40.1 year, 16.7% smokers) Outcome: Peripheral lymphocytes (blood sampled at end of 8-hour shift), analysis blind to exposure. MN using modified	Personal air monitoring (8-hr sample); Exposure categories: High: ≥ 0.026 mg/m³, Low: < 0.026 mg/m³ Mean concentration:	<table><tr><th colspan="3">Micronuclei Frequency by Exposure Level (mean ± SD)</th></tr><tr><th></th><th><0.026 mg/m³</th><th>≥0.026 mg/m³</th></tr><tr><td>MN</td><td>0.26 ± 0.24</td><td>0.31 ± 0.17</td></tr></table>	Micronuclei Frequency by Exposure Level (mean ± SD)				<0.026 mg/m ³	≥0.026 mg/m ³	MN	0.26 ± 0.24	0.31 ± 0.17
Micronuclei Frequency by Exposure Level (mean ± SD)											
	<0.026 mg/m ³	≥0.026 mg/m ³									
MN	0.26 ± 0.24	0.31 ± 0.17									

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																																		
cytokinesis-blocked method, Fenech and Morley (1986) ; stain 3% Giemsa, 2,000 cells/ subject	Low (<i>n</i> = 25): 0.015 mg/m ³ (range 0.005–0.0254) High (<i>n</i> = 9): 0.056 mg/m ³ (range 0.026–0.269) Duration of exposure: NR	Means ratio (95% CI) 1.43 (0.26–7.81), Poisson regression adjusted for gender, age, smoking and other exposures																																		
Orsiere et al. (2006) <i>France</i> Prevalence Population: 59 hospital pathology workers from 5 labs (81% female, mean age 44.7 year, 20% smokers) compared to 37 unexposed workers (76% female, mean age 44 year, 24% smokers). Outcome: MN in peripheral lymphocytes. Subgroups selected randomly from initial groups. Assays conducted blinded. Cytokinesis-blocked micronucleus assay Sari-Minodier et al. (2002) ; stain 5% Giemsa, scoring criteria Fenech (2000) , 1,000 binucleated cells/ subject; FISH with a pan-centromeric DNA probe, same operator scored exposed and referent blinded Related reference: Iarmarcovai et al. (2006) .	Personal sampling; Short-term: 15 min, Long-term 8 hrs during typical work-day. Concentration ¹ : Mean 15-min: 2.46 mg/m ³ , range <0.12–25. 1 mg/m ³ Mean 8-hour 0.123 (range <0.123–0.86 mg/m ³) Duration exposure 13.2 years, range 0.5–34 years	Binucleated micronucleated cell rate (BMCR) in peripheral lymphocytes (mean ± SD) <table><tr><th></th><th>Unexposed (<i>n</i>=37)</th><th>Exposed (<i>n</i>=59)</th></tr><tr><td>% BMCR</td><td>11.1 ± 6.0</td><td>16.9 ± 9.3*</td></tr></table> *Number BMCR per 1,000 binucleated cells, <i>p</i> <0.05, Mann-Whitney U-test. Linear regression of BMCR, increase of 0.263 per 1,000 binucleated cells in exposed, <i>p</i> =0.003, adjusting for gender, age, smoking and alcohol. FISH Analysis of MN in peripheral lymphocytes by exposure (mean ± SD) <table><tr><th>FISH Results¹</th><th>Unexposed (<i>n</i>= 18)</th><th>Exposed (<i>n</i> = 18)</th><th><i>p</i>-Value</th></tr><tr><td>% BMCR</td><td>11.9 ± 5.6</td><td>19.1 ±10.1</td><td>0.021</td></tr><tr><td>% MN</td><td>14.4 ± 8.1</td><td>21.0 ± 12.6</td><td>0.084</td></tr><tr><td>C + MN (%)</td><td>10.3 ± 7.1</td><td>17.3 ± 11.5</td><td>0.059</td></tr><tr><td>C – MN (%)</td><td>4.1 ± 2.7</td><td>3.7 ± 4.2</td><td>0.338</td></tr><tr><td>C1 + MN (%)</td><td>3.1 ± 2.4</td><td>11.0 ± 6.2</td><td><i>p</i><0.001</td></tr><tr><td>Cx + MN (%)</td><td>7.8 ± 5.5</td><td>6.3 ± 6.3</td><td>0.163</td></tr></table> ¹ Results expressed as frequency per 1,000 binucleated cells, mean ± SD; analyzed using Mann-Whitney U-test Linear regression of C1 + MN, increase of 0.586 MN containing one centromere per 1,000 binucleated cells in exposed, <0.001, adjusting for gender, age, smoking and alcohol		Unexposed (<i>n</i> =37)	Exposed (<i>n</i> =59)	% BMCR	11.1 ± 6.0	16.9 ± 9.3*	FISH Results ¹	Unexposed (<i>n</i> = 18)	Exposed (<i>n</i> = 18)	<i>p</i> -Value	% BMCR	11.9 ± 5.6	19.1 ±10.1	0.021	% MN	14.4 ± 8.1	21.0 ± 12.6	0.084	C + MN (%)	10.3 ± 7.1	17.3 ± 11.5	0.059	C – MN (%)	4.1 ± 2.7	3.7 ± 4.2	0.338	C1 + MN (%)	3.1 ± 2.4	11.0 ± 6.2	<i>p</i> <0.001	Cx + MN (%)	7.8 ± 5.5	6.3 ± 6.3	0.163
	Unexposed (<i>n</i> =37)	Exposed (<i>n</i> =59)																																		
% BMCR	11.1 ± 6.0	16.9 ± 9.3*																																		
FISH Results ¹	Unexposed (<i>n</i> = 18)	Exposed (<i>n</i> = 18)	<i>p</i> -Value																																	
% BMCR	11.9 ± 5.6	19.1 ±10.1	0.021																																	
% MN	14.4 ± 8.1	21.0 ± 12.6	0.084																																	
C + MN (%)	10.3 ± 7.1	17.3 ± 11.5	0.059																																	
C – MN (%)	4.1 ± 2.7	3.7 ± 4.2	0.338																																	
C1 + MN (%)	3.1 ± 2.4	11.0 ± 6.2	<i>p</i> <0.001																																	
Cx + MN (%)	7.8 ± 5.5	6.3 ± 6.3	0.163																																	
Ye et al. (2005) <i>China</i> Prevalence study	Formaldehyde sampling: TWA Concentration Controls 0.011 ± 0.0025 mg/m ³	MN frequency in nasal cells <table><tr><th></th><th>Referent</th><th>Wait Staff</th><th>HCHO Workers</th></tr><tr><td></td><td></td><td></td><td></td></tr></table>		Referent	Wait Staff	HCHO Workers																														
	Referent	Wait Staff	HCHO Workers																																	

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results						
Population: 18 workers at a formaldehyde plant at least 1 year (38.9% female, mean age 29 year, and 16 workers exposed to indoor air formaldehyde via building materials (75% female, mean age 22 year) compared to 23 students with no known source of formaldehyde exposure (dormitories) (48% female, mean age 19 year); all nonsmokers Outcome: MN in nasal cells, stain Wright's, scoring criteria Fenech et al. (2003) , per 3,000 cells, blinding not stated.	Max. 0.015 mg/m ³ Wait staff 0.107 ± 0.067 mg/m ³ Max. 0.30 mg/m ³ Workers 0.985 ± 0.286 mg/m ³ Max. 1.694 mg/m ³ Exposure duration: Workers 8.5 (1–15) years Waiters 12 wks	<table><tr><td>MN</td><td>1.25 ± 0.65</td><td>1.75 ± 1.00</td><td>2.70 ± 1.50*</td></tr></table> <i>P</i> <0.05, one-way ANOVA, values estimated from figure	MN	1.25 ± 0.65	1.75 ± 1.00	2.70 ± 1.50*		
MN	1.25 ± 0.65	1.75 ± 1.00	2.70 ± 1.50*					
Burgaz et al. (2002) Turkey Prevalence study Population: 28 pathology workers (46.4% female, mean age 29.7 year, 43% smokers) and 18 unexposed male employees (mean age 31.1 year, 25% smokers), may overlap with study population from Burgaz et al. (2001) Outcome: MN frequency in buccal mucosal cells, stain Feulgen's reaction plus Fast Green, MN, 3,000 cells/ subject counted, coded slides, scoring criteria Tolbert et al. (1992) ; Sarto et al. (1987)	Concentration: Range:2.46–4.92 mg/m ³ Duration: 4.7 ± 3.33 (1–13) years	MN frequency (%) in buccal mucosal cells (mean ± SD) <table><tr><td></td><td>Referent</td><td>Exposed</td></tr><tr><td>MNF Frequency</td><td>0.33 ± 0.30</td><td>0.71 ± 0.56*</td></tr></table> * <i>p</i> <0.05, multifactorial ANOVA adjusting for age, smoking, and gender MN frequency was not associated with duration of exposure		Referent	Exposed	MNF Frequency	0.33 ± 0.30	0.71 ± 0.56*
	Referent	Exposed						
MNF Frequency	0.33 ± 0.30	0.71 ± 0.56*						
Burgaz et al. (2001) Turkey Prevalence study Population: 23 pathology workers (12 male, 11 female) occupationally exposed 5 d, 8 hrs/ wk, mean age 30.6 year, 39% smokers compared to 25 male university and hospital staff, mean age 35.4 year, 76% smokers Outcome: MN frequency in nasal cells. Previously coded slides, stain Feulgen's reaction plus Fast Green, MN, 3,000 cells/ subject counted, scoring criteria Tolbert et al. (1992) ; Sarto et al. (1987)	Exposure based on occupation and duration of employment and quantified via stationary air monitors Exposure conc.: 2.46–4.92 mg/m ³ (converted from ppm by EPA) Exposure duration: Mean: 5.06 ± 3.47 years Range: (1–13) years	MN frequency (%) in nasal epithelial cells (mean ± SD) <table><tr><td></td><td>Referent</td><td>Exposed</td></tr><tr><td>MN frequency</td><td>0.61 ± 0.27</td><td>1.01 ± 0.62*</td></tr></table> * <i>p</i> <0.05, nonparametric test MN frequency was not associated with duration of exposure. MN frequency higher in male exposed, similar between smokers and nonsmokers in referent.		Referent	Exposed	MN frequency	0.61 ± 0.27	1.01 ± 0.62*
	Referent	Exposed						
MN frequency	0.61 ± 0.27	1.01 ± 0.62*						
He et al. (1998) China Prevalence study	Breathing zone air samples during dissection.	MN frequency (%) in peripheral blood lymphocytes (mean ± SD)						

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results			
Population: 13 anatomy students exposed during a 12-wk course (10 hr/ wk) compared to 10 students from same school. Age and gender similar between groups, all non-smokers. Outcome: MN assay, Fenech and Morley (1985) , scored 1,000 cells per individual, blinding not described	Measurements limited to location of exposed students. Concentration in breathing zone: Mean 3.17 mg/m ³ Duration: 12 wks (10 hrs/wk)	Referent		Exposed	
		Lymphocyte MN	3.15 ± 1.46	6.38 ± 2.50*	
		* <i>p</i> <0.01, analytic test not described			
Kitaeva et al. (1996) Russia Prevalence study Population: anatomy instructors (8 female, 5 male), mean age 41 year) compared to 6 female unexposed (mean age 28.5 year); students (6 female, 6 male) Outcome: MN in buccal cells, 1994–1995. MN in mucosal cells compared between exposed and referent instructors, and before and after a 40-min exposure for students at 24 and 48 hrs. Blinding not described, stain Feulgen and light green, analyzed 2,000 cell/ subject	No quantitative exposure assessment. Duration of employment among instructors, females 23.6 years; males 25.6 years 40-min exposures	MN frequency (%) in buccal mucosa cells			
		Referent		Exposed	
		Female instructors	0.64 (N=6)	2.94* (N=8)	
			Before	24 Hr Post	48 Hr Post
		Female students	0.58	2.50**	2.64**
		Male students	0.77	2.02*	1.86
		Male instructors, no Referent (MN frequency = 1.18%)			
		* <i>p</i> <0.05, ** <i>p</i> <0.01, Student's <i>t</i> -test			
Ballarin et al. (1992) Italy Prevalence study Population: 15 plywood factory workers (46.7% female, mean age 31 years,) compared to 15 university or hospital clerks matched for age and sex (mean age 31 year). All nonsmokers. Outcome: MN in nasal mucosal cells, stain feulgen's plus Fast Green, analysis blinded by one reader, 6,000 cells/ subject, scoring criteria Sarto et al. (1987) .	Personal sampling; 8-hr TWA (NIOSH, 1977) Warehouse (<i>N</i> = 3) 0.39 ± 0.20 mg/m ³ , range 0.21–0.6 mg/m ³ Shearing-press (<i>N</i> = 8) 0.1 ± 0.02 mg/m ³ , range 0.08–0.14 mg/m ³ Sawmill (<i>N</i> = 1), 0.09 mg/m ³ Inspirable wood dust: 0.11–0.69 mg/m ³ , 0.73 in sawmill Employment duration 6.8 years	Mean frequency micronuclei per 1000 cells in nasal mucosal cells by exposure group			
		Referent		Exposed	
		MN (%) (SD)	0.25 (0.22)	0.9 (0.47)*	
		* <i>p</i> <0.01, Mann-Whitney U test			
		Short-term Studies			

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																											
Lin et al. (2013) China Cross-shift change Population: 62 plywood workers (17.7% female, mean age 34 year, 17.7% smokers) Outcome: Peripheral lymphocytes, cytokinesis-block micronucleus assay, Fenech (1993), analyzed 1,000 binucleated cells/ subject, scoring criteria Fenech (1993), Fenech et al. (2003); blinded analysis	Air sampling and job function. Mean exposure: 0.27 ± 0.20 mg/m³, range: 0.012–0.67 mg/m³ Mean exposure duration 2.53 ± 2 year	Frequency micronuclei in binucleated cells in peripheral lymphocytes <table> <tr> <th></th><th>Before exposure</th><th>After exposure</th></tr> <tr> <td>MN (%)</td><td>2.29 ± 1.21</td><td>2.29 ± 1.65</td></tr> </table> $p = 0.754$, paired Wilcoxon test Regression coefficients for formaldehyde level, before shift 0.73 (–0.46, 1.92); after shift –0.01 (–1.38, 1.35) Poisson regression adjusted for age, gender, smoking, and alcohol		Before exposure	After exposure	MN (%)	2.29 ± 1.21	2.29 ± 1.65																					
	Before exposure	After exposure																											
MN (%)	2.29 ± 1.21	2.29 ± 1.65																											
Ying et al. (1997) China Panel study Population: 25 non-smoking anatomy students (13 males, 12 females, mean age 18.8 year, Han nationality) exposed during 8-wk course, 3-hr session, 3 times/ wk. Outcome: MN Nasal and Buccal cells, assessed before the start of the course and at the end of 8-wk period. Blinded analysis, one observer; Wright's stain, scored 4,000 cells/ subject; MN blood lymphocytes, stain 4% Giemsa, scored mean of 2870–3167 cells/ subject; MN scoring criteria Sarto et al. (1987)	Air sampling, estimated TWA and peak levels during class and in the dorms. Anatomy labs: Mean TWA: 0.51 ± 0.299 mg/m³, range: 0.07–1.28 mg/m³ Dormitories: Mean TWA: 0.012 ± 0.003 mg/m³, range: 0.011–0.016 mg/m³ Duration: 8 wks	Micronucleated Cell Frequency (Mean+SEM), Change over 8 weeks <table> <tr> <th></th><th>Before exposure</th><th>After exposure</th></tr> <tr> <td>Oral Mucosa</td><td>0.57 ± 0.32</td><td>$0.86 \pm 0.56^*$</td></tr> <tr> <td>Nasal Mucosa</td><td>1.20 ± 0.67</td><td>$3.84 \pm 1.48^*$</td></tr> <tr> <td>Lymphocytes</td><td>0.91 ± 0.39</td><td>1.11 ± 1.54</td></tr> </table> *$p < 0.01$, paired t-test		Before exposure	After exposure	Oral Mucosa	0.57 ± 0.32	$0.86 \pm 0.56^*$	Nasal Mucosa	1.20 ± 0.67	$3.84 \pm 1.48^*$	Lymphocytes	0.91 ± 0.39	1.11 ± 1.54															
	Before exposure	After exposure																											
Oral Mucosa	0.57 ± 0.32	$0.86 \pm 0.56^*$																											
Nasal Mucosa	1.20 ± 0.67	$3.84 \pm 1.48^*$																											
Lymphocytes	0.91 ± 0.39	1.11 ± 1.54																											
Titenko-Holland et al. (1996) USA Panel study Population: same subjects as in Suruda et al. (1993); 35 mortuary students intermittently exposed for 90 d (28 students (with adequate samples, 22 males, 6 females)), age 20–33 years. Outcome: MN analysis on buccal and nasal cells using FISH; blinded analysis Related study: Suruda et al. (1993), same subjects	See Suruda et al. (1993) Subjects with complete MN data from buccal mucosa cells ($n = 19$): Lagged (7–10 d before the last sampling): 1.2 ± 2.1 ppm-hrs; 90-d cumulative (90 d): 14.8 ± 7.2 ppm-hrs;	Micronuclei before and after embalming class (per 1,000 cells) by cell type <table> <tr> <th></th><th>Preexposure</th><th>Postexposure</th></tr> <tr> <td colspan="3"><i>Buccal Cells (N = 19)</i></td></tr> <tr> <td>MN Total</td><td>0.6 ± 0.5</td><td>$2.0 \pm 2.0^*$</td></tr> <tr> <td>MN⁺</td><td>0.4 ± 0.4</td><td>1.1 ± 1.3</td></tr> <tr> <td>MN[–]</td><td>0.1 ± 0.2</td><td>$0.9 \pm 1.1^*$</td></tr> <tr> <td colspan="3"><i>Nasal Cells (N = 13)</i></td></tr> <tr> <td>MN Total</td><td>2.0 ± 1.3</td><td>2.5 ± 1.3</td></tr> <tr> <td>MN⁺</td><td>1.2 ± 1.3</td><td>1.0 ± 0.8</td></tr> <tr> <td>MN[–]</td><td>0.5 ± 0.5</td><td>$1.0 \pm 0.6^*$</td></tr> </table>		Preexposure	Postexposure	<i>Buccal Cells (N = 19)</i>			MN Total	0.6 ± 0.5	$2.0 \pm 2.0^*$	MN ⁺	0.4 ± 0.4	1.1 ± 1.3	MN [–]	0.1 ± 0.2	$0.9 \pm 1.1^*$	<i>Nasal Cells (N = 13)</i>			MN Total	2.0 ± 1.3	2.5 ± 1.3	MN ⁺	1.2 ± 1.3	1.0 ± 0.8	MN [–]	0.5 ± 0.5	$1.0 \pm 0.6^*$
	Preexposure	Postexposure																											
<i>Buccal Cells (N = 19)</i>																													
MN Total	0.6 ± 0.5	$2.0 \pm 2.0^*$																											
MN ⁺	0.4 ± 0.4	1.1 ± 1.3																											
MN [–]	0.1 ± 0.2	$0.9 \pm 1.1^*$																											
<i>Nasal Cells (N = 13)</i>																													
MN Total	2.0 ± 1.3	2.5 ± 1.3																											
MN ⁺	1.2 ± 1.3	1.0 ± 0.8																											
MN [–]	0.5 ± 0.5	$1.0 \pm 0.6^*$																											

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results												
	Subjects with complete MN data from nasal cells (n = 13): Lagged (7–10 d): 1.9 ± 2.5 ppm-hrs; 90-d cumulative (90 d): 16.5 ± 5.8 ppm-hrs	*<i>p</i> < 0.05, Wilcoxon sign-rank test, two-tailed Association with 90-d cumulative exposure for change in total MN frequency in buccal cells, <i>r</i> = 0.44, <i>p</i> = 0.06; no association with 7–10 d lagged exposure, Spearman rank order correlation												
Suruda et al. (1993) USA Panel study Population: 29 students (with adequate samples) (24.1% female, mean age 23.6 year, 17.2% smokers) exposed to formaldehyde for 9 wks during embalming course, with baseline samples taken. Mean duration of embalming 125 min. Possible exposure prior to course. Outcome: MN assay, nasal, buccal and micronucleated peripheral blood lymphocytes. Analysis blinded to exposure status; MN assay buccal and nasal cells, Stich et al. (1982), stain Feulgen/ Fast Green, 1,500 cell/ subject; MN lymphocytes Fenech and Morley (1985), stain Feulgen 2,000 cells/ subject	Personal sampling for 121 of 144 embalming; cumulative exposure estimated using sampling data and time-activity data; Continuous area samples over embalming tables for short-term peaks; Concentration¹: Mean: 1.72 mg/m³, range 0.18–5.29 mg/m³ Duration: 9 wks Average cumulative exposure 18.2 mg/m³-hr, range 5.3–41.3 mg/m³-hr 8-hr TWA Mean 0.41 mg/m³, range 0.123–1.2 mg/m³ Measurements of glutaraldehyde, phenol, & methanol all < LOD, isopropyl alcohol < LOD or very low.	Micronuclei before and after embalming class (per 1,000 cells) <table> <tr> <th>Cell type</th><th>Before exposure</th><th>After 9 weeks</th></tr> <tr> <td>Buccal</td><td>0.046 ± 0.17</td><td>0.60 ± 1.27*</td></tr> <tr> <td>Nasal</td><td>0.41 ± 0.52</td><td>0.50 ± 0.67</td></tr> <tr> <td>Micronucleated lymphocytes</td><td>4.95 ± 1.72</td><td>6.36 ± 2.03*</td></tr> </table> *<i>p</i> < 0.05, Wilcoxon sign-rank test Buccal MN in males associated with cumulative exposure, Spearman coefficient, not nasal MN or micronucleated lymphocytes	Cell type	Before exposure	After 9 weeks	Buccal	0.046 ± 0.17	0.60 ± 1.27*	Nasal	0.41 ± 0.52	0.50 ± 0.67	Micronucleated lymphocytes	4.95 ± 1.72	6.36 ± 2.03*
Cell type	Before exposure	After 9 weeks												
Buccal	0.046 ± 0.17	0.60 ± 1.27*												
Nasal	0.41 ± 0.52	0.50 ± 0.67												
Micronucleated lymphocytes	4.95 ± 1.72	6.36 ± 2.03*												
Zeller et al. (2011) Germany Controlled human exposure study Subjects: 41 healthy volunteers exposed 4 hr/d for 5 d, all male, nonsmokers	12 groups of 2 to 4 persons in a chamber, exposures randomly assigned. Formaldehyde	Frequency of micronuclei and NDI in lymphocytes and nasal mucosa before and after 4-hr exposure over 5 d (N = 40)												

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results		
Outcome: MN in peripheral blood lymphocytes and nasal mucosa cells assessed before and after exposure. Lymphocytes: CBMN test, scored 1,000 binucleated cells/ subject on coded slides. Nuclear division index (NDI) = # cells with 1–4 micronuclei/ Total cells scored. Nasal cells: scored 2,000 cells/ subject on coded slides. Difference in means analyzed using Cochran Mantel Haentzel test and ANOVA.	concentrations: 0 (i.e., background level of 0.01 ppm); 0.3 ppm (0.37 mg/m ³) ^a with four peaks of 0.6 ppm (0.74 mg/m ³); 0.4 ppm (0.49 mg/m ³) with 4 peaks of 0.8 ppm (0.98 mg/m ³); 0.5 ppm (0.67 mg/m ³); and 0.7 ppm (0.86 mg/m ³) Peaks were 15 min each, Four 15-min exercise sessions during exposure.	Cells with micronuclei/ 1,000	Nuclear Division Index	
		Lymphocytes		
		Before	6.5 ± 3.226	2.0 ± 0.232
		After	5.7 ± 3.339 ^a	2.0 ± 0.176
		Nasal mucosa ^b		
		Before	0.21 ± 0.35	
		After	0.27 ± 0.42	
		1-wk after	0.24 ± 0.43	
		2-wks after	0.24 ± 0.45	
		3-wks after	0.17 ± 0.41	
		^a <i>p</i> = 0.11 ^b Several slides could not be analyzed, hence only 1,000 cells scored for several individuals (9–13 subjects per sampling time).		
Speit et al. (2007a) Germany Controlled human exposure study Subjects: 21 healthy volunteers exposed to formaldehyde for 4 hrs/d for 10 d, 11 males, nonsmokers, aged 19–36 years. Outcome: MN in buccal mucosal cells assessed prior to controlled exposure and then during postexposure period. Blinded analysis at end of study by one person, stain DAPI/ propidium iodide, Analyzed 2,000 cells/ subject	Source: para-formaldehyde. Exposure duration: 10 consecutive d, 5 groups of 3–6 persons in chamber, 4-hr exposures, some exposures masked with ethyl acetate (EA), 3 15-min exercise sessions during exposure. Cumulative exposure 16.6 mg/m ³ – hrs; Target concentrations: 0, 0.15, 0.3, 0.5, 0 + EA, 0.3 + EA, 0.5 + EA, 0.3 + 4 x 0.6, 0.5 + 4 x 1.0, and 0.4 + 4 x 1.0 + EA	MN Frequency (per 1,000 cells) in Buccal Mucosa, mean ± SD		
		Immediately before exposure	End of 10-d exposure	
		Mean MN	0.86 ± 0.84	1.33 ± 1.45
		<i>p</i> = 0.052, Wilcoxon signed rank test		
		DNA Damage		
		Prevalence Studies		

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results												
Zendehdel et al. (2017) Iran Prevalence study Population: Workers in 3 melamine dinnerware manufacturing workshops (n = 49) and referents matched by age and sex (n = 34) who worked in food industries, # smokers higher in referent (26% versus 16%), >90% male. Recruitment and participation were not described. Outcome: Peripheral blood cells, Comet assay, alkaline conditions, according to Tice et al. (2000) blinding not described; minimum of 50 randomly selected cells per sample; tail moment and Olive moment	Personal air sampling, NIOSH method 3500, whole shift for each worker. Median TWA in 3 workshops, 0.086 mg/m ³ ; range, 0.02–0.22 mg/m ³ ; authors state that 2/3 of sample were exposed to < 0.1 mg/m ³ Work duration: Exposed 2.5 (1–22) years Referent 2.0 (1–25) years	Comparison of DNA damage (comet assay) between exposed and referent <table><tr><td></td><td>Olive moment Median (min-max)</td><td>Tail moment Median (min-max)</td></tr><tr><td>Exposed (N = 49)</td><td>13 (7.4-36.7)</td><td>22.2 (12.3-65)</td></tr><tr><td>Referent (N = 34)</td><td>8.4 (6.4-31.7)</td><td>14.8 (6.4-57.7)</td></tr></table> p value = 0.001; Mann-Whitney test		Olive moment Median (min-max)	Tail moment Median (min-max)	Exposed (N = 49)	13 (7.4-36.7)	22.2 (12.3-65)	Referent (N = 34)	8.4 (6.4-31.7)	14.8 (6.4-57.7)			
	Olive moment Median (min-max)	Tail moment Median (min-max)												
Exposed (N = 49)	13 (7.4-36.7)	22.2 (12.3-65)												
Referent (N = 34)	8.4 (6.4-31.7)	14.8 (6.4-57.7)												
Costa et al. (2015) Portugal Prevalence study Population: 83 anatomy pathology workers from 9 hospital laboratories, exposed to formaldehyde for at least 1 year, compared to 87 unexposed employees from administrative offices in same geographic area. Exclusions: cancer history, radiation therapy or chemotherapy, surgery with anesthesia or blood transfusion in last year. Exposed and referent similar for mean age 39 years, 77% females, 25% smokers. Outcome: Peripheral blood samples, coded, analyses blinded to exposure status. Comet assay: alkaline conditions according to Singh et al. (1988) ; Scored blind 100 cells/ donor from two gels; % DNA in comet tail. Exposed compared to unexposed using Student's t-test for ln % tDNA; linear regression of ln %tDNA	Exposure assessed via air sampling and deriving an 8-hr TWA for each subject. Exposure concentration: Mean: 0.38 ppm (0.47 mg/m ³) Range: 0.28–0.85 ppm (0.34-1.05 mg/m ³) Exposure duration 12.0 ± 8.2 years	Comparison of % DNA in tail (comet assay) between exposed and referent <table><tr><td></td><td>Mean</td><td>SD</td><td>Mean Ratio (95% CI)</td></tr><tr><td>Exposed (N = 83)</td><td>11.67^a</td><td>0.72</td><td>1.5 (1.14–1.96)^b</td></tr><tr><td>Referent (N = 87)</td><td>7.5</td><td>0.47</td><td>1.0</td></tr></table> ^aStudent's t-test, p<0.001. ^bmodel adjusted for age, gender, smoking habit, and fruit consumption (# pieces consumed/d).		Mean	SD	Mean Ratio (95% CI)	Exposed (N = 83)	11.67 ^a	0.72	1.5 (1.14–1.96) ^b	Referent (N = 87)	7.5	0.47	1.0
	Mean	SD	Mean Ratio (95% CI)											
Exposed (N = 83)	11.67 ^a	0.72	1.5 (1.14–1.96) ^b											
Referent (N = 87)	7.5	0.47	1.0											
Peteffi et al. (2015) Brazil Prevalence study Population: 46 workers in furniture manufacturing facility (mean age 34.5 year, 56.5% male, 1 smoker) and unexposed	Monitoring in 7 sections in facility; referent monitoring in 5 areas of university; breathing zone 8 hr samples collected on	Comparisons of DNA damage (comet assay) in peripheral blood cells, median (interquartile range) <table><tr><td></td><td>Referent</td><td>Exposed</td><td>p-Value</td></tr><tr><td></td><td></td><td></td><td></td></tr></table>		Referent	Exposed	p-Value								
	Referent	Exposed	p-Value											

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results			
group (n = 45) recruited from employees and students of local university with no history of occupational exposure to potentially genotoxic agents or substances metabolized to formic acid. (mean age 35.4 year, 33.3% male, 0 smokers) Outcome: Peripheral blood processed within 4 hr. Comet assay, alkaline conditions according to Tice et al. (2000) ; silver nitrate staining according to Nadin et al. (2001) ; 100 cells/ person read by two independent observers (50 cells each), classified by visual scoring according to Anderson et al. (1994) ; 5 categories based on tail migration (0–IV) and frequency of damaged cells (sum of I–IV), damage index (Pitarque et al., 1999) Nonparametric tests used because data were not normally distributed. Exposed and referent compared using Mann-Whitney test	same day as biological samples. Urine samples collected at end of work day on 5 th day of work; correlation of formaldehyde concentration in air with urinary formic acid concentration, r = 0.626, p<0.001 UV painting, lamination/press, packaging, edge lamination 0.03–0.04 ppm (0.037–0.05 mg/m ³) Edge painting, machining and drilling center, board cutting 0.06–0.09 ppm (0.07–0.11 mg/m ³) Referent mean (SD) 0.012 (0.008) ppm (0.015 (0.01) mg/m ³) Formic acid median Exposed 20.47 mg/L Referent 4.57 mg/L Correlation formaldehyde concentration and formic acid r = –0.626, p <0.001 Exposure duration 5.76 year	Damage index	2.0 (0–4.0)	6.5 (1.0–12.5)	0.007
		Damage frequency (%)	2.0 (0–4.0)	6.0 (1.0–12.5)	0.003
		No differences between men and women for measures of DNA damage in either exposed or referent. No correlation between urinary formic acid and measures of DNA damage.			
(Aydin et al., 2013) Turkey Prevalence study	24 area samples in workplaces; personal samples in breathing zone over 8 hrs.	Comparison of Comet assay results in peripheral blood lymphocytes by exposure UnexposedExposed			

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																								
Population: 46 male workers from 2 MDF plants (mean age 33.4 year, 39.1% smokers) compared to 46 non-exposed male workers in same area (mean age 38.4 year, 50% smokers) (administrative government offices and maintenance services). Half of workers used personal protective equipment. Outcome: DNA damage, Comet assay, tail intensity, tail moment, and tail migration, alkaline conditions, 100 cells/subject	Mean: 0.25 ± 0.07 mg/m ³ Range (0.12–0.41) Duration: Mean: 7.3 years Range (0.33–30)	<table><tr><td>Tail intensity</td><td>5.28 ± 0.22</td><td>4.25 ± 0.29*</td></tr><tr><td>Tail moment</td><td>0.816 ± 0.002</td><td>0.624 ± 0.003*</td></tr><tr><td>Tail migration</td><td>2.16 ± 0.007</td><td>1.68 ± 0.005*</td></tr></table> *ANOVA, <i>P</i> <0.05. Comparisons by smoking strata indicate similar pattern.	Tail intensity	5.28 ± 0.22	4.25 ± 0.29*	Tail moment	0.816 ± 0.002	0.624 ± 0.003*	Tail migration	2.16 ± 0.007	1.68 ± 0.005*															
Tail intensity	5.28 ± 0.22	4.25 ± 0.29*																								
Tail moment	0.816 ± 0.002	0.624 ± 0.003*																								
Tail migration	2.16 ± 0.007	1.68 ± 0.005*																								
Lin et al. (2013) China Prevalence study Population: 96 plywood workers exposed to formaldehyde (13.5% female, mean age 33 year, 30.2% smokers) compared to referent group (N=82) (4% female, mean age 31 year, 40% smokers). Outcome: Blood lymphocytes: DNA damage, Comet assay, olive tail moment, alkaline conditions (pH = 13), 50 cells/sample, blinded analysis.	Exposure assessed by air monitoring and job assignment. Average concentration: High Exposure, <i>N</i> =38 (making glue): 1.48 mg/m ³ (0.914–2.044) Low exposure, <i>N</i> =58 (sanding boards, pressing wood scraps with glue at high temp): 0.68 mg/m ³ (0.455–0.792) Referent group, <i>N</i> =82 (providing & grinding wood scraps): 0.13 mg/m ³ (0.019–0.252) Exposure duration: 2.52 years	Comparison of Comet assay results in peripheral blood lymphocytes by exposure and duration of employment. <table><tr><td></td><th colspan="3">By Exposure</th></tr><tr><td></td><th>Referent</th><th>Low</th><th>High</th></tr><tr><td>Tail moment (Ln)</td><td>0.67 ± 0.55</td><td>0.88 ± 0.55*</td><td>1.01 ± 0.56*</td></tr></table> *ANOVA <i>p</i> -value = 0.006; linear regression model, trend <i>p</i> -value = 0.002, adjusted for age, gender, smoking status, alcohol consumption, duration of employment <table><tr><td></td><th colspan="3">By Number of Work Years</th></tr><tr><td></td><th><1 (<i>N</i> = 57)</th><th>1–3 (<i>N</i> = 64)</th><th>>3 (<i>N</i> = 57)</th></tr><tr><td>Tail moment (Ln)</td><td>0.76 ± 0.56</td><td>0.73 ± 0.59</td><td>0.99 ± 0.52</td></tr></table> *ANOVA <i>p</i> -value = 0.131; trend <i>p</i> -value = 0.059, Adjusted for age, gender, smoking status, alcohol consumption, and formaldehyde levels		By Exposure				Referent	Low	High	Tail moment (Ln)	0.67 ± 0.55	0.88 ± 0.55*	1.01 ± 0.56*		By Number of Work Years				<1 (<i>N</i> = 57)	1–3 (<i>N</i> = 64)	>3 (<i>N</i> = 57)	Tail moment (Ln)	0.76 ± 0.56	0.73 ± 0.59	0.99 ± 0.52
	By Exposure																									
	Referent	Low	High																							
Tail moment (Ln)	0.67 ± 0.55	0.88 ± 0.55*	1.01 ± 0.56*																							
	By Number of Work Years																									
	<1 (<i>N</i> = 57)	1–3 (<i>N</i> = 64)	>3 (<i>N</i> = 57)																							
Tail moment (Ln)	0.76 ± 0.56	0.73 ± 0.59	0.99 ± 0.52																							
Gomaa et al. (2012) Egypt Prevalence study	No formaldehyde measurements; exposure defined by job type	Comparisons of Comet assay results by exposure <table><tr><td></td><th>Unexposed</th><th>Exposed</th></tr></table>		Unexposed	Exposed																					
	Unexposed	Exposed																								

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results										
Population: 30 workers in pathology, histology and anatomy laboratories at a university (30% female, mean age 42.5 year) compared to 15 referents (46.7% female, mean age 39.3 year). Source of referent was not described. Outcome: Comet assay, alkaline conditions according to Singh et al. (1988) ; tail length & tail moment; blinding not described; analyzed 50 cells per subject	Exposure duration: mean 14.3 year	<table><tr><td>Tail length (mm)</td><td>12.5 ± 1.5 (7.2–14.7)</td><td>47.3 ± 8.5* (16.5–74.2)</td></tr><tr><td>Tail moment</td><td>10.8 ± 1.2 (5.8–13.6)</td><td>56.1 ± 16.5* (11.4–88.1)</td></tr></table> *Student's <i>t</i>-test, <i>p</i> <0.05; Mean value per 50 comets ± SE, distribution in parentheses. Results comparable between males and females.	Tail length (mm)	12.5 ± 1.5 (7.2–14.7)	47.3 ± 8.5* (16.5–74.2)	Tail moment	10.8 ± 1.2 (5.8–13.6)	56.1 ± 16.5* (11.4–88.1)				
Tail length (mm)	12.5 ± 1.5 (7.2–14.7)	47.3 ± 8.5* (16.5–74.2)										
Tail moment	10.8 ± 1.2 (5.8–13.6)	56.1 ± 16.5* (11.4–88.1)										
Costa et al. (2011) Portugal Prevalence study Population: 48 pathology workers from 5 hospital laboratories, exposed for at least 1 year (28% female, mean age 40 year, 21% smokers), compared to 50 unexposed employees matched by age, gender, lifestyle, smoking habits, and work area (25% female, mean age 37 year, 14% smokers). Outcome: DNA damage, comet assay, tail length and % tail DNA; alkaline conditions, 100 cells/ subject; analysis blind to exposure	Air sampling in breathing zone; 8-hr TWA derived for each subject. Concentration: ppm converted to mg/m ³ by EPA. Mean: 0.53 mg/m ³ Range: (0.05–1.94) Duration: Mean: 13.6 years Range: (1–31)	Comparisons of Comet assay results by exposure <table><tr><td></td><td>Unexposed</td><td>Exposed</td></tr><tr><td>Tail length</td><td>42.00 ± 1.6</td><td>54.55 ± 2.02*</td></tr><tr><td>% DNA Tail</td><td>8.01 ± 0.64</td><td>11.76 ± 0.74*</td></tr></table> ANOVA, Student's <i>t</i>-test, <i>p</i> <0.05, compared to referent group. Tail length and % tail DNA did not vary by gender, age, or smoking. Comet assay parameters were not associated with exposure duration.		Unexposed	Exposed	Tail length	42.00 ± 1.6	54.55 ± 2.02*	% DNA Tail	8.01 ± 0.64	11.76 ± 0.74*	
	Unexposed	Exposed										
Tail length	42.00 ± 1.6	54.55 ± 2.02*										
% DNA Tail	8.01 ± 0.64	11.76 ± 0.74*										
Jiang et al. (2010) China Prevalence study Population: 151 male workers from 2 plywood plants (mean age 27.4 year, 52.3% smokers) compared to 112 unexposed workers at a machine manufacturer in same town (mean age 28.7 year, 42.9% smokers). Outcome: Peripheral blood lymphocytes, Comet assay, olive tail moment, alkaline conditions; blinded analysis, analyzed > 100 cells/ subject Related reference: Yu et al. (2005) in Chinese	Exposure assessed by job title and personal air monitoring. 4 exposure groups based on 8-hr TWA: 0.135, 0.344, 0.479, 3.141 mg/m ³ . Concentration: ppm converted to mg/m ³ by EPA. Mean: 1.02 mg/m ³ Range: (0.1–0.75) Duration:	Comparison of Comet assay results in peripheral blood lymphocytes by exposure and duration of employment Ln tail moment (TM), geometric mean (95% CI) <table><tr><td>Referent (n=112)</td><td>0.93 (95%CI: 0.78–1.10)</td></tr><tr><td>0.135 mg/m³ (n = 60)</td><td>2.85 (95%CI: 2.37–3.43)*</td></tr><tr><td>0.344 mg/m³ (n=35)</td><td>3.01 (95%CI: 2.48–3.64)*</td></tr><tr><td>0.479 mg/m³ (n=43)</td><td>4.37 (95%CI: 3.78–5.05)*</td></tr><tr><td>3.141 mg/m³ (n=13)</td><td>8.86 (95%CI: 6.50–12.07)**</td></tr></table> *TM compared to referent group, ANOVA, <i>p</i><0.05; **TM compared to referent and other exposure groups, ANOVA <i>p</i><0.05 Tail moment by exposure history (years)*	Referent (n=112)	0.93 (95%CI: 0.78–1.10)	0.135 mg/m ³ (n = 60)	2.85 (95%CI: 2.37–3.43)*	0.344 mg/m ³ (n=35)	3.01 (95%CI: 2.48–3.64)*	0.479 mg/m ³ (n=43)	4.37 (95%CI: 3.78–5.05)*	3.141 mg/m ³ (n=13)	8.86 (95%CI: 6.50–12.07)**
Referent (n=112)	0.93 (95%CI: 0.78–1.10)											
0.135 mg/m ³ (n = 60)	2.85 (95%CI: 2.37–3.43)*											
0.344 mg/m ³ (n=35)	3.01 (95%CI: 2.48–3.64)*											
0.479 mg/m ³ (n=43)	4.37 (95%CI: 3.78–5.05)*											
3.141 mg/m ³ (n=13)	8.86 (95%CI: 6.50–12.07)**											

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results						
	Mean: 2.51 years Range: (0.6–25)	<table><tr><td>0.6–1 (n=33)</td><td>2.27 (2.91–3.71)</td></tr><tr><td>1–2 (n=68)</td><td>2.69 (3.50–4.13)</td></tr><tr><td>3–25 (n=50)</td><td>3.53 (4.11–4.78)**</td></tr></table> *ANOVA, <i>p</i> = 0.03, adjusted for age, formaldehyde exposure history and concentration, current smoking status, alcohol consumption **Dunnett-Hsu test, compared to 0.6–1 year subgroup, <i>p</i> = 0.01	0.6–1 (n=33)	2.27 (2.91–3.71)	1–2 (n=68)	2.69 (3.50–4.13)	3–25 (n=50)	3.53 (4.11–4.78)**
0.6–1 (n=33)	2.27 (2.91–3.71)							
1–2 (n=68)	2.69 (3.50–4.13)							
3–25 (n=50)	3.53 (4.11–4.78)**							
Costa et al. (2008) Portugal Prevalence Study Population: 30 pathology lab workers (4 hospitals), (70% female, mean age 38 year, 27% smokers) compared to 30 administrative employees matched by age, gender, lifestyle, smoking habits and work area (63.3% female, mean age 37 years, 23% smokers). Outcome: Peripheral lymphocytes; blood samples collected 10–11 am; Scored blind to exposure status; Comet assay, tail length, alkaline conditions (pH = 13), 100 cells/ subject	Air sampling in breathing zone, 8-hr TWA derived for each subject Mean: 0.54 mg/m ³ Range: (0.05–1.94) Years employed: Mean ± SD: 11 ± 7 years Range: (0.5–27)	Comparisons of Comet assay results in peripheral blood lymphocytes by exposure <table><tr><td></td><td>Unexposed</td><td>Exposed</td></tr><tr><td>Tail Length</td><td>41.85 ± 1.97</td><td>60.00 ± 2.31*</td></tr></table> *<i>p</i> <0.05, Student’s <i>t</i>-test Tail length was also significantly longer among exposed females compared to males. No difference noted by smoking status. No difference by duration of exposure (data not provided).		Unexposed	Exposed	Tail Length	41.85 ± 1.97	60.00 ± 2.31*
	Unexposed	Exposed						
Tail Length	41.85 ± 1.97	60.00 ± 2.31*						
Short-term Exposure								
Lin et al. (2013) China Cross-shift change Population: 62 plywood workers (17.7% female, mean age 34 year, 17.7% smokers) assessed in 2011. Outcome: Peripheral blood lymphocytes, change over 8-hr shift; Comet assay, olive tail moment, alkaline conditions (pH = 13), blinded analysis, 50 cells/ subject.	Exposure assessed by air sampling and job function. Mean exposure: 0.27 ± 0.20 mg/m ³ Range: 0.012–0.67 mg/m ³	Comet assay results before and after work-shift <table><tr><td></td><td>Before exposure (n=60)</td><td>After exposure (n= 62)</td></tr><tr><td>Ln-transformed Tail moment</td><td>1.47 ± 0.72</td><td>2.30 ± 1.28*</td></tr></table> * <i>p</i> = < 0.001, paired <i>t</i>-test Regression coefficients for formaldehyde level, before shift -0.69 (–2.11, 0.73); after shift 3.64 (1.36, 5.92)		Before exposure (n=60)	After exposure (n= 62)	Ln-transformed Tail moment	1.47 ± 0.72	2.30 ± 1.28*
	Before exposure (n=60)	After exposure (n= 62)						
Ln-transformed Tail moment	1.47 ± 0.72	2.30 ± 1.28*						
Zeller et al. (2011) Germany Controlled human exposure study Subjects: 41 healthy volunteers exposed 4 hr/d for 5 d, all male, nonsmokers	12 groups of 2 to 4 persons in a chamber, exposures randomly assigned. Formaldehyde	Results of Comet assay in lymphocytes before and after 4-hr exposure (N = 37) <table><tr><td></td><td>Before exposure</td><td>After exposure</td></tr></table>		Before exposure	After exposure			
	Before exposure	After exposure						

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																																
Outcome: peripheral lymphocytes. Comet assay: alkaline conditions (pH 13). Analyzed 100 cells/ subject on coded slides.	concentrations: 0 (i.e., background level of 0.01 ppm); 0.3 ppm (0.37 mg/m ³) ^a with four peaks of 0.6 ppm (0.74 mg/m ³); 0.4 ppm (0.49 mg/m ³) with four peaks of 0.8 ppm (0.98 mg/m ³); 0.5 ppm (0.67 mg/m ³); and 0.7 ppm (0.86 mg/m ³) Peaks were 15 min each, Four 15-min exercise sessions during exposure.	<table><tr><td>Tail Moment</td><td>0.30 ± 0.117</td><td>0.33 ± 0.118</td></tr><tr><td>Tail Intensity</td><td>2.28 ± 0.492</td><td>2.66 ± 0.646*</td></tr></table> *p = 0.002, Wilcoxon signed rank test, compared to preexposure level.	Tail Moment	0.30 ± 0.117	0.33 ± 0.118	Tail Intensity	2.28 ± 0.492	2.66 ± 0.646*																										
Tail Moment	0.30 ± 0.117	0.33 ± 0.118																																
Tail Intensity	2.28 ± 0.492	2.66 ± 0.646*																																
DNA Adducts																																		
Bono et al. (2010) Italy (Prevalence study) Population: 20 pathologists from 3 pathology wards who worked in tissue fixation rooms (production rooms) and 20 students and workers from a university's science labs Outcome: M ₁ dG adducts in DNA extracted from whole blood, methods described in van Helden et al. (2009) ; compared mean log-transformed M ₁ dG adducts by exposure tertile or exposure status, using ANCOVA adjusting for sex, age, smoking	Personal sampling over an 8-hr shift in each subject; LOD 0.05 µg/m ³ ; questionnaire data on job-specific work (work in production room where slides were fixed or other areas) & use of personal protection Mean formaldehyde in production room 0.212 ± 0.047 mg/m ³ , other areas 0.0324 ± 0.0061 mg/m ³ , referents 0.028 ± 0.0025 mg/m ³	<table><tr><td colspan="4">Mean levels M₁dG adducts per 10⁸ NNs by exposure group</td></tr><tr><td></td><td>N</td><td>Mean ± SE</td><td>p-Value</td></tr><tr><td>Referent</td><td>20</td><td>2.4 ± 0.3</td><td></td></tr><tr><td>Exposed</td><td>20</td><td>5.7 ± 1.3</td><td>0.045¹</td></tr><tr><td colspan="4">8-hr TWA</td></tr><tr><td><22 µg/m³</td><td>13</td><td>2.3 ± 0.44</td><td></td></tr><tr><td>23–66 µg/m³</td><td>13</td><td>2.7 ± 0.55</td><td>0.775</td></tr><tr><td>>66 µg/m³</td><td>13</td><td>7.3 ± 1.9</td><td>0.018²</td></tr></table> ¹ compared to referent. ² compared to <22 µg/m ³ .	Mean levels M ₁ dG adducts per 10 ⁸ NNs by exposure group					N	Mean ± SE	p-Value	Referent	20	2.4 ± 0.3		Exposed	20	5.7 ± 1.3	0.045 ¹	8-hr TWA				<22 µg/m ³	13	2.3 ± 0.44		23–66 µg/m ³	13	2.7 ± 0.55	0.775	>66 µg/m ³	13	7.3 ± 1.9	0.018 ²
Mean levels M ₁ dG adducts per 10 ⁸ NNs by exposure group																																		
	N	Mean ± SE	p-Value																															
Referent	20	2.4 ± 0.3																																
Exposed	20	5.7 ± 1.3	0.045 ¹																															
8-hr TWA																																		
<22 µg/m ³	13	2.3 ± 0.44																																
23–66 µg/m ³	13	2.7 ± 0.55	0.775																															
>66 µg/m ³	13	7.3 ± 1.9	0.018 ²																															
DNA-Protein Crosslinks																																		
Prevalence Studies																																		
Lin et al. (2013) China (Prevalence)	Exposure categories by air monitoring and job assignment.	DPX levels in peripheral blood lymphocytes by formaldehyde exposure and years of employment DPX by Formaldehyde Level																																

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results			
Population: 96 plywood workers exposed to formaldehyde (13.5% female, mean age 33 year, 30.2% smokers) compared to referent group (N=82) (4% female, mean age 31 year, 40% smokers). Outcome: Peripheral blood lymphocytes: DNA-protein cross-links (DPX), KCl- SDS assay. blinded analysis	Average concentration: High exposure, N=38 (making glue): 1.48 mg/m ³ (range 0.914–2.044) Low exposure, N=58 (sanding boards, pressing wood scraps with glue at high temp): 0.68 mg/m ³ (range 0.455–0.792) Referent group, N=82 (providing & grinding wood scraps): 0.13 mg/m ³ (range 0.019–0.252) Exposure duration: 2.52 years	Referent	Low	High	
		DPX	22.73 ±	22.53 ±	20.37 ±
		(%)	21.47	22.26	20.52
		*ANOVA <i>p</i> -value = 0.894; trend <i>p</i> -value = 0.682, adjusted for age, gender, smoking status, alcohol use and duration of employment			
		DPX by Number of Work Years			
		<1 (N= 57)	1–3 (N= 64)	>3 (N= 57)	
		DPX	19.34 ±	22.10 ±	25.06 ±
		(%)	20.77	20.98	20.57
		ANOVA, ^a <i>p</i> -value = 0.577; ^b trend <i>p</i> -value = 0.376. ^a adjusted for age, gender, smoking status, alcohol use, formaldehyde exposure levels ^b Calculated using linear regression models with adjustment for age, gender, smoking status, alcohol use and formaldehyde exposure levels.			
Shaham et al. (2003) Israel Prevalence study Population: 186 workers from 14 hospital pathology departments (mean age 45.8 year, 68.3% female, 36.6% smokers) compared to 213 administrative workers from the same hospitals (mean age 42.1 year, 40.4% female, 44.6% smokers). Age distribution, gender, origin (ethnicity), and years of education differed significantly between the groups but were adjusted for in the analysis. Outcome: peripheral blood lymphocytes. Mean percent DPX of total DNA in quantity white blood cells, K-SDS method, double blinded.	Field and personal air sampling, sample duration 15 min, multiple times during work-day (# not reported). Concentration Low exposure: 0.49 (range 0.049–0.86) mg/m ³ High exposure: 2.8 (range 0.89–6.9) mg/m ³ Duration: Mean: 15.9 years Range: 1–51 years	Comparison of DNA-protein crosslinks by exposure			
		Referent	Exposed		
		Mean DPX/total DNA ± SE	0.14 ± 0.006	0.21 ± 0.006**	
		** <i>p</i> <0.01, adjusted for age, gender, smoking, education and region of origin.			
		Mean frequency DNA-protein crosslinks by level of exposure			
		Referent	Low	High	
		Mean DPX/ total DNA ¹	0.14	0.19	0.20
		¹ SE was not provided. Trend by exposure level was not statistically significant.			
Shaham et al. (1997) Israel Prevalence study	Field and personal air sampling, sample duration	Frequency of DPX by Exposure			
		Unexposed	Exposed		

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																				
Population: 12 pathology workers (mean age 44 year) compared to 8 age-matched controls (mean age 41 year). Outcome: Mean percent DPX, K-SDS method, double blinded Related references: Shaham et al. (1996)	15 min, multiple times during work-day (# not reported). Concentration: Mean: NR Range: 3.4–3.8 mg/m ³ Exposure duration mean 13 years (range 2–31 years)	<table><tr><td>Mean DPX %</td><td>23 ± 7</td><td>29 ± 6*</td></tr></table> *<i>p</i> = 0.03, ANOVA adjusting for smoking status. Years of exposure linearly correlated with DPX levels.	Mean DPX %	23 ± 7	29 ± 6*																	
Mean DPX %	23 ± 7	29 ± 6*																				
Short-term Studies																						
Lin et al. (2013) China Cross-shift change Population: 62 plywood workers (17.7% female, mean age 34 year, 17.7% smokers) assessed in 2011. Outcome: Blood lymphocytes: % cross links measured before and after 8-hr shift, blinded analysis.	Air sampling and job function. Mean exposure: 0.27 ± 0.20 mg/m ³ Range: 0.012–0.67 mg/m ³	DPX frequency before and after work-shift <table><tr><td></td><td>Before exposure (<i>n</i>= 62)</td><td>After exposure (<i>n</i>= 60)</td></tr><tr><td>DPX (%)</td><td>27.22 ± 10.07</td><td>31.68 ± 14.19*</td></tr></table> * <i>p</i> = 0.019, paired <i>t</i>-test. Regression coefficients for formaldehyde level, before shift 1.70 (–17.84, 21.24); after shift –6.04 (–31.23, 19.15).		Before exposure (<i>n</i> = 62)	After exposure (<i>n</i> = 60)	DPX (%)	27.22 ± 10.07	31.68 ± 14.19*														
	Before exposure (<i>n</i> = 62)	After exposure (<i>n</i> = 60)																				
DPX (%)	27.22 ± 10.07	31.68 ± 14.19*																				
DNA Repair																						
Schlink et al. (1999) Germany Population: Anatomy students, Group 1, 41 students from one university course, 3-hr labs, 2 times per wk (43.9% female, ages 21–30 year, 39% smokers); Group 2, 16 students from a different university course (50% female, ages 21–27 year, 37.5% smokers), and Referent, 10 unexposed students (60% female, ages 22–44 year, 30% smokers); no previous formaldehyde exposure Outcome: O ⁶ -alkylguanine DNA alkyl-transferase activity in peripheral blood lymphocytes (modification of Klein and Oesch (1990)), expressed as fmol MGMT/ 10 ⁶ cells (LOD 1 fmol MGMT/ 10 ⁶ cells), blind to period of sample (before or after); Blood samples collected before 1 st class and after days 50 and 111	Personal sampling near breathing zone once per week, sampling period not reported. formaldehyde exposed, Mean ± SD, 0.2 ± 0.05 mg/m ³ , 0.14–0.3 mg/m ³	MGMT activity change compared (U-test, paired data) before and after exposure; as well as between exposure groups (Wilcoxon, Mann and Whitney U-test) Mean MGMT activity by exposure group (fmol MGMT/ 10 ⁶ cells) <table><tr><td></td><td>N</td><td>Day 0</td><td>Day 50</td><td>Day > 90</td></tr><tr><td>Group 1</td><td>41</td><td>133.2</td><td>131.1¹</td><td>128.2¹</td></tr><tr><td>Group 2</td><td>16</td><td></td><td></td><td>146.9²</td></tr><tr><td>Referen</td><td>10</td><td>138.9</td><td></td><td></td></tr></table> ¹<i>p</i> >0.05 compared to Day 0. ²<i>p</i> >0.05 compared to referent.		N	Day 0	Day 50	Day > 90	Group 1	41	133.2	131.1 ¹	128.2 ¹	Group 2	16			146.9 ²	Referen	10	138.9		
	N	Day 0	Day 50	Day > 90																		
Group 1	41	133.2	131.1 ¹	128.2 ¹																		
Group 2	16			146.9 ²																		
Referen	10	138.9																				

Reference and study design	Exposure	Results												
		MGMT activity did not differ by gender, smoking, allergy status, or alcohol consumption.												
Hayes et al. (1997) USA Panel study Population: 29 students (with adequate samples) exposed to formaldehyde for 9 wks during embalming course 16 male, 7 females, 6 smokers. Mean duration of embalming 125 min. 15 with previous embalming exposure within previous 90 da Outcome: O ⁶ -alkylguanine DNA alkyltransferase activity in peripheral lymphocytes, expressed as pmol AGT/ mg protein (LOD 0.006 pmol AGT/ mg protein), blind to period of sample (before or after); blood samples collected in morning before 1 st class and after 9 wks Related reference: Suruda et al. (1993)	Personal sampling for 121 of 144 embalmings; Exposure concentration: Mean: 1.72 mg/m ³ Range: (0.18–5.29) mg/m ³ Duration: 9 wks (0.173 years) Total number of reported embalmings correlated with estimated cumulative formaldehyde exposure (<i>r</i> = 0.59, <i>p</i> < 0.01).	Individual data pre- and postcourse AGT activity in peripheral blood lymphocytes depicted in graphs by embalming experience during previous 90 d (yes/ no), decreased in 17 students, increased in 6 students (ANOVA adjusting for age, sex and smoking, <i>p</i> < 0.05).												
P53 protein levels in blood														
Attia et al. (2014) Egypt Prevalence study Population: 40 employees at cosmetic manufacturing company (23% male, mean age 25.8 years, 20% smokers) randomly selected, compared to referent (<i>N</i> =20) selected from hospital administrative department with comparable SES & no history of occupational exposure to formaldehyde (35% male, mean age 34 years, 15% smokers) Outcome: Peripheral blood; plasma MDA (commercial kit), plasma p53 (p53 enzyme-linked immunosorbent assay kit). Blinding not stated. Statistical analyses of coded data (blinded assumed). Exposed compared to referent, means (Student's <i>t</i> -test), correlation between urinary formate and MDA or p53 using linear regression	Urine formic acid according to Hopner & Knappe, 1974; unclear how to relate urine formic acid levels to air concentrations Urinary formate Exposed: 53.4 ± 15.01 mg/L Referent: 12.7 ± 4.57 mg/L <i>P</i> <0.05	Comparison of plasma p53 and plasma MDA concentrations in exposed and referent groups <table><thead><tr><th></th><th>Referent</th><th>Exposed</th><th><i>p</i>-Value</th></tr></thead><tbody><tr><td>Plasma p53 (U/mL)</td><td>2.78 ± 0.48</td><td>13.34 ± 4.67</td><td><0.05</td></tr><tr><td>Plasma MDA (nmol/ml)</td><td>3.59 ± 0.83</td><td>9.73 ± 2.72</td><td><0.05</td></tr></tbody></table> Correlations in exposed group: Urinary formate & p53, <i>r</i> =0.91 <i>p</i> <0.001 Urinary formate & MDA, <i>r</i> =0.79, <i>p</i> <0.001 Plasma MDA & plasma p53, <i>r</i> =0.81, <i>p</i> <0.001 Age and gender were not associated with plasma p53, plasma MDA or urinary formate.		Referent	Exposed	<i>p</i> -Value	Plasma p53 (U/mL)	2.78 ± 0.48	13.34 ± 4.67	<0.05	Plasma MDA (nmol/ml)	3.59 ± 0.83	9.73 ± 2.72	<0.05
	Referent	Exposed	<i>p</i> -Value											
Plasma p53 (U/mL)	2.78 ± 0.48	13.34 ± 4.67	<0.05											
Plasma MDA (nmol/ml)	3.59 ± 0.83	9.73 ± 2.72	<0.05											

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																																
Shaham et al. (2003) Israel Prevalence study Population: 186 workers from 14 hospital pathology departments (mean age 42.1 year, 59.6% male, 36.6% smokers) compared to 213 administrative workers from the same hospitals (mean age 45.8 year, 31.7% male, 44.6% smokers). Age distribution, gender, origin (ethnicity), and years of education differed significantly between the groups but were adjusted for in the analysis. Outcome: p53 proteins (wild type and mutant) in serum, p53 quantitative ELISA kit immunoassay, mutant p53 in serum using quantitative ELISA kit immunoassay. Categorical analysis of p53 levels (>pg/mL), exposure groups compared using chi-square test; logistic regression of p53 >150 pg/mL	Field and personal air sampling, sample duration 15 min, multiple times during work-day (# not reported). Concentration Low exposure: 0.49 (range 0.049–0.86) mg/m ³ High exposure: 2.8 (range 0.89–6.9) mg/m ³ Duration: Mean: 15.9 years Range: (1–51) years	Comparisons of exposure, serum total p53, serum mutant p53 and DPXs (OR, 95% CI) <table><thead><tr><th></th><th>Total</th><th>Male</th><th>Female</th></tr></thead><tbody><tr><td colspan="4">Total p53 protein > 150 pg/mL^a</td></tr><tr><td>Referent</td><td>1.0</td><td>1.0</td><td>1.0</td></tr><tr><td>Exposed</td><td>1.6 (0.8–3.1)</td><td>2.0 (0.9–4.4)</td><td>0.8 (0.2–2.7)</td></tr><tr><td colspan="4">Total p53 protein > 150 pg/mL^b</td></tr><tr><td>DPX ≤ 0.187^b</td><td>1.0</td><td>1.0</td><td>1.0</td></tr><tr><td>DPX > 0.187</td><td>2.5 (1.2–5.4)</td><td>1.9 (0.5–7.2)</td><td>2.8 (1.1–7.1)</td></tr></tbody></table> ^a Logistic regression models adjusted for sex, age and smoking. ^b In the exposed group, logistic regression models adjusted for sex, age and smoking. ^b DPX expressed as % of total DNA. Correlations: Total p53 protein and mutant p53 protein, <i>r</i> =0.75, <i>p</i> <0.01 Proportion p53 > 150 pg/mL among exposed <table><tbody><tr><td>DPX ≤ 0.187</td><td>33.3%</td></tr><tr><td>DPX > 0.187</td><td>55.7% (<i>p</i> <0.01)</td></tr></tbody></table>		Total	Male	Female	Total p53 protein > 150 pg/mL ^a				Referent	1.0	1.0	1.0	Exposed	1.6 (0.8–3.1)	2.0 (0.9–4.4)	0.8 (0.2–2.7)	Total p53 protein > 150 pg/mL ^b				DPX ≤ 0.187 ^b	1.0	1.0	1.0	DPX > 0.187	2.5 (1.2–5.4)	1.9 (0.5–7.2)	2.8 (1.1–7.1)	DPX ≤ 0.187	33.3%	DPX > 0.187	55.7% (<i>p</i> <0.01)
	Total	Male	Female																															
Total p53 protein > 150 pg/mL ^a																																		
Referent	1.0	1.0	1.0																															
Exposed	1.6 (0.8–3.1)	2.0 (0.9–4.4)	0.8 (0.2–2.7)																															
Total p53 protein > 150 pg/mL ^b																																		
DPX ≤ 0.187 ^b	1.0	1.0	1.0																															
DPX > 0.187	2.5 (1.2–5.4)	1.9 (0.5–7.2)	2.8 (1.1–7.1)																															
DPX ≤ 0.187	33.3%																																	
DPX > 0.187	55.7% (<i>p</i> <0.01)																																	
Genetic Susceptibility																																		
Costa et al. (2015) ; Costa et al. (2019) Portugal Prevalence study Population: 84 anatomy pathology workers from 9 hospital laboratories, exposed to formaldehyde for at least 1 year, compared to 87 non-exposed employees from administrative offices in same geographic area. Exclusions: cancer history, radiation therapy or chemotherapy, surgery with anesthesia or blood transfusion in last year. Exposed and referent similar for mean age 39 years, 77% females,	Exposure assessed via air sampling and deriving an 8-hr TWA for each subject. Exposure concentration: Mean: 0.38 ppm (0.47 mg/m ³) Range: 0.28–0.85 ppm (0.34–1.05 mg/m ³)	Effect modification by genetic polymorphisms on associations of formaldehyde with markers of genotoxicity (mean ratio, 95% CI) <table><thead><tr><th></th><th colspan="2">Referent</th><th colspan="2">Exposed</th></tr><tr><th></th><th>N</th><th>MR (95% CI)</th><th>N</th><th>MR (95% CI)</th></tr></thead><tbody><tr><td colspan="5">CYP2E1 rs6413432 (% tDNA)</td></tr><tr><td>T/T</td><td>53</td><td>1.00</td><td>51</td><td>1.61 (1.20–2.16)</td></tr><tr><td>T/A + A/A</td><td>15</td><td>0.84 (0.54–1.30)</td><td>7</td><td>0.42 (0.20–0.89)</td></tr></tbody></table>		Referent		Exposed			N	MR (95% CI)	N	MR (95% CI)	CYP2E1 rs6413432 (% tDNA)					T/T	53	1.00	51	1.61 (1.20–2.16)	T/A + A/A	15	0.84 (0.54–1.30)	7	0.42 (0.20–0.89)							
	Referent		Exposed																															
	N	MR (95% CI)	N	MR (95% CI)																														
CYP2E1 rs6413432 (% tDNA)																																		
T/T	53	1.00	51	1.61 (1.20–2.16)																														
T/A + A/A	15	0.84 (0.54–1.30)	7	0.42 (0.20–0.89)																														

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																																															
25% smokers. Outcome: Peripheral blood samples, coded, analyses blinded to exposure status. Differences in genotype distribution evaluated using Pearson’s chi-square test, effect modification by genotype in regression models of exposure on ln % tDNA (comet assay) and chromosome aberrations, CYP2E1 rs6413432, GSTM1 deletion, GSTT1 deletion, GSTP1 rs1695, XRCC1 rs1799782, XRCC1 rs25487, PARP1 rs1136410, MUTYH rs3219489, XRCC3 rs861539	Exposure duration 12.0 ± 8.2 years	GSTP1 rs1695 (CSAs)																																															
		Ile/Ile	32	1.00	37	5.43 (2.04–14.46)																																											
		Ile/Val + Val/Val	55	1.79 (1.14–7.94)	47	0.26 (0.97–3.27)																																											
		XRCC1 rs1799782 (% tDNA)																																															
		Arg/Arg	67	1.00	53	1.46 (1.10-1.93)																																											
		Arg/Trp	2	0.19 (0.06–0.57)	6	4.93 (1.33–18.32)																																											
		PARP1 rs1136410 (Multiaberrant cells)																																															
		Val/Val	60	1.00	50	5.97 (2.34–15.25)																																											
		Val/Ala	8	3.00 (0.55–16.4)	9	0.09 (0.01–0.95)																																											
		Regression models adjusted for age, gender, smoking habit, and fruit consumption.																																															
Micronuclei frequency (%/1,000 cells) by genetic polymorphisms in formaldehyde exposed and unexposed workers																																																	
<table><tr><td></td><td colspan="2">Controls</td><td colspan="2">Exposed</td></tr><tr><td>Gene site</td><td>N</td><td>Mean ± SE</td><td>N</td><td>Mean (SE)</td></tr><tr><td colspan="5">CYP2E1 rs6413432</td></tr><tr><td colspan="5">BNbud</td></tr><tr><td>T/T</td><td>53</td><td>0.36 ± 0.077</td><td>51</td><td>0.80 ± 0.12</td></tr><tr><td>T/A + A/A</td><td>15</td><td>0.20 ± 0.11</td><td>7</td><td>1.57 ± 0.20*</td></tr><tr><td colspan="5">GSTP1 rs1695</td></tr><tr><td colspan="5">MNB</td></tr><tr><td>Ile/Ile</td><td>28</td><td>0.14 ± 0.07</td><td>29</td><td>0.45 ± 0.11</td></tr></table>						Controls		Exposed		Gene site	N	Mean ± SE	N	Mean (SE)	CYP2E1 rs6413432					BNbud					T/T	53	0.36 ± 0.077	51	0.80 ± 0.12	T/A + A/A	15	0.20 ± 0.11	7	1.57 ± 0.20*	GSTP1 rs1695					MNB					Ile/Ile	28	0.14 ± 0.07	29	0.45 ± 0.11
	Controls		Exposed																																														
Gene site	N	Mean ± SE	N	Mean (SE)																																													
CYP2E1 rs6413432																																																	
BNbud																																																	
T/T	53	0.36 ± 0.077	51	0.80 ± 0.12																																													
T/A + A/A	15	0.20 ± 0.11	7	1.57 ± 0.20*																																													
GSTP1 rs1695																																																	
MNB																																																	
Ile/Ile	28	0.14 ± 0.07	29	0.45 ± 0.11																																													

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																																																																																																									
		<table><tr><td>Ile/Val +</td><td>41</td><td>0.20 ± 0.07</td><td>33</td><td>0.82 ± 0.15*</td></tr><tr><td>Val/Val</td><td></td><td></td><td></td><td></td></tr><tr><td>FANCA rs7190823</td><td></td><td></td><td></td><td></td></tr><tr><td>MNL</td><td></td><td></td><td></td><td></td></tr><tr><td>Thr/Thr</td><td>9</td><td>2.33 ± 0.93</td><td>12</td><td>2.33 ± 0.57</td></tr><tr><td>Thr/Ala +</td><td>77</td><td>2.84 ± 0.32</td><td>70</td><td>4.74 ± 0.44*</td></tr><tr><td>Ala/Ala</td><td></td><td></td><td></td><td></td></tr><tr><td colspan="5">* <i>p</i>-values CYP2E1 rs6413432 A variant, 0.022; GSTP1 rs1695 Val variant 0.05; FANCA rs7190823 Ala variant 0.019</td></tr></table>	Ile/Val +	41	0.20 ± 0.07	33	0.82 ± 0.15*	Val/Val					FANCA rs7190823					MNL					Thr/Thr	9	2.33 ± 0.93	12	2.33 ± 0.57	Thr/Ala +	77	2.84 ± 0.32	70	4.74 ± 0.44*	Ala/Ala					* <i>p</i> -values CYP2E1 rs6413432 A variant, 0.022; GSTP1 rs1695 Val variant 0.05; FANCA rs7190823 Ala variant 0.019																																																																					
Ile/Val +	41	0.20 ± 0.07	33	0.82 ± 0.15*																																																																																																							
Val/Val																																																																																																											
FANCA rs7190823																																																																																																											
MNL																																																																																																											
Thr/Thr	9	2.33 ± 0.93	12	2.33 ± 0.57																																																																																																							
Thr/Ala +	77	2.84 ± 0.32	70	4.74 ± 0.44*																																																																																																							
Ala/Ala																																																																																																											
* <i>p</i> -values CYP2E1 rs6413432 A variant, 0.022; GSTP1 rs1695 Val variant 0.05; FANCA rs7190823 Ala variant 0.019																																																																																																											
Ladeira et al. (2013) Portugal Prevalence study Population: 54 hospital workers in histopathology labs compared to 82 administrative staff. Outcome: Genotyping XRCC3 Met241Thr, ADH5 Val309Ile, ADH5 Asp353Glu; associations of polymorphism with mean micronuclei, nucleoplasmic bridges and nuclear buds in lymphocytes and buccal cells within exposed and referent groups, Kruskal-Wallis test Related references: Ladeira et al. (2011)	Personal air sampling, 6-8 hours, estimated 8-hr TWA Exposure conc.: Mean TWA 8 hr 0.2 ± 0.14 mg/m ³ Mean ceiling value: 1.4 ± 0.91 mg/m ³ , range 0.22–3.6 mg/m ³ Exposure duration: 14.5 (1–33) years	<table><tr><td colspan="5">Frequency of micronuclei and nuclear buds (mean ± SE) in lymphocytes by exposure and genotype (number in parentheses)</td></tr><tr><td>Endpoint</td><td colspan="4">Genotypes</td></tr><tr><td>MN</td><td colspan="4">XRCC3</td></tr><tr><td></td><td>Met/Met</td><td>Thr/Met</td><td colspan="2">Thr/Thr</td></tr><tr><td>Exposed</td><td>2.92 ± 0.93</td><td>5.05 ± 0.98</td><td colspan="2">3.53 ± 0.80</td></tr><tr><td>(<i>p</i>=0.37)</td><td>(13)</td><td>(22)</td><td colspan="2">(19)</td></tr><tr><td>Referent</td><td>1.15 ± 0.46</td><td>0.70 ± 0.30</td><td colspan="2">0.74 ± 0.23</td></tr><tr><td>(<i>p</i>=0.62)</td><td>(20)</td><td>(27)</td><td colspan="2">(35)</td></tr><tr><td></td><td colspan="4">ADH5</td></tr><tr><td></td><td>Val/Val</td><td colspan="3">Val/Ile</td></tr><tr><td>Exposed</td><td>2.57 ± 0.65</td><td colspan="3">4.91 ± 0.75</td></tr><tr><td>(<i>p</i>=0.02)</td><td>(21)</td><td colspan="3">(33)</td></tr><tr><td>Referent</td><td>0.97 ± 0.28</td><td colspan="3">0.75 ± 0.23</td></tr><tr><td>(<i>p</i>=0.17)</td><td>(29)</td><td colspan="3">(53)</td></tr><tr><td></td><td colspan="4">ADH5</td></tr><tr><td></td><td>Asp/Asp</td><td colspan="3">Asp/Glu</td></tr><tr><td>Exposed</td><td>4.08 ± 0.91</td><td colspan="3">3.93 ± 0.67</td></tr><tr><td>(<i>p</i>=0.70)</td><td>(24)</td><td colspan="3">(30)</td></tr><tr><td>Referent</td><td>0.86 ± 0.23</td><td colspan="3">0.81 ± 0.26</td></tr><tr><td>(<i>p</i>=0.21)</td><td>(35)</td><td colspan="3">(47)</td></tr><tr><td colspan="5">NBUD</td></tr></table>	Frequency of micronuclei and nuclear buds (mean ± SE) in lymphocytes by exposure and genotype (number in parentheses)					Endpoint	Genotypes				MN	XRCC3					Met/Met	Thr/Met	Thr/Thr		Exposed	2.92 ± 0.93	5.05 ± 0.98	3.53 ± 0.80		(<i>p</i> =0.37)	(13)	(22)	(19)		Referent	1.15 ± 0.46	0.70 ± 0.30	0.74 ± 0.23		(<i>p</i> =0.62)	(20)	(27)	(35)			ADH5					Val/Val	Val/Ile			Exposed	2.57 ± 0.65	4.91 ± 0.75			(<i>p</i> =0.02)	(21)	(33)			Referent	0.97 ± 0.28	0.75 ± 0.23			(<i>p</i> =0.17)	(29)	(53)				ADH5					Asp/Asp	Asp/Glu			Exposed	4.08 ± 0.91	3.93 ± 0.67			(<i>p</i> =0.70)	(24)	(30)			Referent	0.86 ± 0.23	0.81 ± 0.26			(<i>p</i> =0.21)	(35)	(47)			NBUD				
Frequency of micronuclei and nuclear buds (mean ± SE) in lymphocytes by exposure and genotype (number in parentheses)																																																																																																											
Endpoint	Genotypes																																																																																																										
MN	XRCC3																																																																																																										
	Met/Met	Thr/Met	Thr/Thr																																																																																																								
Exposed	2.92 ± 0.93	5.05 ± 0.98	3.53 ± 0.80																																																																																																								
(<i>p</i> =0.37)	(13)	(22)	(19)																																																																																																								
Referent	1.15 ± 0.46	0.70 ± 0.30	0.74 ± 0.23																																																																																																								
(<i>p</i> =0.62)	(20)	(27)	(35)																																																																																																								
	ADH5																																																																																																										
	Val/Val	Val/Ile																																																																																																									
Exposed	2.57 ± 0.65	4.91 ± 0.75																																																																																																									
(<i>p</i> =0.02)	(21)	(33)																																																																																																									
Referent	0.97 ± 0.28	0.75 ± 0.23																																																																																																									
(<i>p</i> =0.17)	(29)	(53)																																																																																																									
	ADH5																																																																																																										
	Asp/Asp	Asp/Glu																																																																																																									
Exposed	4.08 ± 0.91	3.93 ± 0.67																																																																																																									
(<i>p</i> =0.70)	(24)	(30)																																																																																																									
Referent	0.86 ± 0.23	0.81 ± 0.26																																																																																																									
(<i>p</i> =0.21)	(35)	(47)																																																																																																									
NBUD																																																																																																											

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																																				
		<table> <tr> <th colspan="3">XRCC3</th></tr> <tr> <th></th><th>Met/Met</th><th>Thr/Met Thr/Thr</th></tr> <tr> <td>Exposed (<i>p</i>=0.00)</td><td>0.38 ± 0.18 (13)</td><td>1.5 ± 0.33 (22) 0.21 ± 0.12 (19)</td></tr> <tr> <td>Referent (<i>p</i>=0.04)</td><td>0.2 ± 0.09 (20)</td><td>0.04 ± 0.04 (27) 0.03 ± 0.29 (35)</td></tr> <tr> <th colspan="3">ADH5</th></tr> <tr> <th></th><th>Val/Val</th><th>Val/Ile</th></tr> <tr> <td>Exposed (<i>p</i>=0.27)</td><td>0.62 ± 0.28 (21)</td><td>0.88 ± 0.21 (33)</td></tr> <tr> <td>Referent (<i>p</i>=0.06)</td><td>0.00 ± 0.0 (29)</td><td>0.11 ± 0.04 (53)</td></tr> <tr> <th colspan="3">ADH5</th></tr> <tr> <th></th><th>Asp/Asp</th><th>Asp/Glu</th></tr> <tr> <td>Exposed (<i>p</i>=0.74)</td><td>0.71 ± 0.23 (24)</td><td>0.83 ± 0.25 (30)</td></tr> <tr> <td>Referent (<i>p</i>=0.63)</td><td>0.06 ± 0.04 (35)</td><td>0.09 ± 0.04 (47)</td></tr> </table> No differences noted for nucleoplasmic bridges or micronuclei in buccal cells (data provided in article)	XRCC3				Met/Met	Thr/Met Thr/Thr	Exposed (<i>p</i> =0.00)	0.38 ± 0.18 (13)	1.5 ± 0.33 (22) 0.21 ± 0.12 (19)	Referent (<i>p</i> =0.04)	0.2 ± 0.09 (20)	0.04 ± 0.04 (27) 0.03 ± 0.29 (35)	ADH5				Val/Val	Val/Ile	Exposed (<i>p</i> =0.27)	0.62 ± 0.28 (21)	0.88 ± 0.21 (33)	Referent (<i>p</i> =0.06)	0.00 ± 0.0 (29)	0.11 ± 0.04 (53)	ADH5				Asp/Asp	Asp/Glu	Exposed (<i>p</i> =0.74)	0.71 ± 0.23 (24)	0.83 ± 0.25 (30)	Referent (<i>p</i> =0.63)	0.06 ± 0.04 (35)	0.09 ± 0.04 (47)
XRCC3																																						
	Met/Met	Thr/Met Thr/Thr																																				
Exposed (<i>p</i> =0.00)	0.38 ± 0.18 (13)	1.5 ± 0.33 (22) 0.21 ± 0.12 (19)																																				
Referent (<i>p</i> =0.04)	0.2 ± 0.09 (20)	0.04 ± 0.04 (27) 0.03 ± 0.29 (35)																																				
ADH5																																						
	Val/Val	Val/Ile																																				
Exposed (<i>p</i> =0.27)	0.62 ± 0.28 (21)	0.88 ± 0.21 (33)																																				
Referent (<i>p</i> =0.06)	0.00 ± 0.0 (29)	0.11 ± 0.04 (53)																																				
ADH5																																						
	Asp/Asp	Asp/Glu																																				
Exposed (<i>p</i> =0.74)	0.71 ± 0.23 (24)	0.83 ± 0.25 (30)																																				
Referent (<i>p</i> =0.63)	0.06 ± 0.04 (35)	0.09 ± 0.04 (47)																																				
Santovito et al. (2011) Italy Prevalence study Population: 20 pathology workers (mean age 45.7 year) compared to 16 workers from the same hospital (mean age 42.1 year); similar age and gender distribution. All subjects were non-smokers and had not consumed alcohol in 1 year. Outcome: Genotypes GSTT, GSTM; associations of polymorphisms with CA per cell and % of cells with aberrations within exposed and referent groups; generalized linear models with Poisson distribution errors adjusted for gender and age	Exposure conc: Personal air sampling, 8-hr duration. Referent: Mean: 0.036 ± 0.002 mg/m ³ Pathologists: Mean: 0.073 ± 0.013 mg/m ³ Exposure duration: Mean: 13 years Range: 2–27 years	Frequency of chromosomal aberrations per cell (mean ± SE) in lymphocytes by exposure and genotype (number in parentheses) <table> <tr> <th></th><th>Exposed</th><th>Referent</th></tr> <tr> <td>GSTT-pos</td><td>0.028 ± 0.003 (16)</td><td>0.01 ± 0.004 (12)</td></tr> <tr> <td>GSTT-null</td><td>0.04 ± 0.015 (4)</td><td>0.013 ± 0.009 (4)</td></tr> <tr> <td>GSTM-pos</td><td>0.031 ± 0.004 (17)</td><td>0.01 ± 0.004 (10)</td></tr> <tr> <td>GSTM-null</td><td>0.023 ± 0.003 (3)</td><td>0.012 ± 0.008 (6)</td></tr> </table> No differences also were found for the % of cells with chromosomal aberrations (data provided in article).		Exposed	Referent	GSTT-pos	0.028 ± 0.003 (16)	0.01 ± 0.004 (12)	GSTT-null	0.04 ± 0.015 (4)	0.013 ± 0.009 (4)	GSTM-pos	0.031 ± 0.004 (17)	0.01 ± 0.004 (10)	GSTM-null	0.023 ± 0.003 (3)	0.012 ± 0.008 (6)																					
	Exposed	Referent																																				
GSTT-pos	0.028 ± 0.003 (16)	0.01 ± 0.004 (12)																																				
GSTT-null	0.04 ± 0.015 (4)	0.013 ± 0.009 (4)																																				
GSTM-pos	0.031 ± 0.004 (17)	0.01 ± 0.004 (10)																																				
GSTM-null	0.023 ± 0.003 (3)	0.012 ± 0.008 (6)																																				

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results																																																						
Jiang et al. (2010) China Prevalence Population: 151 male workers from 2 plywood plants (mean age 27.4 year, 52.3% smokers) compared to 112 unexposed workers at a machine manufacturer in same town (mean age 28.7 year, 42.9% smokers). Outcome: genotypes GSTM1, GSTT1, GSTP1; associations with olive TM and CBMN frequency within exposed and referent; ANCOVA adjusted for age, smoking and alcohol	Exposure assessed by job title and personal air monitoring. Exposure concentration ppm converted to mg/m³ by EPA. 1.08 mg/m³, range 0.1–7.75 mg/m³ Duration: Mean 2.51 years Range: (0.5–25) years	Frequency of olive TM (geometric mean (95% CI) in lymphocytes by exposure and genotype (number in parentheses) <table> <tr> <th></th><th>Exposed</th><th>Referent</th></tr> <tr> <td>GSTM1-pos</td><td>3.27 (2.83–3.78) (74)</td><td>1.01 (0.77–1.32) (46)</td></tr> <tr> <td>GSTM1-null</td><td>3.86 (3.31–4.5) (77)</td><td>0.87 (0.69–1.1) (66)</td></tr> <tr> <td></td><td><i>P</i> = 0.07</td><td><i>P</i> = 0.43</td></tr> <tr> <td>GSTT1-pos</td><td>3.72 (3.26–4.25) (83)</td><td>1.04 (0.82–1.31) (63)</td></tr> <tr> <td>GSTT1-null</td><td>3.36 (2.83–3.99) (68)</td><td>0.8 (0.61–1.04) (49)</td></tr> <tr> <td></td><td><i>P</i> = 0.47</td><td><i>P</i> = 0.11</td></tr> <tr> <td>GSTP1-Ile/Ile</td><td>3.64 (3.19–4.16) (90)</td><td>0.96 (0.74–1.23) (58)</td></tr> <tr> <td>GSTP1-Val pos</td><td>3.43 (2.87–4.1) (61)</td><td>0.89 (0.7–1.14) (54)</td></tr> <tr> <td></td><td><i>P</i> = 0.49</td><td><i>P</i> = 0.83</td></tr> </table> Frequency of ln CBMN (mean ± SD) in lymphocytes by exposure and genotype (number in parentheses) <table> <tr> <th></th><th>Exposed</th><th>Referent</th></tr> <tr> <td>GSTM1-pos</td><td>5.57 ± 3.45 (74)</td><td>2.91 ± 1.5 (46)</td></tr> <tr> <td>GSTM1-null</td><td>5.5 ± 3.32 (77)</td><td>2.5 ± 1.15 (66)</td></tr> <tr> <td></td><td><i>P</i> = 0.84</td><td><i>P</i> = 0.18</td></tr> <tr> <td>GSTT1-pos</td><td>5.59 ± 3.51 (83)</td><td>2.75 ± 1.41 (63)</td></tr> <tr> <td>GSTT1-null</td><td>5.46 ± 3.22 (68)</td><td>2.57 ± 1.19 (49)</td></tr> <tr> <td></td><td><i>P</i> = 0.70</td><td><i>P</i> = 0.47</td></tr> <tr> <td>GSTP1-Ile/Ile</td><td>5.01 ± 2.98 (90)</td><td>2.79 ± 1.36 (58)</td></tr> </table>		Exposed	Referent	GSTM1-pos	3.27 (2.83–3.78) (74)	1.01 (0.77–1.32) (46)	GSTM1-null	3.86 (3.31–4.5) (77)	0.87 (0.69–1.1) (66)		<i>P</i> = 0.07	<i>P</i> = 0.43	GSTT1-pos	3.72 (3.26–4.25) (83)	1.04 (0.82–1.31) (63)	GSTT1-null	3.36 (2.83–3.99) (68)	0.8 (0.61–1.04) (49)		<i>P</i> = 0.47	<i>P</i> = 0.11	GSTP1-Ile/Ile	3.64 (3.19–4.16) (90)	0.96 (0.74–1.23) (58)	GSTP1-Val pos	3.43 (2.87–4.1) (61)	0.89 (0.7–1.14) (54)		<i>P</i> = 0.49	<i>P</i> = 0.83		Exposed	Referent	GSTM1-pos	5.57 ± 3.45 (74)	2.91 ± 1.5 (46)	GSTM1-null	5.5 ± 3.32 (77)	2.5 ± 1.15 (66)		<i>P</i> = 0.84	<i>P</i> = 0.18	GSTT1-pos	5.59 ± 3.51 (83)	2.75 ± 1.41 (63)	GSTT1-null	5.46 ± 3.22 (68)	2.57 ± 1.19 (49)		<i>P</i> = 0.70	<i>P</i> = 0.47	GSTP1-Ile/Ile	5.01 ± 2.98 (90)	2.79 ± 1.36 (58)
	Exposed	Referent																																																						
GSTM1-pos	3.27 (2.83–3.78) (74)	1.01 (0.77–1.32) (46)																																																						
GSTM1-null	3.86 (3.31–4.5) (77)	0.87 (0.69–1.1) (66)																																																						
	<i>P</i> = 0.07	<i>P</i> = 0.43																																																						
GSTT1-pos	3.72 (3.26–4.25) (83)	1.04 (0.82–1.31) (63)																																																						
GSTT1-null	3.36 (2.83–3.99) (68)	0.8 (0.61–1.04) (49)																																																						
	<i>P</i> = 0.47	<i>P</i> = 0.11																																																						
GSTP1-Ile/Ile	3.64 (3.19–4.16) (90)	0.96 (0.74–1.23) (58)																																																						
GSTP1-Val pos	3.43 (2.87–4.1) (61)	0.89 (0.7–1.14) (54)																																																						
	<i>P</i> = 0.49	<i>P</i> = 0.83																																																						
	Exposed	Referent																																																						
GSTM1-pos	5.57 ± 3.45 (74)	2.91 ± 1.5 (46)																																																						
GSTM1-null	5.5 ± 3.32 (77)	2.5 ± 1.15 (66)																																																						
	<i>P</i> = 0.84	<i>P</i> = 0.18																																																						
GSTT1-pos	5.59 ± 3.51 (83)	2.75 ± 1.41 (63)																																																						
GSTT1-null	5.46 ± 3.22 (68)	2.57 ± 1.19 (49)																																																						
	<i>P</i> = 0.70	<i>P</i> = 0.47																																																						
GSTP1-Ile/Ile	5.01 ± 2.98 (90)	2.79 ± 1.36 (58)																																																						

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Exposure	Results	
		GSTP1 Val pos	6.32 ± 3.78 (61) 2.54 ± 1.27 (54)
			$P = 0.05$ $P = 0.26$

ADH, alcohol dehydrogenase; AGT, O⁶-alkylguanine-DNA alkyltransferase; ANOVA, analysis of variance; C–, centromere negative; C+, centromere positive; CA, chromosomal aberration; CB-MN or CBMN, cytokinesis block-micronucleus; CFU-GM, colony forming unit-granulocyte/macrophage; CI, class interval; CSA, chromosome-type aberration; CSG, centromere separation general; CTA, chromatid-type aberration; DAPI, diamidinophenylindole; DPX, DNA-protein crosslink; EA, ethyl acetate; ELISA, enzyme-linked immunosorbent assay; FISH, fluorescence *in situ* hybridization; GST, glutathione S-transferase; HCHO, formaldehyde; HF, high frequency; IRR, incidence rate ratio; K-SDS/KCl-SDS, potassium chloride-sodium dodecyl sulfate; LOD, level of detection; LTR, lymphocyte transformation rate; M₁dG, malondialdehyde-deoxyguanosine; MAK, maximum permissible concentration (German); MDA, malondialdehyde; MGMT, O⁶-methylguanine methyl transferase; MN, micronucleus; MR, mean ratio; NSM, number of scored metaphases; OR, odds ratio; PARP, poly (ADP-ribose) polymerase; PCD, premature centrosome division; PI, proliferation index; SCE, sister chromatid exchange; SD, standard deviation; SE, standard error; SEM, standard error of the mean; tDNA, tail DNA; TWA, total weighted average; XRCC, X-ray repair cross complementing.

C.3.7. Summary Table by Genotoxicity Endpoint

A text summary of the available genotoxicity data that emphasizes genotoxicity studies incorporating inhalation formaldehyde exposure and related experiments (i.e., given the known toxicokinetics of inhaled formaldehyde) is provided in Section 3.2.5 (*Evidence on Mode of Action*) in the Toxicological Review. Table C-21 below provides a summary of the most relevant data organized by genotoxicity endpoint, as compared to the organization by test system in the previous sections. In addition, when possible, this table separates the summary into investigations of respiratory- versus nonrespiratory-related tissues or systems. Thus, observations of genotoxicity in the upper respiratory tract (URT) and in peripheral blood lymphocytes (PBLs) following inhalation exposure or in related in vitro systems are presented in Table C-21 in order of their importance and relevance to cancer risk beginning with gene mutations, DPXs and DDCs, DNA adducts, CAs, MN, DNA strand breaks, SCE, and other effects. Overall, the evidence supports the conclusion that formaldehyde is genotoxic. Particular weight is placed on the following observations:

- 1) Consistent observations of mutations in exposed rodents and various in vitro systems;
- 2) Observations of CAs, MNs, and SSBs in exposed humans across a range of studies, occupations, and exposure scenarios, with supporting, similar findings in exposed rodents and in vitro systems; and
- 3) Consistent observations of DPX detected in multiple experimental systems, showing a concentration-dependent increase, and concordance of DPX distribution with sites of tumors in the nose.

Table C-21. Genotoxicity summary table

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
Gene Mutations	Respiratory tract tissues or in vitro systems	+ (1/2) In vivo, rodent (inhalation); + 1/1 chronic; 0/1 subchronic studies + (5/5) In vitro, human cell lines, acute studies + (8/10) In vitro, rodent cell lines, acute studies + (13/17) Nonmammalian systems	In vivo rodent studies analyzed SCCs from a chronic study and non-neoplastic nasal mucosa from a subchronic study at 18.45 mg/m ³ All in vitro studies assume MeOH co-exposure; cellular sources both POE and systemic sites Negative in vitro rodent data for HPRT; + results include colony formation and mutation frequency	Mutations induced by formaldehyde across a range of in vitro systems. Mutations observed in SSC in nasal tissues of exposed rodents at 18.45 mg/m ³ in one chronic inhalation study.	Observation of gene mutations in nasal SSC in one chronic-duration rodent study (which only tested high formaldehyde levels), with confirmatory evidence from in vitro test systems across several species. No mutations in subchronic-duration rodent study. No studies of exposed humans or primates.
	Other tissues	+ (1/2) in vivo, rodent (inhalation); dominant lethal studies + (1/2) in vivo, rodent (i.p.); dominant lethal mutation studies	Formalin inhalation exposure at 200 mg/m ³ prevents interpretation; another inhalation study at 1.5 mg/m ³ was equivocal i.p. exposure with MeOH co-exposure caused + DLM in rats (0.125 mg/kg), but not in mice (20 mg/kg) at much higher levels	Results are interpreted as equivocal; the available studies do not provide evidence of mutations in other tissues	
Chromosomal aberrations (CA)	Respiratory tract tissues or in vitro systems	+ (1/1) in vivo, rodent (inhalation): short term study + (4/4) In vitro, human cells/cell lines, acute studies + (5/6) In vitro, rodent cell lines, acute studies	In vivo rat study at 18.45 mg/m ³ with 4-wk exposure In vitro studies assume co-exposure to MeOH; cell sources both POE and systemic sites 1 equivocal CA study in a rodent cell line	CAs were observed in the only in vivo rodent study, which is supported by positive results in human and rodent cells in vitro.	Evidence from exposed humans across several different occupations is consistent with the induction of CAs. These results are supported by observations of CAs in the only available in vivo rodent study (4 wks at high levels), which was consistent with findings from multiple in vitro studies of human and rodent cells lines

Supplemental Information—Formaldehyde (Inhalation)

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
	Other tissues	+(11/16) in vivo, human (inhalation): PBLs +(1/5) in vivo, rodent (inhalation): short term studies +(2/2) in vivo, rodent (gavage, p.o.): acute studies +(1/4) in vivo, rodent (i.p.): acute or short term studies	In humans, half + CAs were observed in pathologists and half among industrial workers; often, these studies involved relatively higher formaldehyde exposure levels (e.g., average >0.2 mg/m³) and longer employment duration (e.g., average >10 year) The only positive rodent inhalation study involved MeOH co-exposure*; 4 studies used PFA Oral exposure in rats and mice involved MeOH co-exposure, although 1 study indicated it takes >10× MeOH to cause a similar level of CAs The + i.p. study was in rat bone marrow cells after 4-wk exposure; – studies were acute, mice studies	Most of the human studies interpreted with higher confidence observed increased CA in PBLs; Lower exposure levels may explain null findings. Rodent results are interpreted as equivocal. The rodent studies do not provide evidence that CAs are induced in other tissues; however, the data suggest the possibility that rats might be more sensitive and that exposure duration is important.	
Micronuclei (MN)	Respiratory tract tissues or in vitro systems	+(11/13) in vivo, human (inhalation); +(0/1) in vivo, rodent (inhalation); short term study +(5/5) In vitro, human cell line; acute study +(4/4) in vitro, rodent cell lines; acute studies +(1/3) nonmammalian studies	MN reported in buccal and nasal cells, occupational (average >0.5 mg/m³), anatomy or embalming courses (average >0.5 mg/m³ with intermittent peaks). No increase after 5–10 d in 2 controlled human exposure studies, In vivo rat study at 18.45 mg/m³ for 4 wk (in BAL) MN observed in primary human blood cultures, and in 3 in vitro rodent studies with no MeOH co-exposure; remaining cell studies assume MeOH; cellular sources both POE and systemic sites	Consistently increased frequency of MN or related endpoint in buccal and/ or nasal cells of exposed individuals Consistent evidence of MN across a range of in vitro mammalian cells, but not in a short term rodent inhalation study.	Available evidence suggests increased MN levels associated with cumulative exposure; the pattern of chromosomal loss (monocentromeric and multi-centromeric micronuclei) was consistent with aneuploidy in exposed individuals

Supplemental Information—Formaldehyde (Inhalation)

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
	Other tissues	+ (11/16) in vivo, human (inhalation) PBLs, + (1/2) in vivo, rodent (inhalation); short-term studies + (1/5) in vivo, rodent (i.p., i.v., p.o. or gavage); acute studies	MN reported in PBLs of workers from plywood and formaldehyde production industry, and pathology, anatomy, and mortuary lab students, at exposure concentrations of 0.1–0.5 mg /m ³ . Null results in studies with low sensitivity. No increase after 5 days in controlled human exposure study. Prevalence increases with longer exposure duration. In rodents, MN were in bone marrow erythrocytes at 12.8 mg/m ³ with 10-wk exposure, but not in peripheral blood at 18.45 mg/m ³ with 4-wk exposure. The + non-inhalation study was an oral rat study of gastric epithelial cells; all – studies were in mice	Most of a large set of studies that measured MN in PBLs reported increased levels among exposed participants working in diverse exposure settings and in several countries. The two rodent inhalation studies suggest the possibility that MN induction may require longer exposure duration, but results were mixed; data suggest the possibility that rats might be more sensitive.	
Aneuploidy	Respiratory tract tissues or in vitro systems	+ (1/3) In vitro, human cell lines; short-term studies + (1/3) in vitro, rodent cell lines; short-term studies	All negative in vitro studies have co-exposure with MeOH	Inconsistent results from in vitro human or rodent cell lines; Methanol co-exposure is likely to influence the aneuploidy in cultured cells	Chromosome aneuploidies are consistent with study findings of CA and mono-centromeric and multicentromeric micronuclei in PBLs of exposed humans
	Other tissues	+ (3/4) in vivo, human (inhalation) + (1/3) in vitro, rodent cell lines + (1/3) in vitro, human cell lines	An occupational study in humans reported monosomy 7 and trisomy 8 in cultured CFU-GM colony cells from peripheral blood. Analysis of same cohort with bigger sample size detected aneuploidy in several chromosomes. Two in vitro studies each from rodent and human cell lines used MeOH-free HCHO, one positive study in human cells has co-exposure with MeOH.	Significant increase in chromosome aneuploidy in cultured CFU-GM colony cells among subset of highly exposed workers compared to matched controls	

Supplemental Information—Formaldehyde (Inhalation)

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
DNA adducts	Respiratory tissues or in vitro systems*	+ (2/2) in monkeys (inhalation) hm-DNA adducts + (3/4) in rats (inhalation) hm-DNA adducts + (2/2) in vitro human cell lines, hm-DNA adducts + (1/1) in vitro rodent cell lines, hm-DNA adducts + (10/10) in cell-free systems, hm-DNA adducts	No in vivo studies in humans showing hm-DNA adducts with a direct exposure to formaldehyde. Detectable hm-DNA adducts in all nasal passages, but not in lungs of rats. High endogenous hm-DNA adduct levels rats and monkeys, but monkeys > rats	All tissues in nasal passages demonstrated hm-DNA adducts except lung tissue of rodents. Endogenous levels of hm-DNA adducts are very high in both rats and monkeys compared to exogenous hm-DNA adducts. Monkeys have much higher endogenous hm-DNA adduct levels compared to rats.	Formaldehyde readily forms hm-DNA adducts in tissues at POE. However, available evidence does not show their formation in distal tissues.
	Other tissues	+ (1/1) in vivo, human, M1G adduct + (0/2) in vivo, monkeys (inhalation), acute studies + (0/2) in vivo, rodent (inhalation), acute studies	One study reported M1G adducts in peripheral blood of pathologists, uncertainties with regard to site of DNA interactions. hm-DNA adducts were not found in distal tissues of exposed monkeys or rodents	Absence of hm-DNA adducts in distal tissues suggest lack of formaldehyde transport to distal sites. Limited evidence of formaldehyde-induced oxidative DNA damage.	
DDC	Respiratory tissues or in vitro systems*	+ (1/1) in vivo, rat (inhalation), acute study + (3/3) in vitro, cell-free systems	Only one in vivo study reports DDC. But DDC are unstable and could be generated as an artifact.	Limited evidence of DDC formation by formaldehyde in vivo.	Limited evidence that formaldehyde inhalation results in DDC although artifacts were not ruled out.
	Other tissues	+ (0/1) in vivo monkey (inhalation) short-term study + (0/1) in vivo rat (inhalation) short-term study	DDC were not detectable in distal tissues.	DDC have not been detected in distal tissues	
DNA-Protein Crosslinks	Respiratory tissues or in vitro systems*	+ (1/1) in vivo, monkeys (inhalation), acute study + (7/11) in vivo, rodents (inhalation), acute studies + (30/30), in vitro, human cell lines, acute studies + (21/21) in vitro, rodent cell lines, acute studies + (3/3) nonmammalian	Concentration-dependent increase in DPX in rodents (0.37–12.1 mg/m ³) and monkeys (0.86–7.37 mg/m ³); DPX demonstrated in nasal mucosa of rats but absent from olfactory mucosa and lung; a negative study in BAL cells used formalin vapors	Consistent evidence of DPX across multiple test systems (two species in vivo, different cell lines, nonmammalian and cell-free test systems)	Anatomical distribution of DPX in rats corresponds to sites of tumor incidence, cell proliferation, and cytotoxicity in the nose. However, no mechanism is identified for DPX formation in PBLs of

Supplemental Information—Formaldehyde (Inhalation)

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
		systems +(4/4) cell-free systems			occupationally exposed individuals.
	Other tissues	+(2/3) in vivo, human (inhalation) PBLs +(4/8) in vivo, rodent (inhalation)	Occupational settings, one null study of plywood workers had low sensitivity (referent group had high exposure), no difference in prevalence by exposure group, but increase in DPX was observed over 8-hr shift. Positive rodent studies have co-exposure with MeOH.	In vivo human studies show exposure duration-dependent increase in DPX in PBLs, but animal in vivo studies are confounded by MeOH coexposure.	
DNA strand breaks	Respiratory tissues or in vitro systems*	+(1/1) in vivo, rodent (inhalation), short-term study +(10/12) in vitro, human cells, acute studies +(3/7), in vitro, rodent cells/cell lines, acute studies +(4/4) nonmammalian systems	Only one in vivo study and several cell culture studies reports SSB formation, but most of these studies have co-exposure with MeOH. Human cells were more sensitive to SSB formation by HCHO exposure (0.005–0.8 mM) Excision-repair deficient yeasts were more sensitive compared to repair-proficient strains.	Single strand breaks in rat study were positively associated with concentration.	Some evidence for SSB with dose-response in respiratory tissues from an inhalation study in rats, and consistent evidence in PBLs from several studies of human exposure and from rodent studies
	Other tissues	+(8/9) in vivo, human (inhalation) PBLs, +(3/4) in vivo, rodent (inhalation), short-term studies	Exposure settings were occupational with means > 0.2 mg/m ³ , 1 controlled human exposure study (4-hr duration). Categorical analysis by one study showed exposure-response trend beginning at 2nd quintile (mean 0.14 mg/m ³) Positive rodent in vivo studies have co-exposure with MeOH.	Consistent evidence of SSB formation in both human and rodent in vivo studies	

Supplemental Information—Formaldehyde (Inhalation)

Genotoxicity endpoint(s)	Experimental system	Genotoxicity evidence (in descending relevance)	Other relevant information or limitations	Endpoint summaries	Endpoint conclusion
Sister chromatid exchange (SCE)	Respiratory tissues or in vitro systems*	+ (6/6) in vitro, human cells/cell lines, short-term studies + (13/14) in vitro hamster cell lines, short-term studies	Positive studies included mostly co-exposure with MeOH, but several studies in both human and animal cell lines, which used methanol-free formaldehyde, were also positive.	Consistent evidence of SCE formation from in vitro human and rodent cell lines	No in vivo studies in animals, and less consistent results in exposed humans
	Other tissues	+ (8/16) in vivo human (inhalation) PBLs + (0/3) in vivo, rat (inhalation) short-term studies	Several studies of occupational exposure showed increased SCE levels. Although MeOH-free or MeOH-co-exposed rat studies were negative, male rats received MeOH-free formaldehyde were positive in bone marrow cells.	Evidence that SCE is induced in some exposed human populations, although the results across studies are not consistent	
Other effects (cell transformation; DNA repair inhibition; unscheduled DNA synthesis; gene conversion, crossing over and translocation)	Respiratory tissues or in vitro systems*	+ (4/7) in vitro, human primary cells/cell lines, (2/5 UDS) and (2/2 DNA repair inhibition, short-term studies + (4/5) in vitro, rodent cell lines, short-term studies (1/1 UDS; 3/4 cell transformation) + (8/8) nonmammalian system; [(1/1) DNA repair inhibition; + (2/2) gene conversion; + (3/3) genetic crossing over/recombination; + (2/2) heritable translocation]	Although most of the in vitro and nonmammalian studies were positive for other genotoxic effects, these studies had co-exposure with MeOH.	Available evidence suggests a variety of other genotoxic endpoints induced by formaldehyde exposure, which may play a supplemental role in overall genotoxicity.	Many of the other genotoxic endpoints support the overall genotoxicity and mutagenicity of formaldehyde across multiple experimental systems.
	Other tissues	+ (1/2) in vivo human (inhalation)	Change in O6-alkylguanine DNA alkyl-transferase activity in PBLs before and after 2- to 3-month exposure in embalming or anatomy labs	Evidence is inadequate to conclude effect on DNA repair inhibition	

C.4. SENSORY IRRITATION (SUPPORTING INFORMATION FOR HAZARD)

Information supporting the evidence synthesis on sensory irritation in Section 3.2.1 of the Toxicological Review is presented in Tables C-22 (human laboratory exposure studies) and C-23 (human occupational studies) below.

Table C-22. Summary of epidemiology studies of laboratory exposures to formaldehyde and human sensory irritation

Reference, study design, exposure levels, confidence	Results																																												
Kriebel et al. (1993) (Massachusetts) Panel study, 24 clinical anatomy students dissecting cadavers during 10-wk lab once a wk, 3 hrs. Outcome: Symptoms recorded before, during and after the lab; ATS questionnaire for baseline and modified brief questionnaire during lab, references provided. Exposure: Personal samples in breathing zone (1- to 1.5-hr duration). Geometric mean 0.73 ppm (SD 1.22 ppm). Range 0.49–0.93 ppm (n=8). No trend in concentrations over semester. Formaldehyde levels in three air samples in the cavities of the cadavers were 3.0, 3.6 and 4.3 ppm. Analysis: Multivariate linear regression models; mean prelab and cross-lab change in symptoms analyzed using random effects models. <i>High confidence</i>	Average symptom prevalence increased from beginning to end of weekly lab session by 43%. Prevalence (%) Before, Midway and After Lab Session <table><tr><th>Symptom</th><th>pre</th><th>mid-</th><th>Post</th></tr><tr><td>Eyes</td><td>16</td><td>66</td><td>59</td></tr><tr><td>Nose</td><td>46</td><td>75</td><td>67</td></tr><tr><td>Throat</td><td>25</td><td>45</td><td>40</td></tr><tr><td>Breathing</td><td>16</td><td>41</td><td>36</td></tr><tr><td>Cough</td><td>15</td><td>26</td><td>20</td></tr></table> Analysis indicated that magnitude of increase in symptom prevalence across lab session decreased as semester advanced (ln week: eye β -0.74, $p = 0.002$; throat β -0.39, $p = 0.03$; nose β -0.64, $p = 0.06$). No trend in prelab symptom severity over 10-week course	Symptom	pre	mid-	Post	Eyes	16	66	59	Nose	46	75	67	Throat	25	45	40	Breathing	16	41	36	Cough	15	26	20																				
Symptom	pre	mid-	Post																																										
Eyes	16	66	59																																										
Nose	46	75	67																																										
Throat	25	45	40																																										
Breathing	16	41	36																																										
Cough	15	26	20																																										
Uba et al. (1989) (California) Panel study, 1984-1985. 103 of 142 medical students in a 7-mo anatomy class, meeting twice a wk for 4 hrs (September 1984–April 1985), mean age (range): 24.3 (21–33) years. Outcome: Persistent symptoms: 103 students completed respiratory questionnaire (ATS) at the beginning (September 1984) and end of course (April 1985). Acute symptoms: 81/103 students completed different questionnaire after anatomy lab with formaldehyde exposure and after microanatomy lab (no formaldehyde) during Nov 1984. Order of questionnaires varied. Exposure: Personal samplers (impingers) in the breathing zone. TWA formaldehyde concentrations (N = 32 samples during different class periods over 7 months). Short-term samples (N = 16) for peak concentrations during dissection and observation. Dissecting room ventilated 24 hrs/d TWA concentrations: range, ≤ 0.05 (LOD) to 0.93 ppm (< 0.06 to 1.1 mg/m³). During dissection: mean 1.9 ppm (2.3 mg/m³); range 0.1 to 5.0 ppm (0.12 to 6.1 mg/m³).	Symptoms during lab session: symptom prevalence in anatomy lab (exposed) compared with microanatomy lab (unexposed) (N = 81) <table><tr><th>Symptom</th><th>Ex-posed</th><th>Unexposed</th><th>Odds Ratio</th></tr><tr><td>Itchy eyes</td><td>33</td><td>1</td><td>33*</td></tr><tr><td>Watery eyes</td><td>36</td><td>3</td><td>12*</td></tr><tr><td>Burning eyes</td><td>47</td><td>0</td><td>infinite</td></tr><tr><td>Burning nose</td><td>19</td><td>0</td><td>infinite</td></tr><tr><td>Sore throat</td><td>21</td><td>4</td><td>5.3**</td></tr><tr><td>Sneezing</td><td>10</td><td>1</td><td>10**</td></tr><tr><td>Rhinorrhea</td><td>13</td><td>3</td><td>4.3**</td></tr><tr><td>Chest tightness</td><td>4</td><td>0</td><td>infinite</td></tr><tr><td>Cough</td><td>5</td><td>4</td><td>1.3</td></tr><tr><td>Wheezing</td><td>2</td><td>0</td><td>infinite</td></tr></table>	Symptom	Ex-posed	Unexposed	Odds Ratio	Itchy eyes	33	1	33*	Watery eyes	36	3	12*	Burning eyes	47	0	infinite	Burning nose	19	0	infinite	Sore throat	21	4	5.3**	Sneezing	10	1	10**	Rhinorrhea	13	3	4.3**	Chest tightness	4	0	infinite	Cough	5	4	1.3	Wheezing	2	0	infinite
Symptom	Ex-posed	Unexposed	Odds Ratio																																										
Itchy eyes	33	1	33*																																										
Watery eyes	36	3	12*																																										
Burning eyes	47	0	infinite																																										
Burning nose	19	0	infinite																																										
Sore throat	21	4	5.3**																																										
Sneezing	10	1	10**																																										
Rhinorrhea	13	3	4.3**																																										
Chest tightness	4	0	infinite																																										
Cough	5	4	1.3																																										
Wheezing	2	0	infinite																																										

Supplemental Information—Formaldehyde (Inhalation)

Reference, study design, exposure levels, confidence	Results																																				
When observing dissection: mean 1.2 ppm (1.5 mg/m³); range 0.2 to 2.0 ppm (0.25 to 2.5 mg/m³). Monthly average in September, October, and May: 0.6, 0.8, 0.1 ppm (0.74, 0.98, and 0.12 mg/m³). Analysis: Symptom prevalence at beginning of course compared to end of course, paired analysis, McNemar’s test; symptom prevalence after lab with formaldehyde compared to lab with no formaldehyde, odds ratios, McNemar’s test paired samples <i>High confidence</i>	<table><tr><td>Dyspnea</td><td>2</td><td>0</td><td>infinite</td></tr></table> McNemar’s test paired samples, * $p<0.001$; **$p<0.05$ Persistent symptoms (Number reporting symptoms only in September 1984 or only in April 1985) <table><tr><td>Symptom</td><td>Sept. 1984</td><td>April 1985</td><td>Odds Ratio</td></tr><tr><td>Cough</td><td>1</td><td>8</td><td>8.0*</td></tr><tr><td>Phlegm</td><td>4</td><td>9</td><td>2.3</td></tr><tr><td>Chronic bronchitis</td><td>4</td><td>2</td><td>0.5</td></tr><tr><td>Chest illnesses</td><td>9</td><td>0</td><td>0**</td></tr><tr><td>Wheezing</td><td>37</td><td>1</td><td>0.03**</td></tr><tr><td>Wheezing with Dyspnea</td><td>4</td><td>0</td><td>0***</td></tr><tr><td>Dyspnea on exertion</td><td>0</td><td>0</td><td>-</td></tr></table> McNemar’s test paired samples, * $p = 0.02$; ** $p < 0.001$; ***$p = 0.05$	Dyspnea	2	0	infinite	Symptom	Sept. 1984	April 1985	Odds Ratio	Cough	1	8	8.0*	Phlegm	4	9	2.3	Chronic bronchitis	4	2	0.5	Chest illnesses	9	0	0**	Wheezing	37	1	0.03**	Wheezing with Dyspnea	4	0	0***	Dyspnea on exertion	0	0	-
Dyspnea	2	0	infinite																																		
Symptom	Sept. 1984	April 1985	Odds Ratio																																		
Cough	1	8	8.0*																																		
Phlegm	4	9	2.3																																		
Chronic bronchitis	4	2	0.5																																		
Chest illnesses	9	0	0**																																		
Wheezing	37	1	0.03**																																		
Wheezing with Dyspnea	4	0	0***																																		
Dyspnea on exertion	0	0	-																																		
Mori et al. (2016) (Japan) Cross-sectional study, Students (2nd year), n=123 (98.4%) enrolled in afternoon gross anatomy classes, April–July 2013, mean age 22.9 years; compared to nonexposed 1st year students, n=114 (91.9%), mean age 21.2 years. 75% males Outcome: Questionnaire, 16 subjective symptoms, frequency never, sometimes, or often; administered April 2013 before, May 2013 during, and January 2014 6 mo after completion of course. Exposure: Area samples at breathing height, 5 locations during class in May 2013 on same day questionnaires were completed. Mean (SD) 0.123 (0.025) mg/m³ (conversion by EPA). Area sample, 5 locations during class on same day questionnaires were completed. Mean (SD) 0.1 (0.02) ppm Analysis: Regression of presence or absence of symptoms in relation to exposure group on day of survey, controlling for doctor-diagnosed allergies, sex and age <i>High confidence</i>	Symptoms reported comparing exposed to unexposed on a day during gross anatomy class (OR (95% CI)) <table><tr><td>Symptom</td><td>OR</td><td>95% CI</td></tr><tr><td>Eye soreness</td><td>2.35</td><td>1.3–4.27</td></tr><tr><td>Eye strain</td><td>1.82</td><td>1.07–3.14</td></tr><tr><td>Itchy eye</td><td>0.75</td><td>0.43–1.31</td></tr><tr><td>Dry eye</td><td>1.11</td><td>0.63–1.96</td></tr><tr><td>Tearing</td><td>2.62</td><td>1.36–5.04</td></tr><tr><td>Itchy nose</td><td>1.76</td><td>1.01–3.06</td></tr><tr><td>Nasal</td><td>0.78</td><td>0.44–1.36</td></tr><tr><td>Runny nose</td><td>0.82</td><td>0.47–1.44</td></tr><tr><td>Sore throat</td><td>1.45</td><td>0.82–2.55</td></tr><tr><td>Dry throat</td><td>0.87</td><td>0.49–1.57</td></tr></table>	Symptom	OR	95% CI	Eye soreness	2.35	1.3–4.27	Eye strain	1.82	1.07–3.14	Itchy eye	0.75	0.43–1.31	Dry eye	1.11	0.63–1.96	Tearing	2.62	1.36–5.04	Itchy nose	1.76	1.01–3.06	Nasal	0.78	0.44–1.36	Runny nose	0.82	0.47–1.44	Sore throat	1.45	0.82–2.55	Dry throat	0.87	0.49–1.57			
Symptom	OR	95% CI																																			
Eye soreness	2.35	1.3–4.27																																			
Eye strain	1.82	1.07–3.14																																			
Itchy eye	0.75	0.43–1.31																																			
Dry eye	1.11	0.63–1.96																																			
Tearing	2.62	1.36–5.04																																			
Itchy nose	1.76	1.01–3.06																																			
Nasal	0.78	0.44–1.36																																			
Runny nose	0.82	0.47–1.44																																			
Sore throat	1.45	0.82–2.55																																			
Dry throat	0.87	0.49–1.57																																			
Kriebel et al. (2001) (Massachusetts) Panel study, 38 anatomy students (of 54 total) during 12-wk class meeting once per week, 2.5 hrs. Mean age 24.9 years, 23.7% male, 2 current smokers, 5 ex-smokers, 4 history of asthma	Mean postlab intensity of eye, nose, and throat irritation decreased over semester. Association of symptom intensity with exposure during lab & interaction with																																				

Supplemental Information—Formaldehyde (Inhalation)

Reference, study design, exposure levels, confidence	Results															
Outcome: Symptom questionnaires before and after each lab session. Scale of symptom intensity ranged from 0 (not at all) to 10 (very, very much). Exposure: Continuous monitoring in 6 homogenous locations (LOD = 0.05 ppm [0.06 mg/m³]. 12-min work-zone concentrations for each student calculated using sampling data and recorded work locations. Geometric mean concentration over all lab sessions and participants: 0.7 ppm [0.9 mg/m³] (GSD 2.13) Peak 12 min concentration 10.91 ppm (13.42 mg/m³) Average ± SD concentration over all weeks and participants: 1.1 ± 0.56 ppm (1.4 ± 0.69 mg/m³) Concentrations decreased over 12-wk semester. Analysis: Generalized estimating equation regression model accounting for lack of independence of repeated measures in individuals. <i>Medium confidence</i> Decline in attendance over time potentially based on exposure and outcome status.	<table><tr><th colspan="3">time (Percent change in intensity per ppm or ppm-weeks)</th></tr><tr><th></th><th>Recent exposure^b</th><th>Recent exposure x ln(week)^c</th></tr><tr><td>Eye Irritation</td><td>1.22*</td><td>−0.35*</td></tr><tr><td>Nose Irritation</td><td>1.09*</td><td>−0.42*</td></tr><tr><td>Throat Irritation</td><td>0.81*</td><td>−0.36*</td></tr></table> *<i>p</i> <0.001 for significant deviation from slope = 0 ^bMean concentration during 2.5-hr lab ^c Interaction between recent exposure and natural log of week number, indicating declining strength of association with time.	time (Percent change in intensity per ppm or ppm-weeks)				Recent exposure ^b	Recent exposure x ln(week) ^c	Eye Irritation	1.22*	−0.35*	Nose Irritation	1.09*	−0.42*	Throat Irritation	0.81*	−0.36*
time (Percent change in intensity per ppm or ppm-weeks)																
	Recent exposure ^b	Recent exposure x ln(week) ^c														
Eye Irritation	1.22*	−0.35*														
Nose Irritation	1.09*	−0.42*														
Throat Irritation	0.81*	−0.36*														
Takahashi et al. (2007) (Japan) Panel study, 2002–2003. 143 medical students (68.5% male, 88.8% 20–24 years of age) who dissected cadavers 15 hours per week for 2 mos and 76 students who had taken same course 2 to 4 years earlier (68.4% male, 77.6% 20–24 years of age). Outcome: Symptom questionnaire administered after 1st day of exposure and at end of course. Exposure: Area formaldehyde samples (> 10 min, 8 locations in room), upon opening of thorax, mean 2.12 ppm (SD 0.23), range 1.7–2.44 ppm (2.6 ± 0.28 mg/m³, range 2.13–3.05 mg/m³). Breathing zone samples (18/143 students), mean 2.4 ppm (SD 0.49), range 1.79–3.78 ppm; (mean 3.0 ± 0.61 mg/m³, range 2.24–4.72 mg/m³) Analysis: Prevalence after first exposure and at end of course compared, McNemar's test <i>Medium confidence</i> Large gap between symptom ascertainment at end of course and exposure measurements. Selection, recruitment and participation was not reported.	Prevalence after first exposure and at end of course estimated from Figure 1 in the paper. Largest increase in symptoms (<i>p</i><0.05) reported for eye soreness (from about 35% to about 68% on 1st day versus end of course), lacrimation (12% to 60%), throat irritation (14% to 42%), eye fatigue (28% to 44%), rhinorrhea (17% to 35%), skin irritation (14% to 28%).															
Takigawa et al. (2005) (Japan) Intervention study, purpose: Evaluate installation of a ventilation system between phases and effects on formaldehyde concentrations and symptoms. 2 phases; 1st phase: 78 volunteer anatomy students in 2001 (mean age 21.6 years); 2nd phase: 79 volunteer anatomy students 3 years later in 2004 (mean age 21.7 years).	Symptom change indexes for 8 of 25 measured symptoms were significantly less comparing the second phase results with the first phase results. <table><tr><th colspan="4">Symptom Change Index</th></tr><tr><th></th><th>Symptom</th><th>1st (N=78)</th><th>2nd (N=79)</th></tr><tr><td>Skin</td><td>Eczema</td><td>0.13</td><td>-0.09</td></tr></table>	Symptom Change Index					Symptom	1 st (N=78)	2 nd (N=79)	Skin	Eczema	0.13	-0.09			
Symptom Change Index																
	Symptom	1 st (N=78)	2 nd (N=79)													
Skin	Eczema	0.13	-0.09													

Supplemental Information—Formaldehyde (Inhalation)

Reference, study design, exposure levels, confidence	Results																											
Outcome: Self-administered questionnaires on health complaints before and during each 2-mo course. Symptom frequency: 1 (never), 2 (scarcely), 3 (sometimes), and 4 (always). Symptom change index: Symptom frequency score during session subtracted from score before course. Exposure: Area formaldehyde samples (>10 min, 9 locations in room); upon opening of thorax (represents highest concentration over 2 mos). Phase I: Median (range) 2.59 (2.1–3.0) mg/m³ (concentration reported as 0.259 mg/m³ in Table 3 of the paper must be an error). Phase II: Median (range) 0.729 (0.291–0.971) mg/m³ Personal samples (measured with gas sampler on 24 students in first phase (42–962 min) and 46 in second phase (100–540 min)): Phase I: Median (range) 3.313 (2.238–8.909) mg/m³ Phase II: Median (range) 0.878 (0.396–3.386) mg/m³ Analysis: Symptom change index, 1st and 2nd phases compared; Mann-Whitney test, <i>p</i> <0.05. <i>Medium confidence</i> Uncertainty regarding impact of smoking on sensitivity of response to intervention	Eye	Itchy	0.74	0.27																								
		Irritated	0.96	0.52																								
		Watery	1.42	0.46																								
		Poor vision	0.17	-0.27																								
	Nose	Itchy	0.67	0.22																								
		Changed sense smell	0.18	0.33																								
	Throat	Sore	0.69	0.22																								
Wantke et al. (2000) (Austria) Panel study, 27 medical students, participants in Wantke et al. (1996) enrolled in a 2nd dissection class, 55.6% male Outcome: Symptoms standardized questionnaire at beginning, in middle, and at end of 10-wk course. Daily symptom cards during class Exposure: Continuous measurements for formaldehyde and phenol at 2 locations during lab, exposures for 43 d Formaldehyde Mean 0.265 ± 0.07 mg/m³, range 0.133–0.410 mg/m³, Phenol Mean 4.65 ± 2.96 mg/m³, range 0.09–11.8 mg/m³ Analysis: Prevalence in November and December compared to October, McNemar exact test <i>Medium confidence</i> Uncertainty regarding selection and recruitment details. See Wantke et al. (1996b)	Symptom prevalence was not correlated with smoking, or type I allergy, complaints of dizziness occurred only in males Prevalence of Symptoms at Beginning, Middle (5 Wks) and End (10 Wks) of Course <table><tr><th>Symptoms</th><th>Before</th><th>Middle</th><th>End</th></tr><tr><td>Burning eyes</td><td>0.111</td><td>0.481**</td><td>0.333*</td></tr><tr><td>Sneezing</td><td>0.074</td><td>0.037</td><td>0.037</td></tr><tr><td>Nosebleed</td><td>0.185</td><td>0.111</td><td>0.185</td></tr><tr><td>Cough</td><td>0.074</td><td>0.148</td><td>0.074</td></tr><tr><td>Shortness of breath</td><td>0</td><td>0.185</td><td>0.037</td></tr></table> *<i>p</i> <0.05, **<i>p</i> <0.01				Symptoms	Before	Middle	End	Burning eyes	0.111	0.481**	0.333*	Sneezing	0.074	0.037	0.037	Nosebleed	0.185	0.111	0.185	Cough	0.074	0.148	0.074	Shortness of breath	0	0.185	0.037
Symptoms	Before	Middle	End																									
Burning eyes	0.111	0.481**	0.333*																									
Sneezing	0.074	0.037	0.037																									
Nosebleed	0.185	0.111	0.185																									
Cough	0.074	0.148	0.074																									
Shortness of breath	0	0.185	0.037																									
Wantke et al. (1996b) (Austria) Panel study, 1995. 45 medical students enrolled in 1st dissection class, 51.1% male, age 20.9 years, 3 hr sessions, 5 d/wk for 4 wks Outcome: Symptoms, standardized questionnaire at beginning and at end of 4-wk course Exposure: Continuous measurements for formaldehyde, 2 locations during lab; Mean 0.124 ± 0.05 ppm, range 0.059–0.219 ppm	Prevalence of Symptoms During 4 Wk Course <table><tr><th>Symptoms</th><th>Before</th><th>During</th><th>p-Value</th></tr><tr><td>Burning eyes</td><td>0.133</td><td>0.289</td><td>< 0.02</td></tr><tr><td>Sneezing</td><td>0.244</td><td>0.089</td><td>NS</td></tr><tr><td>Nosebleeds</td><td>0.244</td><td>0.044</td><td>NS</td></tr><tr><td>Cough</td><td>0.044</td><td>0</td><td>NS</td></tr></table>				Symptoms	Before	During	p-Value	Burning eyes	0.133	0.289	< 0.02	Sneezing	0.244	0.089	NS	Nosebleeds	0.244	0.044	NS	Cough	0.044	0	NS				
Symptoms	Before	During	p-Value																									
Burning eyes	0.133	0.289	< 0.02																									
Sneezing	0.244	0.089	NS																									
Nosebleeds	0.244	0.044	NS																									
Cough	0.044	0	NS																									

Supplemental Information—Formaldehyde (Inhalation)

Reference, study design, exposure levels, confidence	Results																											
No sampling for phenol Analysis: Compared symptom prevalence during course to before, McNemar exact test <i>Low confidence</i> Low participation, possibility of selection bias away from null; Potential recall issues – symptoms for previous week Potential for differential recall of symptoms. s	<table><tr><td>Shortness of breath</td><td>0</td><td>0.022</td><td>NS</td></tr></table> Symptom prevalence was not correlated with gender, smoking, or type I allergy.	Shortness of breath	0	0.022	NS																							
Shortness of breath	0	0.022	NS																									
Chia et al. (1992) (Singapore) Cross-sectional study. 1st year medical students in anatomy lab, 150 of 164 total (91.5%); referent 189 3rd and 4th year medical students, no recent formaldehyde exposure; matched on age, sex, and ethnicity. Outcome: Symptoms during previous 4 wks of anatomy course (twice per wk, 2.5 hr (or other activities for referent), assessed via a modified MRC standardized questionnaire Exposure: Area samples at dissecting tables, n=6, collected on two occasions, Mean (SD) 0.5 ppm (0.08), range 0.4–0.6 ppm Personal samples, n=14 students, duration 2.5 hrs, Mean (SD) 0.74 (0.18), range 0.41–1.2 ppm LOD 0.05 ppm Analysis: Symptom prevalence in exposed compared to referent <i>Low confidence</i> Questions about dissimilarity of 1st and 4th year students and potential for recall bias during previous 4 weeks of course. No measurements of formaldehyde among referent group.	<table><tr><th colspan="4">Prevalence of Symptoms</th></tr><tr><th>Symptom</th><th>Ex-posed (n = 150)</th><th>Refer-ent (n = 189)</th><th>p-Value</th></tr><tr><td>Decreased ability to smell</td><td>0.127</td><td>0.032</td><td>0.002</td></tr><tr><td>Eye irritation</td><td>0.8</td><td>0.132</td><td>< 0.001</td></tr><tr><td>Throat irritation</td><td>0.313</td><td>0.138</td><td>< 0.001</td></tr><tr><td>Dry mouth</td><td>0.18</td><td>0.058</td><td>< 0.001</td></tr></table> No statistically significant difference for other symptoms (cough with mucus, chest tightness, chest pain, and breathlessness) (data were not reported).	Prevalence of Symptoms				Symptom	Ex-posed (n = 150)	Refer-ent (n = 189)	p-Value	Decreased ability to smell	0.127	0.032	0.002	Eye irritation	0.8	0.132	< 0.001	Throat irritation	0.313	0.138	< 0.001	Dry mouth	0.18	0.058	< 0.001			
Prevalence of Symptoms																												
Symptom	Ex-posed (n = 150)	Refer-ent (n = 189)	p-Value																									
Decreased ability to smell	0.127	0.032	0.002																									
Eye irritation	0.8	0.132	< 0.001																									
Throat irritation	0.313	0.138	< 0.001																									
Dry mouth	0.18	0.058	< 0.001																									
Fleisher (1987) (New York) Cross-sectional study 1st year medical students (N = 89) (43.6% of total 204 surveyed) (71% male) in gross anatomy course (formaldehyde exposed). Referent: Same students (n=60) (72% male) in pathology/microbiology laboratory six months later. 98.9% of all students attended 75–100% of all lab sessions. Outcome: Symptoms questionnaire one month after end of course. Symptom frequency: all of the time, some of the time, rarely or never. Exposure: Area formaldehyde measurements in 6 anatomy labs, one day during semester, 1983; sampling time 188–222 minutes. Personal breathing zone samples (3M Diffusion), 2 instructors, sampling time 180–190 min. Area samples: Drager tubes (all labs): <LOD (1 ppm) NIOSH method (3 labs): LOD (0.02 ppm), 0.03, 0.59 ppm; Breathing zone: 0.18 and 0.69 ppm; Analysis: Within person comparisons; t-test comparing mean symptom scores	Symptoms prevalence (% reporting symptom all or some of the time) among 38 students responding to both questionnaires (N=38) <table><tr><th>Symptom</th><th>Anatomy</th><th>Path/ Micro</th></tr><tr><td>Eye Irritation</td><td>68.4*</td><td>21.0</td></tr><tr><td>Nose Irritation</td><td>61.1*</td><td>13.1</td></tr><tr><td>Sneezing</td><td>37.8*</td><td>15.8</td></tr><tr><td>Tightness in chest</td><td>11.1</td><td>2.6</td></tr><tr><td>Shortness of breath</td><td>8.3*</td><td>0.0</td></tr><tr><td>Cough</td><td>28.6*</td><td>5.3</td></tr><tr><td>Throat Irritation</td><td>38.9*</td><td>7.9</td></tr><tr><td>Sinus problems</td><td>35.1*</td><td>5.3</td></tr></table> *p < 0.05	Symptom	Anatomy	Path/ Micro	Eye Irritation	68.4*	21.0	Nose Irritation	61.1*	13.1	Sneezing	37.8*	15.8	Tightness in chest	11.1	2.6	Shortness of breath	8.3*	0.0	Cough	28.6*	5.3	Throat Irritation	38.9*	7.9	Sinus problems	35.1*	5.3
Symptom	Anatomy	Path/ Micro																										
Eye Irritation	68.4*	21.0																										
Nose Irritation	61.1*	13.1																										
Sneezing	37.8*	15.8																										
Tightness in chest	11.1	2.6																										
Shortness of breath	8.3*	0.0																										
Cough	28.6*	5.3																										
Throat Irritation	38.9*	7.9																										
Sinus problems	35.1*	5.3																										

Reference, study design, exposure levels, confidence	Results
<i>Low confidence</i> Low response to both questionnaires and selection potential; with potential for symptoms to contribute to motivation to participate. Temporal gap in symptom response reduced recall accuracy potential.	

GSD = geometric standard deviation; MRC = Medical Research Council; NIOSH = National Institute of Occupational Safety and Health; ND = not detected.

Table C-23. Summary of epidemiology studies of occupational exposures to formaldehyde and human sensory irritation

(Reference), study design, exposure levels	Results												
Neghab et al. (2011) (Iran) Prevalence study, 70 male exposed workers with ≥2-year history of exposure at a melamine-formaldehyde resin producing plant (mean (SD) age: 38.2 (8.4) years; mean (SD) work duration 13.2 (7.8) years. 24 male, healthy referent employees with no current or history of exposure to formaldehyde or other respiratory toxicants (mean (SD) age: 40.0 (8.2) years); mean (SD) work duration 14.5 (8.1) years. 100% participation. Outcome: Respiratory symptoms ascertained via interview using standardized questionnaire (ATS). Exposure: Area samples (40-minute sampling time) in 7 workshops (N=7) and offices (N=1) Formaldehyde concentration: ppm, mean (SD): Exposed: 0.78 (0.40) (0.96 (0.49) mg/m³) Referent: nondetectable Analysis: Symptom prevalence compared <i>Medium confidence</i> Healthy survivor bias Potential for healthy survivor bias with attenuation in measure of association.	Prevalence Respiratory Symptoms: <table><tr><th>Symptom</th><th>Exposed</th><th>Referent</th></tr><tr><td>Cough</td><td>20%*</td><td>0%</td></tr><tr><td>Phlegm</td><td>28.6%*</td><td>0%</td></tr><tr><td>Chest tightness</td><td>52.9%*</td><td>0%</td></tr></table> *<i>p</i> < 0.05	Symptom	Exposed	Referent	Cough	20%*	0%	Phlegm	28.6%*	0%	Chest tightness	52.9%*	0%
Symptom	Exposed	Referent											
Cough	20%*	0%											
Phlegm	28.6%*	0%											
Chest tightness	52.9%*	0%											
Holness and Nethercott (1989) (Toronto, Canada) Prevalence study, 84 of 97 selected funeral service apprentice workers from funeral homes selected by the Metropolitan District Funeral Director’s Association (mean (SD) age 32.1 (11.1) years, 89% male, work duration 8.2 years (SD 9.9)). 38 service volunteers and paid student volunteers as referent subjects similar in age to the apprentices (mean (SD) age 28.7 (12.7) years, 84% male, work duration 7.2 years (SD 11.9)). Outcome: Questionnaires (ATS) administered before and after an embalming procedure. Exposure: Area samples (N=2) during each embalming procedure, mean sampling duration (range): 85 minutes (30–180 minutes). Mean (SD) formaldehyde: Exposed: 0.36 (0.19) ppm (0.44 (0.23) mg/m³)^a, range 0.08–0.81 ppm. Autopsied cases 0.44 ppm.	Prevalence elevated for 12 of 13 eye, URT, respiratory and cutaneous symptoms, but 5 were significantly higher compared with referent: chronic bronchitis (20% vs. 3%, <i>p</i> = 0.035), shortness of breath (20% vs. 3%, <i>p</i> = 0.043), nasal (44% vs. 16%, <i>p</i> = 0.003) and eye (42% vs. 21%, <i>p</i> = 0.026) irritation and past skin problem (42% vs. 13%, <i>p</i> = 0.003).												

Supplemental Information—Formaldehyde (Inhalation)

(Reference), study design, exposure levels	Results																																																								
Average levels were 0.21 ppm when ventilation units were in operation. Referent: 0.02 ppm (0.025 mg/m³)^a Analysis: Differences evaluated using logistic regression analysis controlling for smoking (pack-years). <i>Medium confidence</i> Groups selected from different source populations Differences in source populations for exposed and referent groups lead to uncertainty in comparability, with no consideration of potential confounding.																																																									
Horvath et al. (1988) (Wisconsin) Prevalence study, 109 of 159 workers at a particleboard manufacturing plant (71% participation); 57% male; mean age 37.4, SD 11.7 years; Mean duration of employment 10.3 years (1 – 20 years); Referent: 254 of 300 workers at 2 food plants (44% male; mean age (SD): 34.2 (10.6) years. Outcome: Respiratory symptoms questionnaire (American Thoracic Society, ATS) completed before and after monitored work shift. Intensity assessed by subjects with visual analog scale. Exposure: Personal and area samples; 8-hr, TWA concentrations measured on each worker on the day of examination. In the particleboard plant, TWA values averaged 1.04 mg/m³; range 0.26 to 4.4 mg/m³. In the food plants, TWA values averaged 0.08 mg/m³, range 0.03 ppm to 0.12 ppm). Other agents sampled in particleboard or molded products plant. <table><tr><th>Compound</th><th>Mean (Range)</th></tr><tr><td>Total particulates^a</td><td>0.38 (0.25-4.4) mg/m³</td></tr><tr><td>Respirable particulates</td><td>0.11 (0.025-1.06) mg/m³</td></tr><tr><td>Phenol</td><td>0.15 (0.11-0.26) ppm</td></tr><tr><td>Carbon monoxide</td><td>7.35 (3.0-11.0) ppm</td></tr><tr><td>Sodium hydroxide</td><td>0.4 – 0.21 mg/m³</td></tr><tr><td>Nitrogen dioxide</td><td>ND</td></tr></table> ^aTotal particulates in food plants were 0.5 and 0.42 mg/m³. Analysis: Prevalence compared using chi-square statistic. Dose-response of end of shift symptoms evaluated using multiple regression models. <i>Medium confidence</i> Uncertain whether analyses adjusted for age or gender, although groups were compared and differences were not large.	Compound	Mean (Range)	Total particulates ^a	0.38 (0.25-4.4) mg/m ³	Respirable particulates	0.11 (0.025-1.06) mg/m ³	Phenol	0.15 (0.11-0.26) ppm	Carbon monoxide	7.35 (3.0-11.0) ppm	Sodium hydroxide	0.4 – 0.21 mg/m ³	Nitrogen dioxide	ND	Symptom Prevalence While at Work Reported in Preshift Questionnaire: <table><tr><th>Symptom</th><th>Exposed</th><th>Referent</th></tr><tr><td></td><td></td><td>t</td></tr><tr><td>Nose/ throat irritation</td><td>43.9%*</td><td>13.0%</td></tr><tr><td>Eye irritation</td><td>49.5%*</td><td>24.0%</td></tr></table> *p < 0.05 Symptom Prevalence Reported at End of Shift: <table><tr><th>Symptom</th><th>Exposed</th><th>Referent</th></tr><tr><td></td><td></td><td>t</td></tr><tr><td>Throat sore/burning</td><td>22.0%*</td><td>3.9%</td></tr><tr><td>Cough</td><td>34.9%*</td><td>18.9%</td></tr><tr><td>Phlegm</td><td>26.6%*</td><td>9.8%</td></tr><tr><td>Nose burning</td><td>28.4%*</td><td>2.0%</td></tr><tr><td>Stuffy nose</td><td>33.9%*</td><td>14.2%</td></tr><tr><td>Itching nose</td><td>21.1%*</td><td>7.9%</td></tr><tr><td>Eyes burning</td><td>39.5%*</td><td>9.1%</td></tr><tr><td>Eyes itching</td><td>19.3%*</td><td>7.1%</td></tr></table> *p < 0.05 Intensity (visual analogue scale, 0 – 100) for burning eyes, mean (SD) 47 (27) Shortness of breath (8.3 vs. 5.1%), wheezing (3.7 vs. 2.8%), and difficulty breathing (6.4 vs. 2.0%) were not significantly increased. Dose-response: formaldehyde a significant predictor of cough, chest complaints, phlegm, burning nose, stuffy nose, burning eyes, itchy nose, sore throat, and itchy eyes in multiple regression models; coefficients were not reported.	Symptom	Exposed	Referent			t	Nose/ throat irritation	43.9%*	13.0%	Eye irritation	49.5%*	24.0%	Symptom	Exposed	Referent			t	Throat sore/burning	22.0%*	3.9%	Cough	34.9%*	18.9%	Phlegm	26.6%*	9.8%	Nose burning	28.4%*	2.0%	Stuffy nose	33.9%*	14.2%	Itching nose	21.1%*	7.9%	Eyes burning	39.5%*	9.1%	Eyes itching	19.3%*	7.1%
Compound	Mean (Range)																																																								
Total particulates ^a	0.38 (0.25-4.4) mg/m ³																																																								
Respirable particulates	0.11 (0.025-1.06) mg/m ³																																																								
Phenol	0.15 (0.11-0.26) ppm																																																								
Carbon monoxide	7.35 (3.0-11.0) ppm																																																								
Sodium hydroxide	0.4 – 0.21 mg/m ³																																																								
Nitrogen dioxide	ND																																																								
Symptom	Exposed	Referent																																																							
		t																																																							
Nose/ throat irritation	43.9%*	13.0%																																																							
Eye irritation	49.5%*	24.0%																																																							
Symptom	Exposed	Referent																																																							
		t																																																							
Throat sore/burning	22.0%*	3.9%																																																							
Cough	34.9%*	18.9%																																																							
Phlegm	26.6%*	9.8%																																																							
Nose burning	28.4%*	2.0%																																																							
Stuffy nose	33.9%*	14.2%																																																							
Itching nose	21.1%*	7.9%																																																							
Eyes burning	39.5%*	9.1%																																																							
Eyes itching	19.3%*	7.1%																																																							

Supplemental Information—Formaldehyde (Inhalation)

(Reference), study design, exposure levels	Results		
Löfstedt et al. (2011b) Prevalence study. Sweden 3 brass foundries producing cores using Hot Box method. 43 of 48 exposed workers; 69 of 84 referents working outside core-production and die-casting halls; not exposed to chemicals. Prevalence of “ever” asthma or childhood allergy lower in exposed than in referent (9% and 19%, respectively versus 14% and 35%, respectively, $p < 0.05$) Outcome: Self-report, questionnaire; existence of symptoms during prior week; nasal signs Exposure: Individual measurements. Monoisocyanates: Mean of 4–5 5-min samples randomly distributed over entire shift. Formaldehyde: sampling over entire 8-hr shift Categorized low and high using LOD as cut-point (LOD not reported). Mean 0.51 mg/m³, SD 0.049 mg/m³, range 0.013–0.19 mg/m³ Low (↑) confidence Could not distinguish effect of formaldehyde from those of other irritants that were strongly associated with symptoms; Questionnaire not described. Potential for information bias (reduced recall accuracy); higher proportion of asthma or allergy among referents raises potential healthy worker survival.	Associations of ocular and nasal symptoms within the previous week and nasal signs with formaldehyde exposure		
		Referent (n=68)	Low (n = 30) High (n = 12)
	Any nasal symptoms	1.0	4.3 (1.7–11.2) 4.7 (1.2–19.1)
	Nasal signs – dry mucosa	1.0	2.8 (1.1–6.9) 2.8 (0.8–10.2)
	Irritated eyes	1.0	NR* 6.3 (1.4–28.4)
	NR: not reported		
	Nasal symptoms included discharge, itch, sneezing and congestion ICA and MIC also associated with these nasal endpoints, nasal symptoms OR 3.9 low and 5.0 in high exposed; nasal signs OR 4.5 low and 1.9 high exposed		
Alexandersson et al. (1982); Alexandersson and Hedenstierna (1989) (Sweden) Prevalence study, 1980, Employees at carpentry works (N=47) for > 1 year, regularly exposed to formaldehyde, and working on the study day, mean age (± SE) 35 (1.8) years, 49% smokers, duration employment 5.9 years. Referent (N=20) not exposed to formaldehyde or other lung irritants, employed at the same plant, mean age (± SE) 35.3 (2.3) years. Asthmatics excluded. Follow-up 5 years later (1984), 34 exposed and 18 referents; 21 remained exposed, 13 transferred to tasks with no exposure to irritants. Outcome: Interviews using standardized questionnaire focused on nose, eyes, upper airways, and lungs, chronic bronchitis defined by British Medical Research Council. Exposure: 1980 study: Personal samplers for formaldehyde, terpenes, and dust, N=31, duration 6–7 hr/d; Mean concentration (range): formaldehyde 0.47 mg/m³, 0.05–1.62 mg/m³, terpenes 0 (0–9) mg/m³, dust 0.5 (0.3–0.7) mg/m³ 1984 study: 3–4 15 min samples per person in the exposed group, estimated TWA Mean TWA concentration (± SD): formaldehyde 0.50 (0.12) mg/m³ Mean Peak concentration (± SD): formaldehyde 0.69 ± 0.68 ppm Analysis: Prevalence of symptoms while at work, change from 1980 to 1984, chi-square	Symptom Prevalence at Work, 1980 (%)		
		Exposed	Referent
	Eye	74	0
	Nose, Throat	36	0
	Symptom Prevalence at Work, 1984 (%)		
		Ex-posed	Trans-ferred
	Eyes		
	Smartin g	45	30
	Itching	40	20
	Running	60	30
	Nose		
	Running	30	10
	Dryness	15	0
	↓ Smell	0	0
	Change from 1980 to 1984 not statistically significant, $p > 0.05$		

Supplemental Information—Formaldehyde (Inhalation)

(Reference), study design, exposure levels	Results																													
Low confidence Evidence of healthy survivor bias; confounding by smoking.																														
Herbert et al. (1994) Prevalence study, 99 oriented strand board (OSB) workers (exposed, 98% participation), mean age 35.4 years, 51.5% smokers; work duration 5.1 years; 165 oil/gas field plant workers (not exposed to formaldehyde or oil and gas vapors) from same geographic area (82% participation), mean age 34.9 years, 27.9% smokers, work duration 10 years. Excluded 14 workers in referent with hydrogen sulfide exposure. Outcome: Respiratory symptoms ascertained via interview using standardized questionnaire. Exposure: Time weighted average formaldehyde and dust concentrations based on 21-hr continuous sampling in the breathing zone at 5 work sites on 2 separate days. Formaldehyde: range 0.07–0.27 ppm (0.09–0.33 mg/m³). Dust mean: 0.27 mg/m³, 2.5 µm diameter Analysis: Symptom prevalence compared Low confidence Different prevalence smoking and duration of employment between exposed and referent; no adjustment in analyses.	Prevalence Respiratory Symptoms (relevant to URT irritation): <table><tr><th>Symptom</th><th>Exposed</th><th>Referent</th></tr><tr><td>Usual Cough</td><td>24.5%*</td><td>11.1%</td></tr><tr><td>Usual Phlegm</td><td>31.3%*</td><td>13.3%</td></tr><tr><td>Chest tightness</td><td>43.4%*</td><td>22.8%</td></tr></table> *<i>p</i> < 0.05	Symptom	Exposed	Referent	Usual Cough	24.5%*	11.1%	Usual Phlegm	31.3%*	13.3%	Chest tightness	43.4%*	22.8%																	
Symptom	Exposed	Referent																												
Usual Cough	24.5%*	11.1%																												
Usual Phlegm	31.3%*	13.3%																												
Chest tightness	43.4%*	22.8%																												
Holmström et al. (1991) Sweden Prevalence study, Group 1: 16 persons exposed to medium density fiberboard (MDF) dust for at least 30% of the workday, mean age 44.1 years, 100% male, 38% smokers. Group 2: 29 exposed to other types of wood dust, mean age 39.3 years, 86.2% male, 31% smokers. Group 3 (Referent), 36 governmental clerks living in same village as chemical plant, mean age 39.9 years, 47.2% male, 28% smokers. (Groups 2 and 3 same as for Holmström and Wilhelmsson, 1988) Outcome: Symptom prevalence; Questionnaire and medical examination Exposure: Personal exposure measurements stable through year, average 0.2–0.3 mg/m³, peaks seldom > 0.5 mg/m³, Formaldehyde Concentration, mean MDF 0.26 mg/m³, range 0.17–0.48 mg/m³ Wood dust 0.25 mg/m³, range 0.3–1.0 mg/m³ Referent 0.09 mg/m³ Analysis: Exposed compared to referent; prevalence rate difference, 95% confidence intervals Low (↓) confidence Potential for healthy survivor bias with attenuation in measure of association.; groups selected from different source populations; Questionnaire not described. Potential confounding and no adjustment in analyses.	Rate Difference (%) in Symptoms, Exposed versus Referent <table><tr><th rowspan="2">Sympto m</th><th colspan="2">MDF</th><th colspan="2">Wood Dust</th></tr><tr><th>%</th><th>95% CI</th><th>%</th><th>95% CI</th></tr><tr><td>Nasal</td><td>66</td><td>47, 85</td><td>3</td><td>–20, 26</td></tr><tr><td>Eye</td><td>38</td><td>13, 64</td><td>1</td><td>–1, 13</td></tr><tr><td>Throat</td><td>19</td><td>–3, 42</td><td>4</td><td>–8, 18</td></tr><tr><td>Lower airway</td><td>36</td><td>9, 63</td><td>3</td><td>–14, 21</td></tr></table> Relief from symptoms during weekends in 80% in MDF group and 67% in wood dust group; and during vacations.	Sympto m	MDF		Wood Dust		%	95% CI	%	95% CI	Nasal	66	47, 85	3	–20, 26	Eye	38	13, 64	1	–1, 13	Throat	19	–3, 42	4	–8, 18	Lower airway	36	9, 63	3	–14, 21
Sympto m	MDF		Wood Dust																											
	%	95% CI	%	95% CI																										
Nasal	66	47, 85	3	–20, 26																										
Eye	38	13, 64	1	–1, 13																										
Throat	19	–3, 42	4	–8, 18																										
Lower airway	36	9, 63	3	–14, 21																										

Supplemental Information—Formaldehyde (Inhalation)

(Reference), study design, exposure levels	Results																				
Alexandersson and Hedenstierna (1988) Supplemental: Alexandersson (1988) (Sweden) Prevalence study, 38 exposed employees working with acid-hardening lacquers for the previous 12 mos (mean age (SD): 34 (10) years, mean duration employment 7.8 years) and at work on the study day. 18 referent employees at the same company (mean age (SD): 37 (9) years). Asthmatics excluded. Outcome: Interviews regarding irritation of eyes, nose, throat, lungs and bronchi were conducted using a standardized questionnaire. Exposure: Formaldehyde measurements in the breathing zone, 3–4 15 min samples per person in the exposed group. No formaldehyde measurements reported for referent group. Formaldehyde TWA: 0.40 mg/m³, range: 0.12–1.32 mg/m³. Peak concentration (15 min): 0.70 mg/m³, range: 0.14–2.6 mg/m³. Additional measurements of solvents and dust (4 hr) Analysis: Group comparisons, chi-square statistic <i>Low confidence</i> Potential for healthy survivor bias with attenuation in measure of association. Confounding and no adjustment in analyses.	Symptom Prevalence at Work <table><tr><th></th><th>Exposed N (%)</th><th>Referent N (%)</th></tr><tr><td>Eye</td><td>25 (65.8)</td><td>3 (16.7)</td></tr><tr><td>Nose, Throat</td><td>15 (39.5)</td><td>0</td></tr><tr><td>Dyspnea</td><td>4 (10.5)</td><td>0</td></tr><tr><td>Chest oppression</td><td>4 (10.5)</td><td>0</td></tr><tr><td>Cough</td><td>2 (5.3)</td><td>0</td></tr></table>		Exposed N (%)	Referent N (%)	Eye	25 (65.8)	3 (16.7)	Nose, Throat	15 (39.5)	0	Dyspnea	4 (10.5)	0	Chest oppression	4 (10.5)	0	Cough	2 (5.3)	0		
	Exposed N (%)	Referent N (%)																			
Eye	25 (65.8)	3 (16.7)																			
Nose, Throat	15 (39.5)	0																			
Dyspnea	4 (10.5)	0																			
Chest oppression	4 (10.5)	0																			
Cough	2 (5.3)	0																			
Wilhelmsson and Holmstrom (1992); Holmström and Wilhelmsson (1988) (Sweden) Prevalence study, three test groups chosen by the Swedish Board of Occ. Safety and Health. Group 1: 70 exposed to formaldehyde at a chemical plant (resins and impregnation of paper for laminate production), mean age 36.9 years, 87% male, work duration 10.4 year (SD 7.3), range 1–36 year. Group 2: 100 exposed to wood dust and formaldehyde, mean age 40.5 years, 93% male, work duration 16.6 year (SD 11.3), range 1–45 year. Group 3 (referent), 36 governmental clerks living in same village as chemical plant, mean age 39.9 years, 56% male, work duration 11.4 (SD 5.4), 4–18 year. Outcome: Questionnaire and medical examination, excluding upper airway infections. Atopics identified and analyzed separately from nonatopics based on a laboratory test utilizing the allergosorbent principle. Exposure: Breathing zone (personal samplers, 1–2 hrs), mean, range 1985: Group 1: 0.26 (SD 0.17) mg/m³; 0.05–0.50 mg/m³. Group 2: 0.25 (SD 0.05) mg/m³; 0.2–0.3 mg/m³ and 1.65 mg/m³ for wood dust. Group 3 Referent: 0.09 mg/m³ Cumulative exposure (dose-years) based on JEM No occupational exposure to solvents; other agents (phenol, ammonia, epichlorhydrin, methanol, and ethanol) less than 1% above PEL. Analysis: Compared symptom prevalence across exposure groups, chi-square <i>Low (↓) confidence</i>	Significantly increased symptom prevalence reported in formaldehyde exposed groups <table><tr><th></th><th colspan="3">Exposure Group</th></tr><tr><th></th><th>1</th><th>2</th><th>3</th></tr><tr><td>Nasal</td><td>64%*</td><td>53%*</td><td>25%</td></tr><tr><td>Eye</td><td>24%*</td><td>21%</td><td>6%</td></tr><tr><td>Deep airway discomfort</td><td>44%*</td><td>39%*</td><td>14%</td></tr></table> *<i>p</i> < 0.05 No significant difference between atopics vs. nonatopics in symptom prevalence. Majority reported symptoms did not change over time		Exposure Group				1	2	3	Nasal	64%*	53%*	25%	Eye	24%*	21%	6%	Deep airway discomfort	44%*	39%*	14%
	Exposure Group																				
	1	2	3																		
Nasal	64%*	53%*	25%																		
Eye	24%*	21%	6%																		
Deep airway discomfort	44%*	39%*	14%																		

Supplemental Information—Formaldehyde (Inhalation)

(Reference), study design, exposure levels	Results																																																																																																									
Potential for healthy survivor bias with attenuation in measure of association.; groups selected from different source populations; Questionnaire not described. Potential confounding and no adjustment in analyses.																																																																																																										
Kilburn et al. (1985a) (Los Angeles) Prevalence study, 76 female histology technicians in 23 hospitals & 2 labs (exposed), 97% of eligible, mean (SD) age 40.8 (11.6) years, work duration 12.8 (9.3) years; 56 women in referent (secretaries and clerks in same institutions) matched with 40 of the technicians for age, cigarette smoking, and ethnicity, mean (SD) age 39.5 (10.5) years. Outcome: Questionnaire for symptoms; composite experience for previous months or years Exposure: Environmental samples for formaldehyde, xylene, toluene, and chloroform by regional NIOSH laboratory in 10 of 25 labs; 1–4 hrs sampling time. Collected information on exposures, work practices and ventilation. Tissue specimen preparation, Formaldehyde 0.2–1.9 ppm (0.25–2.34 mg/m ³) ^a ; rooms with tissue processors, xylene 8.9–12.6 ppm, chloroform 2–19.1 ppm; Staining and cover-slipping, xylene 3.2–102 ppm, toluene 8.9–12.6 ppm. Clerical offices Formaldehyde ND; xylene ND Analysis: Prevalence by hours formaldehyde exposure and xylene exposure (statistical analyses not provided). <i>Low confidence</i> Extended time period for symptom reporting may have reduced accuracy of recall; incomplete matching; results of statistical analyses were not reported.	Formaldehyde, xylene and toluene concentrations were not correlated with symptoms (data not shown). Symptom Prevalence (%) by Duration of Formaldehyde Exposure (hours) <table><tr><td></td><td></td><td colspan="3">Formaldehyde</td><td colspan="2">Xylene: #</td></tr><tr><td></td><td></td><td colspan="3">(Hours)</td><td colspan="2">Slides Cover</td></tr><tr><td>Symptom</td><td>Ref</td><td>0</td><td>1–3</td><td>>4</td><td><100</td><td>>4 hours¹ <100</td></tr><tr><td>Number</td><td></td><td>7</td><td>22</td><td>47</td><td>27</td><td>20</td></tr><tr><td>< odor²</td><td>5</td><td>14</td><td>32</td><td>32</td><td>22</td><td>45</td></tr><tr><td>Eye</td><td>20</td><td>28</td><td>59</td><td>66</td><td>63</td><td>70</td></tr><tr><td>Throat</td><td>12</td><td>14</td><td>36</td><td>49</td><td>37</td><td>65</td></tr><tr><td>Dry Mouth</td><td>20</td><td>43</td><td>50</td><td>47</td><td>41</td><td>55</td></tr><tr><td>Cough</td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>Dry</td><td>9</td><td>14</td><td>23</td><td>34</td><td>22</td><td>50</td></tr><tr><td>Mucous</td><td>9</td><td>14</td><td>0</td><td>19</td><td>7</td><td>35</td></tr><tr><td>Blood</td><td>0</td><td>0</td><td>0</td><td>8.5</td><td>4</td><td>15</td></tr><tr><td>Chest</td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>Tight</td><td>5</td><td>14</td><td>27</td><td>40</td><td>26</td><td>60</td></tr><tr><td>Pain</td><td>5</td><td>14</td><td>23</td><td>40</td><td>37</td><td>40</td></tr></table>			Formaldehyde			Xylene: #				(Hours)			Slides Cover		Symptom	Ref	0	1–3	>4	<100	>4 hours ¹ <100	Number		7	22	47	27	20	< odor ²	5	14	32	32	22	45	Eye	20	28	59	66	63	70	Throat	12	14	36	49	37	65	Dry Mouth	20	43	50	47	41	55	Cough							Dry	9	14	23	34	22	50	Mucous	9	14	0	19	7	35	Blood	0	0	0	8.5	4	15	Chest							Tight	5	14	27	40	26	60	Pain	5	14	23	40	37	40
		Formaldehyde			Xylene: #																																																																																																					
		(Hours)			Slides Cover																																																																																																					
Symptom	Ref	0	1–3	>4	<100	>4 hours ¹ <100																																																																																																				
Number		7	22	47	27	20																																																																																																				
< odor ²	5	14	32	32	22	45																																																																																																				
Eye	20	28	59	66	63	70																																																																																																				
Throat	12	14	36	49	37	65																																																																																																				
Dry Mouth	20	43	50	47	41	55																																																																																																				
Cough																																																																																																										
Dry	9	14	23	34	22	50																																																																																																				
Mucous	9	14	0	19	7	35																																																																																																				
Blood	0	0	0	8.5	4	15																																																																																																				
Chest																																																																																																										
Tight	5	14	27	40	26	60																																																																																																				
Pain	5	14	23	40	37	40																																																																																																				

CI = confidence interval; MDF = medium density fiberboard; OR = odds ratio; OSB = oriented strand board; SE = standard error.

^aConcentrations reported by authors as ppm or ppb converted to mg/m³.

C.5. PULMONARY FUNCTION (SUPPORTING INFORMATION FOR HAZARD)

The acute, controlled human exposure studies involving healthy or asthmatic volunteers generally did not observe changes, even at high concentrations, although two studies by one research team observed small decrements (<5%) when longer exercise components (15 minutes) were included (see Appendix C.5.1). Studies using shorter exercise components (8–10 minutes) reported no changes. Two studies of asthmatic volunteers included an allergen challenge (dust mites, pollen), which resulted in a hyperreactive bronchial response at a lower challenge dose associated with formaldehyde exposure compared to clean air in one study that imposed mouth breathing (nose clips) (see Section 3.2.4 of the Toxicological Review). The studies of occupational

groups or dissection labs that measured pulmonary function declines over the course of the workday or lab (Appendix C.5.2) provide support for formaldehyde-related effects over a short-term exposure; however, many did not account for diurnal changes, limiting the interpretation of these results.

C.5.1. Controlled human exposure studies

Formaldehyde exposures (0.62–3.7 mg/m³), lasting from minutes to up to 5 hours, have not induced pulmonary function deficits in healthy, nonexercising volunteers in controlled human exposure studies (see Appendix B.3.3 for study evaluation summaries). The studies exposed small numbers (<25) of diverse individuals, often including males and females of varying age, and two included current smokers [31% of participants in the study described in Andersen ([1979](#)) and Andersen and Molhave ([1983](#)), and 13% of the participants in Schachter et al. ([1987](#))]. In some studies, the variation around the mean change in lung function was quite large suggesting that the response to exposure was large in some individuals, and in others, the response was small ([Witek et al., 1986](#); [Schachter et al., 1986b](#); [Schachter et al., 1987](#)).

In contrast to the studies of exposure without exercise, small but statistically significant deficits in pulmonary function (e.g., decreased FEV₁, FVC, FEV₃, FEF_{25–75}, specific airways conductance) during formaldehyde exposures of 2.5 or 3.7 mg/m³ were reported in two studies from one research group that included two or more 15-minute exercise regimens within the study protocol ([Green et al., 1987](#); [Green et al., 1989](#)). Although the average decrease in lung function after 55 minutes of exposure was considered small by the authors (2.2–3.3%), 5 of the 38 subjects individuals exhibited clinically significant deficits defined as a 10% or greater change, even after only 2 hours of exposure, suggesting that individual susceptibility may be an important consideration ([Green et al., 1987](#)). These effects were not seen, however, in studies with shorter exercise segments [8–10 minutes; ([Schachter et al., 1986b](#); [Schachter et al., 1987](#); [Kulle et al., 1987](#))].

One of two studies of asthmatic volunteers that included an allergen challenge (dust mites, pollen), reported a hyperreactive bronchial response triggered at a lower challenge dose associated with formaldehyde exposure compared to clean air. This study included the use of nose clips to impose mouth breathing ([Casset et al., 2006](#)) (see Section 3.2.4 of the Toxicological Review).

Table C-24. Formaldehyde effects on pulmonary function in controlled human exposure studies

Study and design	Results
Medium Confidence (Randomized, results fully reported)	
References: Witek et al. (1986) ; Schachter et al. (1986a) Population: N = 15 healthy, age 18–35 years, N=15 asthmatic, age 22 ± 5 years, all nonsmokers. Exposure: 40 min; Clean air and 2 ppm	No decrements in percent change from baseline in resting protocol; FVC, FEV ₁ , MEF50% (shown below), MEF40% or R _{aw} .

Supplemental Information—Formaldehyde (Inhalation)

Study and design	Results																																																									
(2.46 mg/m ³) ^a Protocol: Random assignment to order of exposure, double blinded. Two dose levels, four exposure conditions, 2 d at rest and 2 d with exercise segment (10 min, at 10 min into the exposure period), separated by 4 d. Testing at baseline, and at 4 times during 40-min exposure, and 10 and 30 min postexposure. Change from baseline tested using “standard test” and Bonferroni adjustment.	Exercise protocol showed decrement in MEF50% 30 min after exposure end. Percent Change from Baseline (Mean±SD) <table><tr><th></th><th>Clean Air</th><th>2 ppm</th></tr><tr><td>FVC (L)</td><td colspan="2">During exposure (@ 40 min.)</td></tr><tr><td>rest</td><td>-1.14 ± 4.8</td><td>-0.99 ± 3.5</td></tr><tr><td>exercise</td><td>1.6 ± 7.7</td><td>0.17 ± 6.2</td></tr><tr><td>FEV₁ (L)</td><td></td><td></td></tr><tr><td>rest</td><td>-0.41 ± 5.0</td><td>1.65 ± 4.5</td></tr><tr><td>exercise</td><td>4.87 ± 8.3*</td><td>4.56 ± 5.3**</td></tr><tr><td>MEF50% (L/sec)</td><td></td><td></td></tr><tr><td>rest</td><td>2.74 ± 4.4</td><td>7.4 ± 5.0*</td></tr><tr><td>exercise</td><td>8.72 ± 12.6</td><td>8.8 ± 8.1**</td></tr></table> <table><tr><td>FVC (L)</td><td colspan="2">30 min. postexposure</td></tr><tr><td>rest</td><td>0.31 ± 5.1</td><td>1.75 ± 3.5</td></tr><tr><td>exercise</td><td>-2.53 ± 5.4</td><td>-0.25 ± 5.6</td></tr><tr><td>FEV₁ (L)</td><td></td><td></td></tr><tr><td>rest</td><td>0.5 ± 4.7</td><td>-1.15 ± 5.3</td></tr><tr><td>exercise</td><td>-0.37 ± 4.5</td><td>1.76 ± 4.91</td></tr><tr><td>MEF50% (L/sec)</td><td></td><td></td></tr><tr><td>rest</td><td>-0.87 ± 5.4</td><td>2.65 ± 8.1</td></tr><tr><td>exercise</td><td>1.07 ± 5.3</td><td>-5.74 ± 5.4**</td></tr></table> *p <.05; **p <.01		Clean Air	2 ppm	FVC (L)	During exposure (@ 40 min.)		rest	-1.14 ± 4.8	-0.99 ± 3.5	exercise	1.6 ± 7.7	0.17 ± 6.2	FEV₁ (L)			rest	-0.41 ± 5.0	1.65 ± 4.5	exercise	4.87 ± 8.3*	4.56 ± 5.3**	MEF50% (L/sec)			rest	2.74 ± 4.4	7.4 ± 5.0*	exercise	8.72 ± 12.6	8.8 ± 8.1**	FVC (L)	30 min. postexposure		rest	0.31 ± 5.1	1.75 ± 3.5	exercise	-2.53 ± 5.4	-0.25 ± 5.6	FEV₁ (L)			rest	0.5 ± 4.7	-1.15 ± 5.3	exercise	-0.37 ± 4.5	1.76 ± 4.91	MEF50% (L/sec)			rest	-0.87 ± 5.4	2.65 ± 8.1	exercise	1.07 ± 5.3	-5.74 ± 5.4**
	Clean Air	2 ppm																																																								
FVC (L)	During exposure (@ 40 min.)																																																									
rest	-1.14 ± 4.8	-0.99 ± 3.5																																																								
exercise	1.6 ± 7.7	0.17 ± 6.2																																																								
FEV₁ (L)																																																										
rest	-0.41 ± 5.0	1.65 ± 4.5																																																								
exercise	4.87 ± 8.3*	4.56 ± 5.3**																																																								
MEF50% (L/sec)																																																										
rest	2.74 ± 4.4	7.4 ± 5.0*																																																								
exercise	8.72 ± 12.6	8.8 ± 8.1**																																																								
FVC (L)	30 min. postexposure																																																									
rest	0.31 ± 5.1	1.75 ± 3.5																																																								
exercise	-2.53 ± 5.4	-0.25 ± 5.6																																																								
FEV₁ (L)																																																										
rest	0.5 ± 4.7	-1.15 ± 5.3																																																								
exercise	-0.37 ± 4.5	1.76 ± 4.91																																																								
MEF50% (L/sec)																																																										
rest	-0.87 ± 5.4	2.65 ± 8.1																																																								
exercise	1.07 ± 5.3	-5.74 ± 5.4**																																																								
Reference: Schachter et al. (1987) Population: N = 15 healthy hospital laboratory workers routinely exposed to HCHO as part of their job, age 32 ± 11.3 years, 33.3 % male, N = 2 smokers. Exposure: 40 min; clean air and 2.0 ppm (2.46 mg/m ³) ^a Protocol: Random assignment to order of exposure, double blinded. Two dose levels, four exposure conditions, 2 d at rest and 2 d with exercise. One 10-min exercise segments at 5 min into the 40-min exposure period. Testing at baseline, and at 4 times during exposure, and 10 and 30 min postexposure. Percent change from baseline tested using one sample t-test with Bonferroni adjustment.	Percent Change from Baseline (Mean±SD) <table><tr><th></th><th>Clean Air</th><th>2 ppm</th></tr><tr><td>FVC (L)</td><td colspan="2">During exposure (@ 40 min.)</td></tr><tr><td>rest</td><td>-1.64 ± 5.67</td><td>-1.30 ± 3.64</td></tr><tr><td>exercise</td><td>-1.32 ± 6.94</td><td>-1.60 ± 6.03</td></tr><tr><td>FEV₁ (L)</td><td></td><td></td></tr><tr><td>rest</td><td>-1.25 ± 5.25</td><td>-2.05 ± 3.62</td></tr><tr><td>exercise</td><td>-0.67 ± 6.33</td><td>-1.56 ± 6.02</td></tr></table> <table><tr><td>FVC (L)</td><td colspan="2">30 min. postexposure</td></tr><tr><td>rest</td><td>0.68 ± 4.13</td><td>-0.54 ± 2.51</td></tr><tr><td>exercise</td><td>0.30 ± 4.58</td><td>-0.07 ± 4.25</td></tr><tr><td>FEV₁ (L)</td><td></td><td></td></tr><tr><td>rest</td><td>1.94 ± 5.85</td><td>-0.95 ± 3.0</td></tr><tr><td>exercise</td><td>0.62 ± 3.81</td><td>0.23 ± 4.2</td></tr></table>		Clean Air	2 ppm	FVC (L)	During exposure (@ 40 min.)		rest	-1.64 ± 5.67	-1.30 ± 3.64	exercise	-1.32 ± 6.94	-1.60 ± 6.03	FEV₁ (L)			rest	-1.25 ± 5.25	-2.05 ± 3.62	exercise	-0.67 ± 6.33	-1.56 ± 6.02	FVC (L)	30 min. postexposure		rest	0.68 ± 4.13	-0.54 ± 2.51	exercise	0.30 ± 4.58	-0.07 ± 4.25	FEV₁ (L)			rest	1.94 ± 5.85	-0.95 ± 3.0	exercise	0.62 ± 3.81	0.23 ± 4.2																		
	Clean Air	2 ppm																																																								
FVC (L)	During exposure (@ 40 min.)																																																									
rest	-1.64 ± 5.67	-1.30 ± 3.64																																																								
exercise	-1.32 ± 6.94	-1.60 ± 6.03																																																								
FEV₁ (L)																																																										
rest	-1.25 ± 5.25	-2.05 ± 3.62																																																								
exercise	-0.67 ± 6.33	-1.56 ± 6.02																																																								
FVC (L)	30 min. postexposure																																																									
rest	0.68 ± 4.13	-0.54 ± 2.51																																																								
exercise	0.30 ± 4.58	-0.07 ± 4.25																																																								
FEV₁ (L)																																																										
rest	1.94 ± 5.85	-0.95 ± 3.0																																																								
exercise	0.62 ± 3.81	0.23 ± 4.2																																																								
Reference: Green et al. (1987) Population: n = 22, mean age 26.9 ± 3.6 year, nonsmoking, no history of allergies or hay fever; gender not reported. Exposure: 60 min, clean air or 3.01 ± 0.01 ppm [3.7 ± 0.01 mg/m ³] ^a Protocol: Random assignment to order of exposure; single blinded. Two 15-min exercise segments at 15 and 45 min into the 60-min exposure period. Testing before and during exposure	Declines evident at 47 min, Statistically significant decrements measured in several endpoints at 55 min. Absolute values at 55 min exposure <table><tr><th></th><th>Clean air</th><th>3 ppm</th></tr><tr><td>FVC</td><td>5.04 ± 0.15</td><td>4.92 ± 0.15*</td></tr><tr><td>FEV₁</td><td>4.29 ± 0.12</td><td>4.15 ± 0.13*</td></tr><tr><td>FEV₃</td><td>4.93 ± 0.15</td><td>4.80 ± 0.15*</td></tr></table>		Clean air	3 ppm	FVC	5.04 ± 0.15	4.92 ± 0.15*	FEV ₁	4.29 ± 0.12	4.15 ± 0.13*	FEV ₃	4.93 ± 0.15	4.80 ± 0.15*																																													
	Clean air	3 ppm																																																								
FVC	5.04 ± 0.15	4.92 ± 0.15*																																																								
FEV ₁	4.29 ± 0.12	4.15 ± 0.13*																																																								
FEV ₃	4.93 ± 0.15	4.80 ± 0.15*																																																								

Supplemental Information—Formaldehyde (Inhalation)

Study and design	Results
period (approximate 15 min intervals); paired <i>t</i>-test comparing ratio of exposed value at time(n) to time(0) to ratio of clean air value at time(n) to time(0).	FEF₂₅₋₇₅ 4.74 ± 0.25 4.56 ± 0.29 <i>*p</i> < 0.02, paired <i>t</i>-test
Reference: Green et al. (1989) Population: N = 24, 14 women and 10 men, age 18–35 years, nonsmoking, no history of asthma, no medications, FVC >80%, FEV/FVC >75%. Exposure: 2 hr, clean air, 3 ppm [3.69 mg/m³]^a, 0.5 mg/m³ ACA (activated aerosol carbon), 3 ppm plus 0.5 mg/m³ ACA. Protocol: Randomized block design with 4 2-hr exposure conditions, one per week; double blinded. Four 15-min exercise segments at 15, 45, 75, and 105 min into the 2-hr exposure period. Spirometric testing before and during exposure period (5 times). PEF at 2 hrs, and hourly intervals for 8-hrs postexposure, and at 12 and 16 hrs postexposure.	Results presented in graphs for FEV₁, FVC, FEF₂₅₋₇₅, and FEV₃. During exposure to formaldehyde + ACA, statistically significant changes were measured in FVC and FEV₃ at several intervals and decreased SG_{aw} was measured at the end of exposure; magnitudes of the changes were less than 10% of baseline. No statistically significant (<i>p</i> >0.05) effects were observed on FVC, FEV₁, or FEV₃, at any of 5 intervals during 2-hr exposures; for formaldehyde only exposure, statistically significant decrements were observed for FEF₂₅₋₇₅ and SGaw at 50 and 80 min, magnitudes of the changes were 3–5%, compared with baseline.
Low Confidence (Incomplete reporting of results, or blinding not described with multiple exposure levels)	
References: Andersen (1979); Andersen and Molhave (1983) Population: N = 16 healthy students, age 30–33, 68.8 % male, 31.2% smokers Exposure: 5 hours; 0.3, 0.5, 1.0, and 2.0 mg/m³ Protocol: Formaldehyde exposure order determined by Latin square design; blinding not described. Groups of 4 over 4 d; testing before (during 2 hrs clean air) and 2 times during exposure. No exercise component.	No change in FVC, FEV₁, or FEF₂₅₋₇₅; data presented in graphs Visual inspection indicates decrease in VC at 1 and 2 mg/m³, FEF₂₅₋₇₅ at 0.5 mg/m³ (not statistically significant).
Reference: Kulle et al. (1987) Population: Group 1 (N = 10), Group 2 (N = 9), nonsmoking healthy, age 26.3 ± 4.7 years, 53% male. Exposure: 3 hr, Group 1: 0.0, 0.5, 1.0, or 2.0 ppm at rest (0.0, 0.62, 1.23, 2.46 mg/m³)^a at rest, and an additional 2.0 ppm with exercise; Group 2: 0.0, 1.0, or 3.0 ppm (0.0, 1.23, or 3.69 mg/m³), and an additional 2.0 ppm with exercise. Protocol: Exposure order randomly assigned; blinding not reported. 3-hr exposures each week, at same time on 5 occasions. 8-min exercise segment every half hour during 2 ppm exposure. Pulmonary function tests (FVC, FEV₁, FEF₂₅₋₇₅ and SGaw) at 0, 30, 60, 90, 120, 150, and 180 min during exposure, and 24 hrs postexposure.	No change in pulmonary function (means by testing time, no SD presented).
Reference: Lang et al. (2008) Population: N = 21, age 19–39 years, nonsmoking, healthy volunteers.	No statistically different differences between baseline Day 1 and postexposure on Day 10 (data not presented).

Supplemental Information—Formaldehyde (Inhalation)

Study and design	Results																											
Exposure: 4 hours, clean air, 0.15, 0.3 and 0.5 ppm (0.0, 0.19, 0.37, and 0.62 mg/m³)^a; additional 0.3 and 0.5 ppm with peaks up to 1.0 ppm (1.23 mg/m³)^a; additional 0.0, 0.3, and 0.5 ppm with ethyl acetate to “mask” formaldehyde. Protocol: Exposure order randomly assigned; double blinded. Ten 4-hour exposure conditions, one per day, over 10 days. Airway resistance (R_{tot}, PEF, FEV₁, FEF₂₅₋₇₅, and SGaw measured on first exam and on first and last exposure day, pre and post exposure. No exercise component.																												
Low Confidence (No randomization; blinding not discussed)																												
Reference: Day et al. (1984) Population: 2 groups of 9 adults each. Group 1, N = 9, adversely affected (nonrespiratory) by HCHO fumes emitted by urea foam insulation (UFFI) in their homes. Group 2, N = 9, not affected by UFFI present in their homes, or volunteer with no UFFI exposure. Descriptive data on study subjects was not presented. Exposure: 1.5 hrs in chamber, 1.0 ppm (1.23 mg/m³)^a, 0.5 hr under hood, 1.2 ppm (1.48 mg/m³)^a; no clean air control. Protocol: Testing before, after, and 6.5 hrs after exposure. No exercise component.	No change in FVC, FEV ₁ , or FEF ₂₅₋₇₅ (mean ± SD) paired <i>t</i> -test																											
Reference: Sauder et al. (1986) Population: n = 9, mean age 26 ± 3.6 years, healthy, non allergic (for 6 wks prior to test), nonsmokers. Exposure: 3 hrs; 0, 3 ppm (3.69 mg/m³)^a Protocol: Nonrandom assignment; blinding not described. 8-min bicycle exercise followed by spirometry measurements after each 30-min interval during 3 hr exposures. First day clean air only, second day 3 ppm formaldehyde. Testing again after 24 hrs. Repeated measures ANOVA	<table><tr><th></th><th>Clean air</th><th>3 ppm</th></tr><tr><td></td><td colspan="2">30 minutes</td></tr><tr><td>FVC</td><td>4.61</td><td>4.62</td></tr><tr><td>FEV₁</td><td>3.98</td><td>3.90*</td></tr><tr><td>FEF₂₅₋₇₅</td><td>4.46</td><td>4.16**</td></tr><tr><td></td><td colspan="2">180 minutes</td></tr><tr><td>FVC</td><td>4.71</td><td>4.68</td></tr><tr><td>FEV₁</td><td>4.02</td><td>3.99</td></tr><tr><td>FEF₂₅₋₇₅</td><td>4.45</td><td>4.48</td></tr></table> *<i>p</i> <0.05, ** <i>p</i> <0.01, paired <i>t</i>-test Statistically significant decreases in FEV₁ (2%) and FEF₂₅₋₇₅ (7%) after first 30 minutes; Range in response: FEV₁ -5% to +1% FEF₂₅₋₇₅ -14% to +2% No other changes during exposure or 24 hrs after.		Clean air	3 ppm		30 minutes		FVC	4.61	4.62	FEV ₁	3.98	3.90*	FEF ₂₅₋₇₅	4.46	4.16**		180 minutes		FVC	4.71	4.68	FEV ₁	4.02	3.99	FEF ₂₅₋₇₅	4.45	4.48
	Clean air	3 ppm																										
	30 minutes																											
FVC	4.61	4.62																										
FEV ₁	3.98	3.90*																										
FEF ₂₅₋₇₅	4.46	4.16**																										
	180 minutes																											
FVC	4.71	4.68																										
FEV ₁	4.02	3.99																										
FEF ₂₅₋₇₅	4.45	4.48																										

^aConcentrations reported by authors as ppm or ppb converted to mg/m³.

C.5.2. Change in pulmonary function measures during a work shift or anatomy lab session

Daily changes in pulmonary function measures (e.g., across a work shift or during a lab session lasting a few hours) were assessed in studies among workers employed for several years in exposed jobs or among students enrolled in an anatomy lab. Declines in FEV₁, FVC and FEV₁/FVC were observed in some studies ([Neghab et al., 2011](#); [Horvath et al., 1988](#); [Herbert et al., 1994](#); [Alexandersson et al., 1982](#); [Alexandersson and Hedenstierna, 1989](#)) but not others ([Malaka and](#)

[Kodama, 1990](#); [Löfstedt et al., 2009](#); [Holness and Nethercott, 1989](#); [Alexandersson, 1988](#); [Alexandersson and Hedenstierna, 1988](#)). Most of the studies measured changes only among exposed individuals; measurements in a comparison group would have allowed adjustment for diurnal effects. Daily declines in FEF_{25-75} were more consistently reported by the occupational studies of wood products employees ([Neghab et al., 2011](#); [Malaka and Kodama, 1990](#); [Horvath et al., 1988](#); [Alexandersson et al., 1982](#)), and exposed groups had larger decrements compared to the referent groups among the two studies that reported cross-shift changes in both groups ([Malaka and Kodama, 1990](#); [Horvath et al., 1988](#)). One well-conducted study using repeated peak expiratory flow measures taken by students trained in the procedure at multiple points during dissection lab sessions found that PEF declined over the course of a lab and these daily declines became attenuated over successive weeks ([Kriebel et al., 2001](#)).

Figures C-19–C-21 present study findings for three spirometry measures, FEF_{25-75} , FEV_1 , and FVC, and study details are summarized in Table C-25. For each measure, the mean difference across a work shift or lab session in exposed and referent groups (when reported) is plotted with error bars depicting the standard error. Separate graphs depict the mean before and after difference expressed as absolute value (e.g., FEV_1 in liters) or percent predicted. The third plot shows results for studies that reported changes as a percent of the baseline value.

Supplemental Information—Formaldehyde (Inhalation)

Reference	Setting	Referent	Confidence
Malaka, 1990, N = 55	Wood products	N = 50	Medium
Alexandersson, 1989, N = 21	Wood products	Not measured	Low
Horvath, 1988, N = 109	Wood products	N = 254	High
Alexandersson, 1988, N = 38	Wood products	Not measured	Low
Alexandersson, 1982, N = 47	Wood products	Not measured	Low
Khaliq, 2009, N = 20	Anatomy lab	No referent	Low
Uba, 1989, N = 96	Anatomy lab	Week 2 vs baseline day	High

Reference	Setting	Referent	Confidence
Binawara, 2010, N = 80	Anatomy lab	No referent	Low

Reference	Setting	Referent	Confidence
Akbar-Khanzadeh, 1997, N = 50	Anatomy lab	N = 36	Low
Akbar-Khanzadeh, 1994, N = 34	Anatomy lab	N = 12	Medium

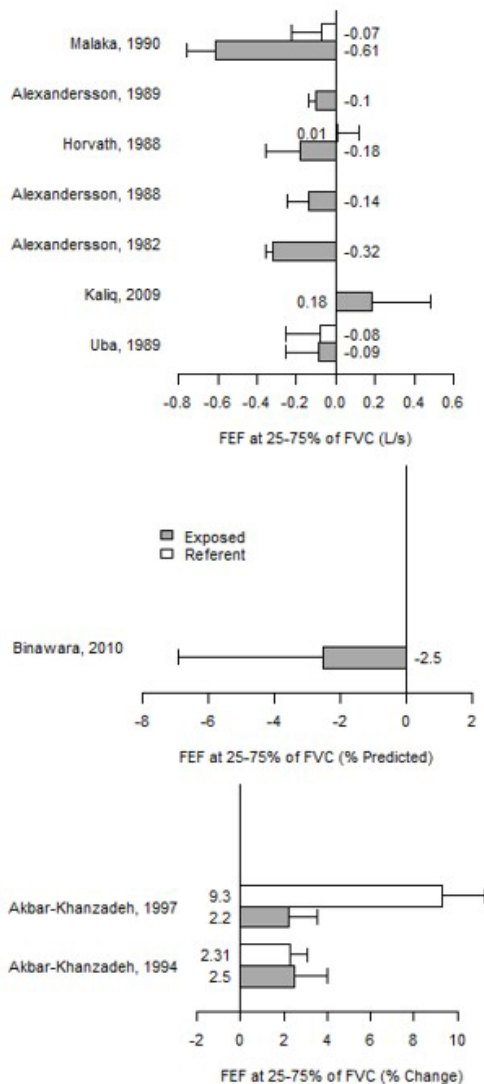


Figure C-19. Plots of change in FEF at 25–75% of FVC across a work shift or anatomy lab session by study with study details. The difference in reported means before and after shift or lab as either liters/second or % predicted are shown, and percent change in FEF across the lab was reported by two studies (3rd panel). Mean difference or percent change and SE are shown. These were calculated by EPA when not reported using SD for before and after means.

Reference	Setting	Referent	Confidence
Malaka, 1990, N = 55	Wood products	N = 50	Medium
Herbert, 1994, N = 99	Wood products	Not measured	Low
Alexandersson, 1989, N = 21	Wood products	Not measured	Low
Horvath, 1988, N = 109	Wood products	N = 254	High
Alexandersson, 1988, N = 38	Wood products	Not measured	Low
Alexandersson, 1982, N = 47	Wood products	Not measured	Low
Khaliq, 2009, N = 20,	Anatomy lab	No referent	Low
Chia, 1992, N = 13	Anatomy lab	Not measured	Low
Uba, 1989, N = 96	Anatomy lab	Week 2 vs baseline day	High

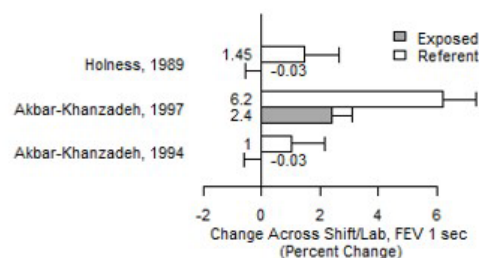
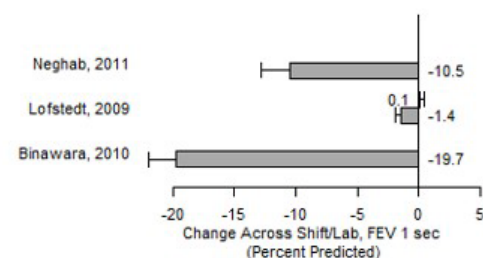
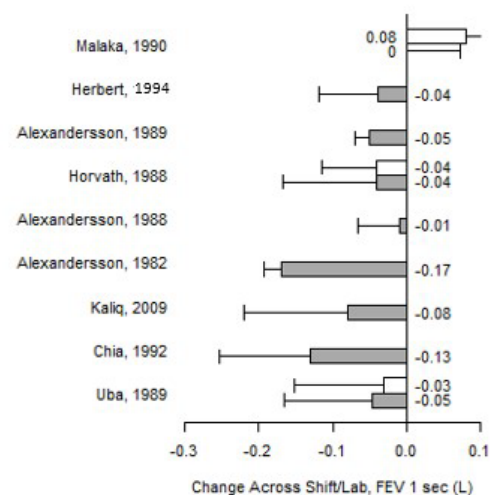


Figure C-20. Plots of change in FEV1 across a work shift or anatomy lab session by study with study details. The difference in reported means before and after shift or lab as either liters or % predicted are shown, or percent change in FEV1 across the lab. Mean difference or percent change and SE are shown. These were calculated by EPA when not reported using SD for before and after means.

Reference	Setting	Referent	Confidence
Malaka, 1990, N = 55	Wood products	N = 50	Medium
Herbert, 1994, N = 99	Wood products	Not measured	Low
Alexandersson, 1989, N = 21	Wood products	Not measured	Low
Horvath, 1988, N = 109	Wood products	N = 254	High
Alexandersson, 1988, N = 38	Wood products	Not measured	Low
Alexandersson, 1982, N = 47	Wood products	Not measured	Low
Khalil, 2009, N = 20,	Anatomy lab	No referent	Low
Chia, 1992, N = 13	Anatomy lab	Not measured	Low
Uba, 1989, N = 96	Anatomy lab	Week 2 vs baseline day	High

Reference	Setting	Referent	Confidence
Neghab, 2011, N = 70	Chemicals	Not measured	Low
Lofstedt, 2009, N = 64,	Chemicals	N = 134	Medium
Binawara, 2010, N = 80	Anatomy lab	No referent	Low

Reference	Setting	Referent	Confidence
Holness, 1989, N = 22	Embalming	N = 13	Medium
Akbar-Khanzadeh, 1997, N = 50	Anatomy lab	N = 36	Low
Akbar-Khanzadeh, 1994, N = 34	Anatomy lab	N = 12	Medium
Demographic information for Holness, 1989 are for entire study groups.			

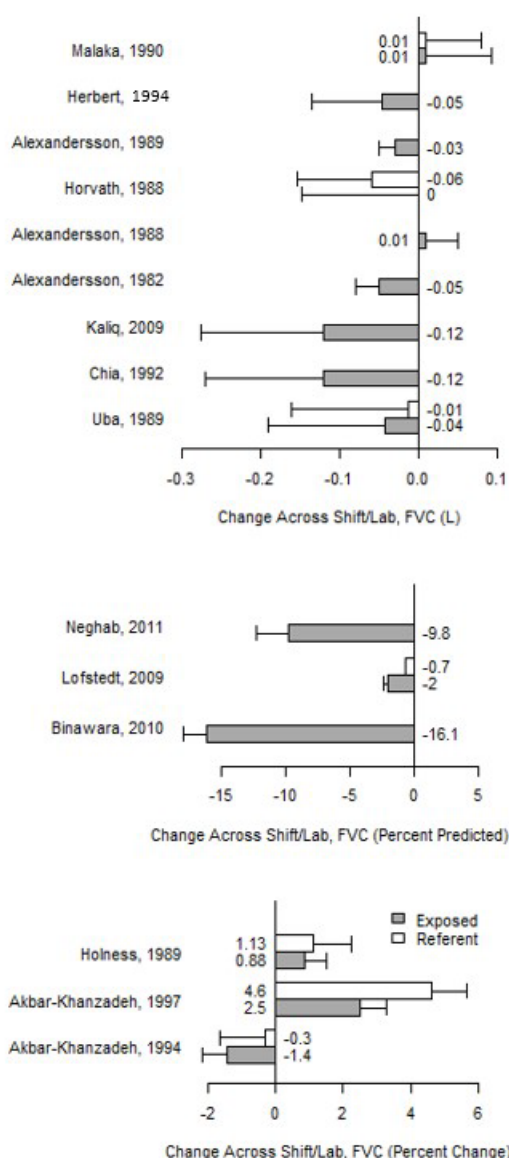


Figure C-21. Plots of change in FVC across a work shift or anatomy lab session by study with study details. The difference in reported means before and after shift or lab as either liters or % predicted are shown, or percent change in FVC across the lab. Mean difference or percent change and SE are shown. These were calculated by EPA when not reported using SD for before and after means.

Table C-25. Studies of pulmonary function measures during a work shift or anatomy lab session

Study information	Group characteristics	Measures reported and analysis methods
Occupational studies		

Supplemental Information—Formaldehyde (Inhalation)

Study information	Group characteristics	Measures reported and analysis methods
(Neghab et al., 2011) Resin production Confidence: Low (No comparison group)	Exposed: N = 70, male, age 38 year, 24% smokers; Referent: Not measured	FEV ₁ , FVC, FEV ₁ /FVC, PEF Mean values (percent predicted) before and after shift compared (paired <i>t</i> -test) in exposed
(Löfstedt et al., 2009) Chemical company Confidence: Medium (Healthy survivor effect)	Exposed: N = 64, 89% male, age 44 year, 25% smokers; Referent: N = 134, 88% male, age 40 year, 22% smokers	VC, FEV ₁ Compared mean difference across shift (percent predicted) between exposed and referent (regression); association with formaldehyde adjusting for isocyanate levels and smoking (regression)
(Malaka and Kodama, 1990) Plywood manufacture Confidence: Medium (healthy survivors)	Exposed: N = 55, male, age 27 year, 53% smokers; Referent: matched by age, ethnicity, and smoking; N = 50, male, age 29 year, 53% smokers	FEV ₁ , FVC, FEV ₁ /FVC, FEF ₂₅₋₇₅ Mean values before and after shift compared (paired <i>t</i> -test) in exposed and referent
(Herbert et al., 1994) Particle board manufacture Confidence: Low (No comparison group)	Exposed: N = 99, sex NR, age 35 year, 52% smokers; Referent: Not measured	FEV ₁ , FVC, FEV ₁ /FVC Mean values before and after shift compared (paired <i>t</i> -test) in exposed
(Alexandersson and Hedenstierna, 1989) Cabinet manufacture, 5-year follow-up of (Alexandersson et al., 1982) Confidence: Low (No comparison group)	Exposed: N = 21, male, age 37 year, 48% smokers; Referent: Not measured	FEV ₁ , FVC, FEV ₁ /FVC, FEF ₂₅₋₇₅ Mean values before and after shift compared, stratified by smoking status (paired <i>t</i> -test) in exposed
(Holness and Nethercott, 1989) Funeral workers (embalming) Confidence: Medium (comparison groups selected from different source populations)	Exposed: N = 22, 89% male, age 32 year, 50% smokers; Referent (community volunteers): N = 13, 84% male, age 28 year, 37% smokers (Demographic information for are for entire study groups)	FEV ₁ , FVC, FEF ₅₀ , FEF ₇₅ Compared mean percent change during embalming (or after 2–3 hr) (percent predicted) between exposed and referent (regression adjusting for age, height, and pack-year smoked)
(Horvath et al., 1988) Particle board manufacture Confidence: High	Exposed: N = 109, 57% male, age 37 year, 53% smokers; Referent (food processing): N = 254, 44% male, age 34 year, 53% smokers	FEV ₁ , FVC, FEV ₁ /FVC, FEF ₂₅₋₇₅ , PEF Mean values before and after shift (percent predicted) compared (paired <i>t</i> -test) in exposed and referent; correlation with formaldehyde concentration
(Alexandersson and Hedenstierna, 1988) ; Alexandersson (1988) Wood products Confidence: Low (No comparison group)	Exposed: N = 38, male, age 34 year, 50% smokers; Referent: Not measured	FEV ₁ , FVC, FEV ₁ /FVC, FEF ₂₅₋₇₅ Mean values before shift on first day and after shift on second day compared, stratified by smoking status (paired <i>t</i> -test) in exposed
(Alexandersson et al., 1982) Cabinet manufacture Confidence: Low (No comparison group)	Exposed: N = 47, male, age 35 year, 51% smokers; Referent: Not measured	FEV ₁ , FVC, FEV ₁ /FVC, FEF ₂₅₋₇₅ Mean values before and after shift compared, stratified by smoking status (paired <i>t</i> -test) in exposed
Anatomy lab (dissection)		

Supplemental Information—Formaldehyde (Inhalation)

Study information	Group characteristics	Measures reported and analysis methods
(Saowakon et al., 2015) Anatomy course Confidence: Low (No comparison group)	N = 36, gender NR, age 19.8 year, nonsmokers; no referent	FVC, FEV ₁ , FEV ₁ /FVC, FEF ₂₅₋₇₅ , PEF Mean values compared before and after dissection session (paired <i>t</i> -test) in exposed
(Binawara et al., 2010) Anatomy course Confidence: Low (No comparison group)	N = 80, male, age 20 year, nonsmokers; referent: No referent	FEV ₁ , FVC, FEV ₁ /FVC, FEF ₂₅₋₇₅ , PEF Mean values (percent predicted) before and after shift compared (paired <i>t</i> -test) in exposed
(Khaliq and Tripathi, 2009) Anatomy course Confidence: Low (No comparison group; small sample size)	Exposed: N = 20, male, age 18 year, nonsmokers; no referent	FEV ₁ , FVC, FEV ₁ /FVC, FEF ₂₅₋₇₅ , PEF Mean values before and after lab compared (repeated measure ANOVA) in exposed
(Akbar-Khanzadeh and Mlynek, 1997) Anatomy course Confidence: Low (Analyses did not account for possible acclimatization to formaldehyde over time)	Exposed: N = 50, 50% male, age 24 year, nonsmokers; referent (physiotherapy students): N = 36, 24% male, age 24 year, nonsmokers	FEV ₁ , FVC, FEF ₂₅₋₇₅ Compared mean percent change (standardized for baseline) over lab in exposed and referent (paired <i>t</i> -test); compared difference between groups (unpaired <i>t</i> -test)
(Akbar-Khanzadeh et al., 1994) Anatomy course, Confidence: Medium (Comparison groups dissimilar; small sample size in referent)	Exposed: N = 34, 71% male, age 26 year, nonsmokers; referent: N = 12, 67% male, age 31 year, nonsmokers	FEV ₁ , FVC, FEV ₁ /FVC, FEF ₂₅₋₇₅ Compared mean percent change (standardized for baseline) over lab in exposed and referent (paired <i>t</i> -test); compared difference between groups (unpaired <i>t</i> -test)
(Chia et al., 1992) Anatomy course Confidence: Low (No comparison group; small sample size)	Exposed: N = 13 male, n = 9 female, age NR, smoking NR; referent: Not measured	FEV ₁ , FVC (means adjusted for age and height); Mean values before and after lab compared (chi-square statistic)
(Uba et al., 1989) Anatomy course Confidence: High	Exposed: N = 96, 74% male, age 24 year, nonsmokers; comparison: Cross-lab change week 2 vs. baseline day	FEV ₁ , FVC, FEV ₁ /FVC, FEF ₂₅₋₇₅ Mean percent change over lab session at 2 weeks compared to baseline (repeated measures ANOVA, adjusted for sex)

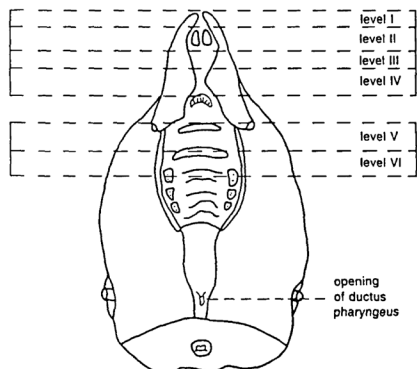
C.6. RESPIRATORY TRACT PATHOLOGY (SUPPORTING INFORMATION FOR HAZARD)

C.6.1. Short-term respiratory tract pathology studies in experimental animals

Due to the abundance of high-quality, longer duration exposure studies on respiratory tract effects in experimental animals, the results of supportive *medium* and *high confidence* short-term studies that did not provide information that was unexamined or inadequately examined in the longer term studies (i.e., species differences; the relative contribution of concentration and duration to lesion development) are summarized below in Table C-26 (note: the details of *low confidence* animal studies are not described for respiratory pathology owing to the large number of *high* and *medium confidence* studies available).

In addition, mechanistic evidence on changes in the respiratory tract, namely effects on mucociliary function and cellular proliferation, informed the interpretation of respiratory tract pathology. These effects are summarized in Appendix C.7.1.

Table C-26. Short-term respiratory pathology studies in animals

Reference and study design	Results																																																																																																																						
RAT																																																																																																																							
High Confidence																																																																																																																							
Reuzel et al. (1990) Wistar rats; male; 10/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers 22 hrs/d for 3 d. Test article: Paraformaldehyde. Actual concentrations were 0, 0.37 (±0.01), 1.4 (±0.0), and 3.8 (±0.1) mg/m³. ¹ This study also evaluated the combined effects of ozone and FA mixtures on nasal epithelium. Data presented here in the Results column are for FA-only exposed rats. Histopathologic evaluation of the respiratory tract included 6 standard sections of the nose.	<table><tr><td></td><td colspan="6">Concentration of FA</td></tr><tr><td></td><td colspan="2">0 mg/m³</td><td colspan="2">0.37 mg/m³</td><td colspan="2">1.4 mg/m³</td></tr><tr><td></td><td>II^a</td><td>III^a</td><td>II</td><td>III</td><td>II</td><td>III</td></tr><tr><td colspan="7">Disarrangement/loss of cilia without hyper/metaplasia</td></tr><tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr><tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr><tr><td colspan="7">Disarrangement/loss of cilia with hyper/metaplasia</td></tr><tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>1/10</td><td>0/10</td><td>2/9</td><td>0/9</td></tr><tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr><tr><td>Marked</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr><tr><td colspan="7">Keratinization</td></tr><tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr><tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr><tr><td colspan="7">Rhinitis</td></tr><tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>2/10</td><td>0/10</td><td>1/9</td><td>0/9</td></tr><tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/9</td><td>0/9</td></tr></table> ^a Level in the nose examined.								Concentration of FA							0 mg/m³		0.37 mg/m³		1.4 mg/m³			II ^a	III ^a	II	III	II	III	Disarrangement/loss of cilia without hyper/metaplasia							Minimal to slight	0/10	0/10	0/10	0/10	0/9	0/9	Moderate	0/10	0/10	0/10	0/10	0/9	0/9	Disarrangement/loss of cilia with hyper/metaplasia							Minimal to slight	0/10	0/10	1/10	0/10	2/9	0/9	Moderate	0/10	0/10	0/10	0/10	0/9	0/9	Marked	0/10	0/10	0/10	0/10	0/9	0/9	Keratinization							Minimal to slight	0/10	0/10	0/10	0/10	0/9	0/9	Moderate	0/10	0/10	0/10	0/10	0/9	0/9	Rhinitis							Minimal to slight	0/10	0/10	2/10	0/10	1/9	0/9	Moderate	0/10	0/10	0/10	0/10	0/9	0/9
	Concentration of FA																																																																																																																						
	0 mg/m³		0.37 mg/m³		1.4 mg/m³																																																																																																																		
	II ^a	III ^a	II	III	II	III																																																																																																																	
Disarrangement/loss of cilia without hyper/metaplasia																																																																																																																							
Minimal to slight	0/10	0/10	0/10	0/10	0/9	0/9																																																																																																																	
Moderate	0/10	0/10	0/10	0/10	0/9	0/9																																																																																																																	
Disarrangement/loss of cilia with hyper/metaplasia																																																																																																																							
Minimal to slight	0/10	0/10	1/10	0/10	2/9	0/9																																																																																																																	
Moderate	0/10	0/10	0/10	0/10	0/9	0/9																																																																																																																	
Marked	0/10	0/10	0/10	0/10	0/9	0/9																																																																																																																	
Keratinization																																																																																																																							
Minimal to slight	0/10	0/10	0/10	0/10	0/9	0/9																																																																																																																	
Moderate	0/10	0/10	0/10	0/10	0/9	0/9																																																																																																																	
Rhinitis																																																																																																																							
Minimal to slight	0/10	0/10	2/10	0/10	1/9	0/9																																																																																																																	
Moderate	0/10	0/10	0/10	0/10	0/9	0/9																																																																																																																	
	<table><tr><td></td><td colspan="4">Concentration of FA</td></tr><tr><td></td><td colspan="2">0 mg/m³</td><td colspan="2">3.8 mg/m³</td></tr><tr><td></td><td>II^a</td><td>III^a</td><td>II</td><td>III</td></tr><tr><td colspan="5">Disarrangement/loss of cilia without hyper/metaplasia</td></tr><tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td></tr><tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td></tr><tr><td colspan="5">Disarrangement/loss of cilia with hyper/metaplasia</td></tr><tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>7/10</td><td>3/10</td></tr><tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>3/10</td><td>5/10</td></tr><tr><td>Marked</td><td>0/10</td><td>0/10</td><td>2/10</td><td>0/10</td></tr><tr><td colspan="5">Keratinization</td></tr><tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>7/10</td><td>0/10</td></tr><tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>1/10</td><td>0/10</td></tr><tr><td colspan="5">Rhinitis</td></tr><tr><td>Minimal to slight</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td></tr><tr><td>Moderate</td><td>0/10</td><td>0/10</td><td>0/10</td><td>0/10</td></tr></table> ^a Level in the nose examined.								Concentration of FA					0 mg/m³		3.8 mg/m³			II ^a	III ^a	II	III	Disarrangement/loss of cilia without hyper/metaplasia					Minimal to slight	0/10	0/10	0/10	0/10	Moderate	0/10	0/10	0/10	0/10	Disarrangement/loss of cilia with hyper/metaplasia					Minimal to slight	0/10	0/10	7/10	3/10	Moderate	0/10	0/10	3/10	5/10	Marked	0/10	0/10	2/10	0/10	Keratinization					Minimal to slight	0/10	0/10	7/10	0/10	Moderate	0/10	0/10	1/10	0/10	Rhinitis					Minimal to slight	0/10	0/10	0/10	0/10	Moderate	0/10	0/10	0/10	0/10																																
	Concentration of FA																																																																																																																						
	0 mg/m³		3.8 mg/m³																																																																																																																				
	II ^a	III ^a	II	III																																																																																																																			
Disarrangement/loss of cilia without hyper/metaplasia																																																																																																																							
Minimal to slight	0/10	0/10	0/10	0/10																																																																																																																			
Moderate	0/10	0/10	0/10	0/10																																																																																																																			
Disarrangement/loss of cilia with hyper/metaplasia																																																																																																																							
Minimal to slight	0/10	0/10	7/10	3/10																																																																																																																			
Moderate	0/10	0/10	3/10	5/10																																																																																																																			
Marked	0/10	0/10	2/10	0/10																																																																																																																			
Keratinization																																																																																																																							
Minimal to slight	0/10	0/10	7/10	0/10																																																																																																																			
Moderate	0/10	0/10	1/10	0/10																																																																																																																			
Rhinitis																																																																																																																							
Minimal to slight	0/10	0/10	0/10	0/10																																																																																																																			
Moderate	0/10	0/10	0/10	0/10																																																																																																																			
Figure 1 from Reuzel et al. (1990) depicting cross levels of the rat nose evaluated for histopathological lesions.	Histopathological changes for Level I not reported.																																																																																																																						
Main limitations: No major limitations.																																																																																																																							

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results																																																																																																																																																							
	Histopathological changes for Levels IV, V, and VI reported together. Only change observed was minimal to slight rhinitis in rats (4/10) exposed to 3.8 mg/m ³ FA.																																																																																																																																																							
Medium Confidence																																																																																																																																																								
Andersen et al. (2008) Fischer 344 rats; male; 8/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for up to 3 wks. Rats sacrificed at end of single 6-hr exposure (day 1), 18 hrs after single 6-hr exposure (day 1 recovery), at end of 5 d of exposure (day 5), at end of 6 d of exposure (day 6), 18 hrs after 6 d of exposure (day 6 recovery), and at end of 15 d of exposure (day 15). Test article: Paraformaldehyde. Actual concentrations were determined on a daily basis and reported in the Results column. Target concentrations were 0, 0.9, 2.5, 7.4, and 18.5 mg/m ³ . ¹ This study also evaluated the effects of a single FA instillation (40 µL, 400 mM per nostril). Data presented here in the Results column are for inhalation exposures. Histopathologic evaluation of the respiratory tract included nasal sections at levels I (front of nose), II (anterior lateral meatus, anterior septum, medial aspect maxilloturbinate), and III (posterior lateral meatus, posterior septum). Main limitations: small sample size; somewhat high variability in chamber concentrations.	Target and Actual FA Concentrations^a <table><tr><th>Target concentration (mg/m³)</th><th>Day 1 (mg/m³)</th><th>Day 5 (mg/m³)</th><th>Day 6 (mg/m³)</th><th>Day 15 (mg/m³)</th></tr><tr><td>0</td><td>0±0</td><td>0±0</td><td>0±0</td><td>0±0</td></tr><tr><td>0.9</td><td>0.74±0.23</td><td>0.79±0.15</td><td>0.75±0.16</td><td>0.7±0.11</td></tr><tr><td>2.5</td><td>2.08±0.46</td><td>2.14±0.43</td><td>2.26±0.49</td><td>2.2±0.31</td></tr><tr><td>7.4</td><td>5.83±1.73</td><td>6.43±0.76</td><td>6.00±1.25</td><td>6.14±0.97</td></tr><tr><td>18.5</td><td>17.7±5.7</td><td>NA</td><td>NA</td><td>NA</td></tr></table> ^aDaily means ± SD. Histopathology Incidence <table><tr><th></th><th colspan="8">FA (mg/m³)</th></tr><tr><th></th><th>0</th><th colspan="2">0.9</th><th colspan="2">2.5</th><th colspan="2">7.4</th><th></th></tr><tr><th>Time point</th><th>InI^a</th><th>InI</th><th>EH</th><th>InI</th><th>EH</th><th>InI</th><th>EH</th><th>SM</th></tr><tr><td>Day 1</td><td>0^b</td><td>1</td><td>0</td><td>6</td><td>0</td><td>8</td><td>0</td><td>0</td></tr><tr><td>Day 1 R^c</td><td>4</td><td>2</td><td>1</td><td>1</td><td>3</td><td>7</td><td>8</td><td>0</td></tr><tr><td>Day 5</td><td>1</td><td>1</td><td>0</td><td>5</td><td>3</td><td>8</td><td>8</td><td>7</td></tr><tr><td>Day 6</td><td>5</td><td>2</td><td>0</td><td>4</td><td>1</td><td>7</td><td>8</td><td>0</td></tr><tr><td>Day 6 R</td><td>6</td><td>1</td><td>0</td><td>3</td><td>2</td><td>7</td><td>8</td><td>0</td></tr><tr><td>Day 15</td><td>3</td><td>1</td><td>0</td><td>0</td><td>2</td><td>5</td><td>7</td><td>0</td></tr></table> 0 ppm: EH and SM were ND; 0.7 ppm: SM was ND; 2 ppm SM was ND ^aInI = inflammatory infiltrate; EH = epithelial hyperplasia; SM = squamous metaplasia. ^bNumber of animals with the lesion (n = 8). ^cRecovery group. Histopathological Incidence <table><tr><th></th><th colspan="7">FA (mg/m³)</th></tr><tr><th></th><th>0</th><th colspan="3">18.5</th><th colspan="3"></th></tr><tr><th></th><th></th><th colspan="3">Level I</th><th colspan="3">Level II</th></tr><tr><th>Time point</th><th>InI^a</th><th>InI</th><th>UcL</th><th>EH</th><th>InI</th><th>UcL</th><th>EH</th></tr><tr><td>Day 1</td><td>0^b</td><td>8</td><td>NR</td><td>NR</td><td>7</td><td>2</td><td>1</td></tr></table> 0 ppm: UcL was NR, EH was ND ^aInI = inflammatory infiltrate; UcL = ulcerative lesions; EH = epithelial hyperplasia. ^bNumber of animals with the lesion (n = 8).	Target concentration (mg/m ³)	Day 1 (mg/m ³)	Day 5 (mg/m ³)	Day 6 (mg/m ³)	Day 15 (mg/m ³)	0	0±0	0±0	0±0	0±0	0.9	0.74±0.23	0.79±0.15	0.75±0.16	0.7±0.11	2.5	2.08±0.46	2.14±0.43	2.26±0.49	2.2±0.31	7.4	5.83±1.73	6.43±0.76	6.00±1.25	6.14±0.97	18.5	17.7±5.7	NA	NA	NA		FA (mg/m ³)									0	0.9		2.5		7.4			Time point	InI ^a	InI	EH	InI	EH	InI	EH	SM	Day 1	0 ^b	1	0	6	0	8	0	0	Day 1 R ^c	4	2	1	1	3	7	8	0	Day 5	1	1	0	5	3	8	8	7	Day 6	5	2	0	4	1	7	8	0	Day 6 R	6	1	0	3	2	7	8	0	Day 15	3	1	0	0	2	5	7	0		FA (mg/m ³)								0	18.5								Level I			Level II			Time point	InI ^a	InI	UcL	EH	InI	UcL	EH	Day 1	0 ^b	8	NR	NR	7	2	1
Target concentration (mg/m ³)	Day 1 (mg/m ³)	Day 5 (mg/m ³)	Day 6 (mg/m ³)	Day 15 (mg/m ³)																																																																																																																																																				
0	0±0	0±0	0±0	0±0																																																																																																																																																				
0.9	0.74±0.23	0.79±0.15	0.75±0.16	0.7±0.11																																																																																																																																																				
2.5	2.08±0.46	2.14±0.43	2.26±0.49	2.2±0.31																																																																																																																																																				
7.4	5.83±1.73	6.43±0.76	6.00±1.25	6.14±0.97																																																																																																																																																				
18.5	17.7±5.7	NA	NA	NA																																																																																																																																																				
	FA (mg/m ³)																																																																																																																																																							
	0	0.9		2.5		7.4																																																																																																																																																		
Time point	InI ^a	InI	EH	InI	EH	InI	EH	SM																																																																																																																																																
Day 1	0 ^b	1	0	6	0	8	0	0																																																																																																																																																
Day 1 R ^c	4	2	1	1	3	7	8	0																																																																																																																																																
Day 5	1	1	0	5	3	8	8	7																																																																																																																																																
Day 6	5	2	0	4	1	7	8	0																																																																																																																																																
Day 6 R	6	1	0	3	2	7	8	0																																																																																																																																																
Day 15	3	1	0	0	2	5	7	0																																																																																																																																																
	FA (mg/m ³)																																																																																																																																																							
	0	18.5																																																																																																																																																						
		Level I			Level II																																																																																																																																																			
Time point	InI ^a	InI	UcL	EH	InI	UcL	EH																																																																																																																																																	
Day 1	0 ^b	8	NR	NR	7	2	1																																																																																																																																																	
Cassee and Feron (1994) Wistar rats; male; 20/group. Exposure: Rats were exposed in dynamic nose-only chambers for 3 d (6 consecutive 12-hr periods of 8 hrs of exposure to FA followed by 4 hrs of nonexposure). Rats sacrificed immediately (i.e., within 30 min) after last exposure.	<table><tr><th></th><th colspan="2">Controls</th><th colspan="2">FA</th></tr><tr><th>Type of lesions</th><th>II^a</th><th>III^a</th><th>II</th><th>III</th></tr><tr><td colspan="5">Disarrangement, flattening and slight basal cell hyperplasia</td></tr><tr><td>Minimal</td><td>0/5</td><td>1/5</td><td>0/5</td><td>0/5</td></tr><tr><td>Slight</td><td>0/5</td><td>0/5</td><td>0/5</td><td>0/5</td></tr><tr><td>Frank necrosis</td><td>0/5</td><td>0/5</td><td>5/5</td><td>5/5</td></tr><tr><td colspan="5">Hyperplasia accompanied by squamous metaplasia</td></tr><tr><td>Slight</td><td>0/5</td><td>0/5</td><td>2/5</td><td>3/5</td></tr></table>		Controls		FA		Type of lesions	II ^a	III ^a	II	III	Disarrangement, flattening and slight basal cell hyperplasia					Minimal	0/5	1/5	0/5	0/5	Slight	0/5	0/5	0/5	0/5	Frank necrosis	0/5	0/5	5/5	5/5	Hyperplasia accompanied by squamous metaplasia					Slight	0/5	0/5	2/5	3/5																																																																																																															
	Controls		FA																																																																																																																																																					
Type of lesions	II ^a	III ^a	II	III																																																																																																																																																				
Disarrangement, flattening and slight basal cell hyperplasia																																																																																																																																																								
Minimal	0/5	1/5	0/5	0/5																																																																																																																																																				
Slight	0/5	0/5	0/5	0/5																																																																																																																																																				
Frank necrosis	0/5	0/5	5/5	5/5																																																																																																																																																				
Hyperplasia accompanied by squamous metaplasia																																																																																																																																																								
Slight	0/5	0/5	2/5	3/5																																																																																																																																																				

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results				
Test article: Paraformaldehyde. Actual concentrations were 0 and 4.4 (SE $\pm$ 0.1) mg/m³ FA. Histopathologic evaluation of the respiratory tract included standard cross sections of the head (see cross sections in Reuzel et al. (1990)). Main limitations: hyperplasia and metaplasia were reported together. This study also evaluated the nasal changes induced by exposures to ozone alone and FA and ozone. Data presented here in the Results column are for FA-only exposures.	Moderate	0/5	0/5	2/5	2/5
	Marked	0/5	0/5	1/5	0/5
	Rhinitis				
	Slight ^b	0/5	0/5	0/5	0/5
	Moderate	0/5	0/5	0/5	4/5
	Marked	0/5	0/5	5/5	1/5
	^a Standard cross section level II and III.				
	^b Influx of neutrophils mainly observed.				
Cassee et al. (1996b) Wistar rats; male; number of animals per group varied but are reported in the Results column. Exposure: Rats were exposed to FA in dynamic nose-only chambers 6 hrs/d for 1 or 3 d. Rats sacrificed immediately after last exposure. Test article: Paraformaldehyde. Actual concentrations were 0, 1.2, 3.9, and 7.9 mg/m³.¹ Histopathologic evaluation of the respiratory tract included standard cross sections at levels II, III, and/or IV of the nose (see Reuzel et al. (1990) for cross-sectional levels). Main limitations: small N; failure to report data for the 7.9 mg/m³ group. This study also evaluated the combined effects of FA, acetaldehyde, and acrolein on nasal epithelium. Data presented here in the Results column are for FA-only exposed rats.	1-day exposure: no treatment-related histopathological nasal lesions observed				
	Histopathological changes from 3 days of exposure ^a				
					FA (mg/m ³)
	Site, type, degree, and incidence of lesions	0	1.2	3.9	
	Number of noses examined	19	5	6	
	Disarrangement, necrosis, thickening, and desquamation of respiratory/transitional epithelium ^b				
	Slight (mainly disarrangement)	0	0	3	
	Moderate	0	0	2	
	Severe (extensive)	0	0	0	
	Basal cell hyperplasia and/or increased number of mitotic figures in respiratory/transitional epithelium				
	Slight (focal)	0	0	4	
	Moderate	0	0	2	
	Severe (extensive)	0	0	0	
	Increased incidence of "single-cell necrosis" in olfactory epithelium ^c				
	A few necrotic cells	0	0	0	
	A moderate number of necrotic cells	0	0	0	
	Many necrotic cells	0	0	0	
	Atrophy of olfactory epithelium				
	Slight (mainly disarrangement)	0	0	0	
	Moderate (focal)	0	0	0	
	Severe (extensive)	0	0	0	
	Rhinitis				
	Slight	2	1	0	
	Moderate	1	0	0	
	Severe	0	0	0	
	^a Data for 7.9 mg/m ³ group NR.				
	^b Changes observed at levels II and III.				
	^c Changes observed at levels III and IV.				

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results				
Monteiro-Riviere and Popp (1986) Fischer 344 rats; male; 3–5/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d for either 1, 2, or 4 d. Interim sacrifices were performed either immediately or 18 hrs after last exposure. Test article: Paraformaldehyde. Actual concentrations were 0, 0.6 (± 0.1), 2.7 (± 0.4), 7.3 (± 0.1), and 18.2 (± 0.4) mg/m³.¹ Histopathologic evaluation of the respiratory tract included transverse sections of the skull that contained the dorsal nasal concha, lateral wall, and ventral nasal concha. Main limitations: small N; (note: only 3 of 5 rats/ treatment group were evaluated in “detail”); failed to report lesion incidence and severity	Cellular occurrence of ultrastructure lesion ^{a,b}	7.3 mg/m ^{3c}	7.3 mg/m ³ (1-day) ^d	7.3 mg/m ³ (2-day)	7.3 mg/m ³ (4-day)
	Cytoplasmic vacuoles	ALL	ALL		NC
	Autophagic vacuoles	BA	BA		BA, CU, NC
	Loss of microvilli	CI	CI	CI	CI, CU, BR
	Hypertrophy		CI, GO	CI, GO	CI, GO
	SER in apical region		NC		NC
	Intracytoplasmic lumen			CI	
	Mitochondrial swelling				CI, BR
	Neutrophils	+	+	+	
	Intercellular edema		+	+	
	Ciliated mucous cells			+	+
	Nonkeratinized squamous cells				+
	^a Abbreviations: BA, basal cells; CI, ciliated cells; CU, cuboidal cells; BR, brush cells; NC, nonciliated columnar cells; GO, goblet cells; SER, smooth endoplasmic reticulum; ALL, all cell types; +, indicates presence. Nucleolar segregation, pyknotic nuclei, and internalized cilia not observed. ^b These lesions were not observed at 0.6 mg/m ³ (1 or 4 d exposure) or 2.7 mg/m ³ (1 or 4 d exposure) FA. ^c Rats in this group were immediately sacrifice after exposure. ^d Number of days of exposure, rats sacrificed 18 hrs later.				
	Cellular occurrence of ultrastructure lesion ^{a,b}	18.2 mg/m ³ (1-d) ^c	18.2 mg/m ³ (2-d)		
	Cytoplasmic vacuoles	CU, NC	NC		
	Autophagic vacuoles	BA, CI, CU, NC	BA, CU, NC		
	Loss of microvilli	BA, CI, CU	CI, CU, NC		
	SER in apical region	NC	NC		
	Nucleolar segregation	BA, CU	BA, CU		
	Pyknotic nuclei	CU	CI		
	Internalized cilia	CI	CI		
	Neutrophils	+			
	Intercellular edema	+			
	Nonkeratinized squamous cells	+	+		
	^a Abbreviations: BA, basal cells; CI, ciliated cells; CU, cuboidal cells; BR, brush cells; NC, nonciliated columnar cells; GO, goblet cells; SER, smooth endoplasmic reticulum; ALL, all cell types; +, indicates presence. Hypertrophy, Intracytoplasmic lumen, mitochondrial swelling, and ciliated mucous cells not observed. ^b These lesions were not observed at 0.6 mg/m ³ (1 or 4 d exposure) or 2.7 mg/m ³ (1 or 4 d exposure) FA.				

Reference and study design	Results																																																																																																																																		
	^c Number of days of exposure, rats sacrificed 18 hrs later.																																																																																																																																		
Speit et al. (2011b) Fischer 344 rats; males; 6/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 4 wks. Test article: Formalin (methanol concentration NR). Actual concentrations were 0, 0.63 (±0.6), 1.23 (±0.14), 2.48 (±0.18), 7.53 (±0.42), 12.3 (±0.48), 18.4 (±0.06) mg/m ³ . ¹ Histopathologic evaluation of the respiratory tract included 4 levels of the nasal cavity: I (nasal septum, lateral meatus [wall], maxilloturbinate, nasoturbinate), II (nasal septum, lateral meatus [wall]), and III and IV (nasopharynx). Main limitations: Formalin; small N	No FA-related histological changes observed in levels I–IV of rats exposed to 0.63, 1.23, 2.48, and 7.53 mg/m ³ . Histopathological analysis of nasal lesions after 4 wks <table><tr><td></td><td colspan="4">Incidence and grading of findings^a</td></tr><tr><td></td><td></td><td colspan="3">FA (mg/m³)</td></tr><tr><td></td><td>Grade^b</td><td>0</td><td>12.3</td><td>18.4</td></tr><tr><td colspan="5">Level I</td></tr><tr><td>Metaplasia, squamous</td><td>1</td><td>0</td><td>1</td><td>0</td></tr><tr><td></td><td>2</td><td>0</td><td>5</td><td>0</td></tr><tr><td></td><td>3</td><td>0</td><td>0</td><td>4</td></tr><tr><td></td><td>4</td><td>0</td><td>0</td><td>2</td></tr><tr><td>Degeneration, (multi) focal</td><td>2</td><td>0</td><td>0</td><td>1</td></tr><tr><td></td><td>3</td><td>0</td><td>0</td><td>3</td></tr><tr><td></td><td>4</td><td>0</td><td>0</td><td>2</td></tr><tr><td>Inflammation, (multi) focal</td><td>2</td><td>0</td><td>0</td><td>1</td></tr><tr><td></td><td>3</td><td>0</td><td>0</td><td>4</td></tr><tr><td colspan="5">Level II</td></tr><tr><td>Metaplasia, squamous</td><td>2</td><td>0</td><td>0</td><td>1</td></tr><tr><td></td><td>3</td><td>0</td><td>0</td><td>5</td></tr><tr><td>Degeneration, (multi) focal</td><td>1</td><td>0</td><td>0</td><td>1</td></tr><tr><td></td><td>2</td><td>0</td><td>0</td><td>2</td></tr><tr><td></td><td>3</td><td>0</td><td>0</td><td>3</td></tr><tr><td>Inflammation, (multi) focal</td><td>2</td><td>0</td><td>0</td><td>1</td></tr><tr><td colspan="5">Level III</td></tr><tr><td>Metaplasia, transitional</td><td>1</td><td>0</td><td>0</td><td>4</td></tr><tr><td></td><td>2</td><td>0</td><td>0</td><td>1</td></tr><tr><td colspan="5">Level IV</td></tr><tr><td>Metaplasia, transitional</td><td>1</td><td>0</td><td>0</td><td>2</td></tr><tr><td></td><td>2</td><td>0</td><td>0</td><td>3</td></tr></table> ^a Number of animal with lesions (6 analyzed per group). ^b 1 = minimal; 2 = slight; 3 = moderate; 4 = severe/marked.		Incidence and grading of findings ^a						FA (mg/m ³)				Grade ^b	0	12.3	18.4	Level I					Metaplasia, squamous	1	0	1	0		2	0	5	0		3	0	0	4		4	0	0	2	Degeneration, (multi) focal	2	0	0	1		3	0	0	3		4	0	0	2	Inflammation, (multi) focal	2	0	0	1		3	0	0	4	Level II					Metaplasia, squamous	2	0	0	1		3	0	0	5	Degeneration, (multi) focal	1	0	0	1		2	0	0	2		3	0	0	3	Inflammation, (multi) focal	2	0	0	1	Level III					Metaplasia, transitional	1	0	0	4		2	0	0	1	Level IV					Metaplasia, transitional	1	0	0	2		2	0	0	3
	Incidence and grading of findings ^a																																																																																																																																		
		FA (mg/m ³)																																																																																																																																	
	Grade ^b	0	12.3	18.4																																																																																																																															
Level I																																																																																																																																			
Metaplasia, squamous	1	0	1	0																																																																																																																															
	2	0	5	0																																																																																																																															
	3	0	0	4																																																																																																																															
	4	0	0	2																																																																																																																															
Degeneration, (multi) focal	2	0	0	1																																																																																																																															
	3	0	0	3																																																																																																																															
	4	0	0	2																																																																																																																															
Inflammation, (multi) focal	2	0	0	1																																																																																																																															
	3	0	0	4																																																																																																																															
Level II																																																																																																																																			
Metaplasia, squamous	2	0	0	1																																																																																																																															
	3	0	0	5																																																																																																																															
Degeneration, (multi) focal	1	0	0	1																																																																																																																															
	2	0	0	2																																																																																																																															
	3	0	0	3																																																																																																																															
Inflammation, (multi) focal	2	0	0	1																																																																																																																															
Level III																																																																																																																																			
Metaplasia, transitional	1	0	0	4																																																																																																																															
	2	0	0	1																																																																																																																															
Level IV																																																																																																																																			
Metaplasia, transitional	1	0	0	2																																																																																																																															
	2	0	0	3																																																																																																																															

¹Study authors originally reported FA concentrations in ppm. These values were converted based on 1 ppm = 1.23 mg/m³, assuming 25°C and 760 mm Hg.

Abbreviations: **FA**—Formaldehyde; **NA**—Not applicable; **ND**—Not detected; **NR**—Not reported; **SD**—Standard deviation; **SE**—Standard error of the mean.

C.7. MECHANISTIC EVIDENCE RELATED TO POTENTIAL NONCANCER RESPIRATORY HEALTH EFFECTS (SUPPORTING INFORMATION FOR HAZARD)

Note: Large sections of this analysis are redundant to synthesis text, figures, and tables presented in the Toxicological Review. However, the entirety of the analyses and discussion is included below to contextualize the conclusions described in the Toxicological Review with the appropriate methodological considerations, supporting analyses, and other information of potential interest.

This evaluation provides an integrated discussion characterizing potential relationships between the mechanistic changes observed following formaldehyde inhalation in the context of potential respiratory effects, but it does not attempt to explicitly define a single mode of action. The methods are described in Section 2 of the Toxicological Review.

The identification of the studies is primarily documented in Appendix B.2.6, although some studies were identified through other health-effect specific searches (e.g., immune-mediated conditions; respiratory tract pathology) as described elsewhere. The evaluation of individual mechanistic studies is documented in Appendix B.3.6.

C.7.1. Synthesis of Changes in the Upper Respiratory Tract (URT)

Data on formaldehyde-induced mechanistic changes in the URT are largely based on studies in experimental animals or acutely exposed human volunteers, as most of these endpoints are difficult to examine in long-term observational epidemiology studies. The specific studies and summary findings supporting the synthesis below are described in Table C-27. While the structure and function of the URT across species is considered similar, interpretation of compensatory or adaptive changes within the human URT following long-term exposure based on findings in experimental animals is difficult to infer.

The majority of the events which are potential initial or direct effects of formaldehyde (see asterisks in Figures C-23 and C-24) occur at the level of the respiratory epithelium, including evidence supporting the involvement of formaldehyde in reactions with cellular macromolecules such as proteins (e.g., detoxifying enzymes) and DNA, effects on the local redox system, and interactions with sensory nerve endings within the respiratory epithelium. While these events are interrelated, they could be caused by formaldehyde independently and simultaneously. Although some studies have reported changes in these initial mechanistic events at formaldehyde concentrations as low as 0.035 mg/m³ following acute or short-term exposure, notable uncertainties remain. For example, tissue alterations that might increase vulnerability to these changes with continued exposure is expected (e.g., decreases in mucociliary clearance). Conversely, gradual tissue changes following exposure might also lead to resilience (e.g., increases in epithelial cell barrier function). More detailed mechanistic studies characterizing the initial molecular interactions of formaldehyde in the URT following long-term exposure would help to clarify potential progressive changes in the ability of formaldehyde inhalation to elicit these initial changes.

Effects on the mucociliary system are likely secondary to the production of reactive byproducts or covalent modification to mucosal structural components following physical interactions of formaldehyde with proteins in the mucus. The effects of formaldehyde on mucus flow patterns appear to include both a concentration and exposure-duration dependency (as well as variability due to humidity), although a mechanism reliant on direct modification of macromolecules alone would be expected to be driven largely by concentration. The impact of this is difficult to define and integrate into the overall mechanistic picture. Persistent changes to the

normally protective mucociliary apparatus or tissue redox capacity are likely to eventually lead to epithelial damage (which has been shown to correlate with inhibited mucociliary function following formaldehyde exposure). To repopulate damaged tissue and cells, and to protect against further insult, damage often leads to cell proliferation or hyperplasia (i.e., an increase in the amount of tissue due to proliferation of normal cells), and/or the damage can eventually lead to epithelial lesions such as squamous metaplasia, where cells transition to a different phenotype. This proliferation, hyperplasia, and/or metaplasia can be adaptive (e.g., response to tissue stress) or maladaptive, and could lead to subsequent effects on pulmonary function through thickening or keratinization of the respiratory epithelium, or thickening of mucus, all of which can restrict airflow. Formaldehyde exposure-induced damage to the URT epithelial cells could also result in an altered release of cytokines or other soluble mediators, which, were they to reach the LRT, could contribute to decreased pulmonary function through airway hyperreactivity and/or hypersensitivity to challenges such as allergen exposure ([Hulsmann and Dejongste, 1996](#)). In general, the plausible initial mechanistic events and changes in mucus flow patterns observed after formaldehyde exposure occur at lower formaldehyde levels than those eliciting URT epithelial lesions (i.e., at ≤ 0.3 mg/m³ in exposed humans and >0.6 mg/m³ in animals).

Inhaled formaldehyde also appears to directly stimulate trigeminal nerve endings in the nasal mucosa. Activation of these chemosensory afferents, likely C fibers, is known to initiate afferent signals that result in the burning sensation characteristic of sensory irritation. This chemosensory activation, which can also occur in the eyes, is enhanced in the anterior third of the nasal cavity and is typically less sensitive than olfaction ([Hummel and Livermore, 2002](#)). These characteristics are consistent with the known distribution of inhaled formaldehyde (see Appendix C.1) and with observations that formaldehyde exposure typically causes chemosensory-related irritation at higher concentrations than those necessary for olfactory detection in naïve individuals (e.g., as demonstrated by ([Berglund et al., 2012](#))). The rapid detection of these sensations in exposed individuals suggests a receptor-mediated event that is dependent on formaldehyde penetration to the nerve endings, which may not have an exposure duration threshold. Based on mechanistic studies in vitro and ex vivo, activation of the trigeminal nerve by formaldehyde is likely mediated, at least in large part, through Transient Receptor Potential A1 (TRPA1) cation channels. To a lesser extent, this activation may also involve TRPV1 channels, which can be coexpressed and coactivated alongside TRPA1 in certain situations ([Salas et al., 2009](#)). Overall, very little is known about changes in chemosensitivity to inhaled formaldehyde with repeated exposure over time, as mechanistic studies of long-term exposure were not identified. With acute, controlled exposure in human volunteers, the initial irritation response to formaldehyde, which is highly variable across individuals, has been shown to plateau (e.g., ([Green et al., 1987](#))) or even decline somewhat ([Bender et al., 1983](#)) when exposure is continued for several minutes to hours; however, this pattern may depend upon concentration ([Andersen and Molhave, 1983](#)), and changes to this response pattern in humans over time, particularly with exposure longer than 1 day, remain unclear. Studies of reflex

bradypnea in rodents (see Appendix C.2), which is dependent on the activation of the trigeminal nerve, show that repeated exposure for up to a month elicits a similar level of activation of this pathway. However, uncertainties with these data include a nonconstant exposure (i.e., short-term rodent studies employed work hour-like exposure periodicity) and testing only at reflex bradypnea-inducing levels (e.g., $>1 \text{ mg/m}^3$). It is unclear how this informs long-term responses to constant oronasal exposure in humans (who do not exhibit this reflex) at lower formaldehyde levels. Enhanced irritation with prolonged exposure could occur directly as a result of sensitization of the receptors (e.g., TRPA1) to formaldehyde or indirectly by increased access of formaldehyde to trigeminal nerve endings following damage to juxtaposed epithelial cells. Electrophilic oxidative stress products such as hydrogen peroxide and 4-hydroxynonenal are also known to be capable of stimulating sensory nerve receptors such as TRPA1 ([Taylor-Clark et al., 2008](#); [Alexandersson, 1988](#); [Alexandersson and Hedenstierna, 1988](#)), and *moderate* evidence exists to support the presence of oxidative stress in both the upper and lower airways. In addition, airway inflammation has been shown to reduce the threshold for activation of afferent fibers, through an unknown mechanism ([Carr and Undem, 2001](#)). Conversely, however, as this action is mediated predominantly by access of formaldehyde to chemoreceptors, changes such as the conversion of normal epithelium to squamous epithelium or damage and destruction of nerve afferents would be expected to reduce or desensitize subsequent irritant responses¹⁵. Taken together, this suggests a complex sequence of interactions that might impact trigeminal nerve chemosensation over time.

Together with the centrally mediated physiological response, stimulation of airway sensory nerves, including the trigeminal nerve, can also cause a more immediate localized release of neuropeptides like substance P and calcitonin gene-related protein (CGRP). These released neuropeptides, particularly substance P, can affect local immune responses by increasing vascular permeability and leukocyte recruitment, among other things ([Sarin et al., 2006](#)), as has been demonstrated with substance P-dependent eosinophil accumulation in the human nasal mucosa after allergen exposure ([Fajac et al., 1995](#)). Observations of neuropeptide changes, including increased substance P, have been reported at slightly higher formaldehyde levels than those shown to activate the trigeminal nerve, generally $>1 \text{ mg/m}^3$. While URT neuropeptide levels have not been examined in great detail following formaldehyde exposure, given that the URT represents the primary region of formaldehyde flux, formaldehyde exposure-induced increases in neuropeptides in model systems and related tissue regions, including the LRT, are inferred to provide support for the few URT-specific studies that observed elevated neuropeptide levels. The formaldehyde-specific

¹⁵ For example, although only indirectly applicable to formaldehyde exposure-induced sensory irritation, dry eye disease is far more prevalent in older individuals (age 50+) than younger individuals (under 18) ([Farrand et al., 2017](#)). This is based, at least in part, on differences in tissue physiology that help regulate access of airborne irritants to sensitive components of the eye (e.g., reduced tear production and changes in lipid composition with aging). While this may also apply to the direct irritant effects of airborne formaldehyde on the eye, it is unclear the extent to which parallel, protective, age-dependent mechanisms exist within the nasal epithelium.

data further indicate that the neuropeptides are released from neuronal rather than nonneuronal sources, at least following short-term exposure, and this release appears to be at least partially dependent on TRPA1 activation. The formaldehyde-specific URT studies have not examined many of the potential consequences of these changes, particularly after long-term exposure. Elevated URT neuropeptides might result in local inflammatory changes ranging from increased histamine and mucus secretion to edema and nasal obstruction during normal or exaggerated attempts to minimize nasal irritation ([Barnes et al., 1991a, b](#)).

The immune response in the URT following formaldehyde exposure has not been thoroughly studied, particularly in exposed humans; however, the available evidence does provide *moderate* support for granulocyte (e.g., eosinophils; neutrophils) involvement. The available data generally indicate that eosinophils are increased in the URT with acute or short-term exposure at ≈ 0.5 mg/m³, although one study suggests the possible increases at much lower levels in exposed humans with longer exposure ([Norback et al., 2000](#)). Although the role for eosinophils in the upper airways of exposed individuals remains unclear, airway eosinophils are known to be tightly regulated and uncommon in normal airways. In addition to their traditional role as immune “effectors” (i.e., releasing toxic molecules to destroy invading pathogens), activation of eosinophils can also cause them to release a number of chemical mediators which damage epithelial cells, stimulate mucus secretion, induce airway hyperresponsiveness, and perpetuate further recruitment of inflammatory mediators into the airway ([Cohn et al., 2004](#)). Eosinophils, which are relatively rare ($\approx 1\%$) blood leukocytes, are a hallmark of allergic asthma ([Howarth et al., 2000](#)); however, no formaldehyde-specific studies meeting the inclusion criteria evaluated the URT for changes in other commonly observed inflammatory markers of allergic individuals such as activated mast cells or histamine. In addition, the data are unable to inform whether this inflammatory change persists in the human URT with long-term exposure. It should be recognized that acute inflammation is a protective response of the tissue to stress or damage; inflammation is more concerning when it becomes dysregulated, recurrent, and/or persistent.

At much higher concentrations (>5 mg/m³), neutrophils also appear to increase within the upper airways, presumably via migration from the blood. Neutrophils, which are the most common ($\geq 50\%$) blood leukocyte, are also relatively uncommon ($\leq 2\%$) in healthy airways. These phagocytic cells, along with eosinophils, are one of the first cells recruited to inflamed tissues shortly after infection. Both eosinophils and neutrophils can release toxic mediators, including lipid-active factors and reactive oxygen species (ROS), for which *moderate* evidence exists to support increased levels in the URT following formaldehyde exposure, and can damage bystander epithelial cells. However, in contrast to eosinophils, neutrophils are not thought to be associated with allergic responses or asthma, although they can be increased in individuals with pulmonary disease ([O'Donnell et al., 2006](#)). Changes in other cells in the URT, including basophils, macrophages, and lymphocytes, were not observed in the few short-term studies examining them.

Exactly how or why eosinophils and neutrophils migrate to the upper airways following formaldehyde exposure remains unclear. One possibility is that this response is related to the *slight* evidence of increased frequency and duration of URT infections in chronically exposed humans. However, while this effect might be caused by loss of barrier function (e.g., from epithelial cell damage or inhibited mucociliary function) leading to increased colonization of the epithelium by bacteria, this is not temporally plausible for the eosinophil increases observed following acute exposure. Evidence of specific changes in chemoattractants known to stimulate recruitment of these cells to the URT (e.g., eotaxin; IL-5; or, indirectly, TNF α or IL-1 β , which can stimulate eotaxin in epithelial cells) was not identified, and thus, the biological explanation for the recruitment of these cells to the upper airways is unknown. Although not examined, it is also possible that formaldehyde could directly or indirectly (e.g., through tissue damage) interact with and modify epithelial components, including pattern recognition receptors, that can trigger release of ROS and lead to immunological responses ([Lambrecht and Hammad, 2012](#); [Kim et al., 2015a](#)). Overall, although *moderate* evidence indicates that inflammatory cells including eosinophils and neutrophils are increased in the URT following formaldehyde exposure, the data are limited in their ability to define the concentration and duration requirements for the effects of formaldehyde exposure on URT immunological processes, which might inform how these changes are initiated.

Table C-27. Summary of changes in the upper respiratory tract (URT) resulting from formaldehyde exposure

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (<u>exposure duration</u>)	Conclusion
Structural Modification of the Upper Airways				
Modification of biological macromolecules [see Appendix C.1 and C.3 for additional detail]	High or Medium	Human: None (note: binding of formaldehyde to albumin and other soluble proteins in human mucus has been demonstrated in vitro; e.g., Bogdanffy et al. (1987) ; hemoglobin adducts at ≈ 0.2 mg/m ³ , Bono et al. (2012))	Consistent with its known chemistry, formaldehyde can modify cellular biological macromolecules, including DNA, and interacts with soluble factors such as albumin and glutathione, shortly after exposure to low concentrations (e.g., <0.5 mg/m ³) across a <u>wide range of exposure durations</u>	Robust
		Animal: Multiple animal studies demonstrate that inhaled formaldehyde can bind and modify biological macromolecules, which is consistent with the known biological reactivity of formaldehyde; evidence includes increased DNA-protein crosslinks (DPXs), hydroxymethyl (hm) DNA adducts, and reactions with glutathione; (e.g., increased DPXs are observed at ≥ 0.37 mg/m ³ , Casanova et al. (1989) ; hmDNA adducts and protein adducts at ≥ 0.86 mg/m ³ , (Lu et al., 2010a ; Lu et al., 2011 ; Edrissi et al., 2013b)		
	Low	Human: N/A (see summary)	Sufficient information for ‘Robust’ from <i>high or medium confidence</i> studies	
		Animal: N/A (see summary)		
Impaired Mucociliary Function	High or Medium	Human: decreased mucus flow at ≥ 0.3 mg/m ³ after acute exposure and pathological changes in mucociliary clearance in workers at mean exposed levels of 0.25 – 0.26 mg/m ³ after chronic exposure (Holmström and Wilhelmsson, 1988 ; Andersen and Molhave, 1983).	Decreased mucus flow and ciliary beat, and impaired clearance, in humans and rats at ≥ 0.25 and ≥ 2.5 mg/m ³ , respectively (observed <u>across exposure durations</u>), eventually leading to cilia loss	Robust
		Animal: mucociliary function was generally unaffected at 0.57 mg/m ³ after short-term exposure—minor changes were notable at 2.46 mg/m ³ ; robust changes were observed at the next highest concentrations tested, ≥ 7.27 mg/m ³ ; a general lack of recovery with longer exposure duration		
	Low	Human: Increases in ciliary activity at 1.23 mg/m ³ in dissociated human nasal epithelial cells (Wang et al., 2014b), with decreased cilia beating frequency in human epithelial cells at ≥ 3.46 mg/m ³ (Wang et al., 2014b ; Schafer et al., 1999): in vitro acute	Suggestive of decreased ciliary beat and ciliastasis at ≥ 5 mg/m ³ in humans and rats with <u>acute</u> exposure, and cilia damage at ≥ 0.5 mg/m ³ with <u>short-term</u> exposure; usually preceded by initial effects including slight increases in activity	
		Animal: Ciliastasis and mucostasis: (Morgan et al., 1986a ; Morgan et al., 1986c) acute 14.76 mg/m ³ (not ≤ 2.46 mg/m ³ ; recovery); Morgan et al. (1984) : acute in vitro (frog palates) ≥ 5.36 mg/m ³ (authors noted early activity increase, even at 1.69 mg/m ³); structural cilia changes: (Monteiro-Riviere and Popp, 1986) short-term ≥ 0.5 mg/m ³ , (Abreu et al., 2016) acute at 0.25 , but not 1.2 – 3.7 mg/m ³		

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (<u>exposure duration</u>)	Conclusion
Structural Change in URT Mucus Membrane or Nasal Obstruction	High or Medium	Human: Membrane hypertrophy, atrophy, rhinitis: (Lyapina et al., 2004) chronic (years) 0.87 mg/m ³	Mucus membrane damage and swelling in humans at 0.87 mg/m ³ with <u>chronic</u> exposure	Moderate particularly in persons with nasal damage
		Animal: None		
	Low	Human: Data suggest increased mucosal swelling, nasal obstruction, and/or rhinitis in workers (Holmström and Wilhelmsson, 1988) chronic at 0.26 mg/m ³ and (Norback et al., 2000): short-term at ≤0.016 mg/m ³ , which did not increase in severity with longer exposure; increase in mucosal swelling in symptomatic nasal distress patients, but not healthy controls: Falk et al. (1994) acute (2 hr) ≥0.073 mg/m ³ Animal: Rhinitis and necrosis in rats after acute or short term (1–3 d) at ≥3.94 or 4.43 mg/m ³	Observations at ≤0.26 mg/m ³ in humans or at >3.5 mg/m ³ in rats support data from the chronic-duration study and suggest increased acute vulnerability of people with a prior nasal condition	
URT Epithelial Damage or Dysfunction [see Toxicological Review Section 3.2.4 for additional data and discussion]	High or Medium	Human: Indirect data indicating epithelial damage, including loss of ciliated cells, in occupational studies at 0.1–>2 mg/m ³ (Holmström and Wilhelmsson, 1988), 1989, 3564; Edling et al. 1987, 4059 (Edling et al., 1988 ; Ballarin et al., 1992), with one with more equivocal findings (Boysen et al., 1990); however, these histopathological symptom scores included hyperplasia and metaplasia, which complicate interpretation Animal: Increased epithelial damage and related nasal lesions: duration-dependent, typically ≥2.46 mg/m ³ in subchronic and chronic studies (e.g., (Andersen et al., 2010) lower in some longer-term studies) and generally correlating with inhibited mucociliary activity; goblet cell loss in monkeys (Monticello et al., 1989) short term (1 wk) at 7.38 mg/m ³	Duration-dependent epithelial damage, typically at ≥2.5 mg/m ³ in <u>subchronic</u> or <u>chronic</u> rat studies, and with supportive indirect findings from human studies at 0.1–0.2 mg/m ³ , generally correlates with inhibited mucociliary activity	Robust
		Human: None		
	Low	Animal: Goblet cell damage and decreased junctional proteins between epithelial cells in rats (Arican et al., 2009): subchronic (12 weeks) at 18.5 mg/m ³ ; mRNA and/or miRNA changes associated with apoptosis (Rager et al., 2014): short term (2 d in macaques or 28 d in rats) or DNA repair (Andersen et al., 2010): short term (1 wk, but not at 4–13 week durations) at ≥2.46 mg/m ³ ; Rhinitis and necrosis in rats after acute or short term (1–3 d) at ≥3.94 or 4.43 mg/m ³	Studies suggest that nasal epithelial damage is increased, even in <u>short-term</u> studies, at ≥2.5 mg/m ³	

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (<u>exposure duration</u>)	Conclusion
URT Cellular (Epithelial) Proliferation [see Toxicological Review Section 3.2.4 for additional data and discussion]	High or Medium	Human: None: indirect data from humans indicating an increase in histopathological scores that sometimes included hyperplasia were not specific enough to independently evaluate proliferation	Increased cell proliferation in rats at <u>all tested durations</u> . Proliferation increases were typically observed in the anterior nasal cavity at tested levels $\geq 3.5\text{--}4\text{ mg/m}^3$, and were generally not observed at $\leq 1.23\text{ mg/m}^3$. Sites of proliferation correlated with the development of hyperplasia and metaplasia, although the temporal and exposure levels specifics of this association are unclear. Indirect data from observations of hyperplasia in exposed animals and humans are consistent with these data.	Robust ↑
		Animal: Acute dose-dependent increases in cell proliferation in rats, measured primarily by DNA labeling during the final days of exposure, were consistently observed following acute, short-term, and subchronic exposure, and generally with a similar magnitude of responses across durations. Proliferation was typically highest in anterior regions (e.g., “level 2”), with little evidence of proliferation at $\leq 1.23\text{ mg/m}^3$, mixed findings between 1.24 and 3.5 mg/m^3 , and studies generally reporting increases with exposure at higher levels, particularly with longer exposure duration. These data are supported by consistent observations of formaldehyde exposure-induced increases in hyperplasia in pathology studies, some of which provided information showing a correlation between acute proliferation and hyperplasia and metaplasia. The only rat study that measured exposure longer than 13 wks suggests that increases in acute proliferation may begin to decrease in magnitude with chronic exposure at $\geq 6\text{ mg/m}^3$ (Monticello et al., 1996). A few studies suggest that mice may exhibit less robust responses than rats, while monkeys may exhibit proliferation in more posterior nasal regions at $>7\text{ mg/m}^3$.		
	Low	Human: N/A (see summary)	Sufficient information for ‘Robust’ from <i>high or medium confidence</i> studies	
		Animal: N/A (see summary)		
Sensory Nerve-Related Changes				
Trigeminal Nerve Stimulation	High or Medium	Human: None	Increased activity of trigeminal nerve afferents at $<0.5\text{ mg/m}^3$ following <u>acute</u> exposure in animals	Robust ↑
		Animal: Increased afferent nerve activity: Tsubone and Kawata (1991) acute $\approx 20\%$ at 0.62 mg/m^3 and $\approx 50\%$ at 2.21 mg/m^3 ; Kulle and Cooper (1975) acute (threshold detection at 25 sec) at 0.31 mg/m^3		
	Low	Human: None	Supportive indirect evidence from ex vivo and in vitro experiments	
		Animal: Indirect evidence: with acute exposure, dose-dependent increase in nerve currents and CI—release in intact rat trachea (Luo et al., 2013), and stimulation using in vitro neuronal preparations (Mcnamara et al., 2007; Kunkler et al., 2011)		
	High	Human: None		

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (<u>exposure duration</u>)	Conclusion
TRPA1 and/or TRPV1 Stimulation		<i>Animal:</i> Formaldehyde and related chemicals such as acrolein activate the trigeminal system in wild-type mice, but not TRPA1 knockout mice following acute exposure, at least at high exposure levels (Yonemitsu et al., 2013); taken together with the established role for TRPA1 in acrolein-induced sensory effects (e.g., (Bautista et al., 2006)); these data indirectly support a role for TRPA1 in sensory nerve-related changes following formaldehyde exposure	Indirect data identify TRPA1 as a molecular target for formaldehyde exposure-induced sensory effects	Moderate (TRPA1); Slight (TRPV1: not shown in Figures C-23 and C-24)
	Low	<i>Human:</i> None <i>Animal:</i> Formaldehyde activates the transient receptor potential cation channels, TRPA1 and TRPV1, in in vitro and ex vivo models relevant to acute inhalation exposure of the URT and upper LRT: (Mcnamara et al., 2007 ; Luo et al., 2013), and in vivo using formalin as a pain stimulus (not shown); Inhibition of TRPA1 and TRPV1 channels localized to sensory nerve endings reduce FA exposure-induced nerve currents in rat trachea (Luo et al., 2013) and immune-related responses in mice (Wu et al., 2013 ; Lu et al., 2005): 1 or 3 mg/m ³ for 2 or 4 wk	Indirect data identify TRPA1 and/or TRPV1, as molecular target(s) of formaldehyde exposure with <u>acute</u> or <u>short-term</u> exposure; inhibitor studies demonstrate that downstream effects of sensory nerve stimulation depend on TRPA1 or TRPV1 stimulation.	
Neuropeptide Release	High or Medium	<i>Human:</i> None <i>Animal: in plasma:</i> Increased substance P in mice with subchronic exposure (Fujimaki et al., 2004b): subchronic at 2.46 mg/m ³	Indirect evidence that Substance P was increased with <u>subchronic</u> exposure in a single mouse study at 2.46 mg/m ³	Moderate ↑ (relevant to both URT and LRT; note: evidence for NK Receptor involvement is Slight)
	Low	<i>Human: in URT:</i> Substance P in nasal lavage is increased in human volunteers with ocular exposure (He et al., 2005): 4 d (5 min/d) at 3 mg/m ³ , but not at 1 mg/m ³ <i>Animal: in URT:</i> Formaldehyde stimulates release of calcitonin gene related-protein (CGRP) in in vitro models relevant to inhalation exposure of the URT (Kunkler et al., 2011); Experiments using the related chemical, acrolein, suggest this is TRPA1-mediated (Kunkler et al., 2011). <i>in LRT:</i> Inhibition of substance P receptor (NK1) inhibited formaldehyde-induced currents in isolated rat trachea (Luo et al., 2013); increased substance P and CGRP in mouse BAL, both amplified with ovalbumin (OVA) sensitization, and both involved TRP activation (Wu et al., 2013): short term at 3 mg/m ³	Data suggest formaldehyde activates TRP channels on sensory neurons, leading to release of CGRP and substance P, with <u>acute</u> or <u>short-term</u> exposure at >1 mg/m ³	
Immune and Inflammation-Related Changes				
URT Oxidative Stress	High or	<i>Human:</i> Increased nasal epithelial M1dG adducts (marker for oxidative stress and lipid peroxidation (Bono et al., 2016): unknown duration (but likely years) at >0.066 mg/m ³	Direct and indirect evidence of elevated reactive oxygen species	Moderate ↑

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (<u>exposure duration</u>)	Conclusion
		<i>Animal</i> : mRNA changes indicating increased stress-response proteins: (Andersen et al., 2008) short-term $\geq 2.46 \text{ mg/m}^3$	(ROS), possibly at very low concentrations (e.g., at $>0.066 \text{ mg/m}^3$, with a maximum of 0.444 mg/m^3) with <u>prolonged</u> human exposure	
	Low	<i>Human</i> : Increased nasal lavage nitrites (Priha et al., 2004): acute (8 hr shift) 0.19 mg/m^3	Data suggest elevated oxidative stress at very low formaldehyde concentrations with <u>acute</u> and <u>short-term</u> exposure.	
		<i>Animal</i> : Increased glutathione peroxidase and/or nonprotein sulfhydryl groups (Cassee et al., 1996b) and (Cassee and Feron, 1994): short-term (3 d) 3.94 and 4.43 mg/m^3 , respectively		
Nasal Cellular Inflammatory Response	High or Medium	<i>Human</i> : None	Cellular infiltration observed by histology, primarily neutrophils, but indirectly supporting other immune cell infiltration, in <u>short-term</u> animal studies at 7.38 mg/m^3 . Indirect evidence of increases in granulocytes (and possibly lymphocytes) at 2.46 mg/m^3 with short term exposure.	Moderate ↑ granulocytes (neutrophils, eosinophils); Note: data on lymphocytes considered Indeterminate
		<i>Animal</i> : Increased inflammatory response, mostly neutrophils but also mention of lymphocytes and other inflammatory cells (e.g., assumed monocytes, basophils and eosinophils): (Monticello et al., 1989) short-term (1 or 6 wk) 7.38 mg/m^3 ; “inflammatory cell” infiltration: (Andersen et al., 2008) acute or short-term (1 d–3 wk) 7.38 mg/m^3 ; mRNA and miRNA changes associated with inflammation in rats and nonhuman primates: (Rager et al., 2013 ; Rager et al., 2014) short-term (1 or 4 wk, with some miRNA changes reversible with 1 wk recovery) at 2.46 mg/m^3 ; 35 formaldehyde-responsive transcripts altered in the nose known to be related to immune cells indirectly indicated increases in granulocytes (i.e., eosinophil and neutrophil markers) and lymphocyte changes, and (Andersen et al., 2010): short-term (1 wk, but not ≥ 4 wk) at $\geq 12.3 \text{ mg/m}^3$		
	Low	<i>Human</i> : N/C in nasal lavage cell counts, but increased total protein: Priha et al. (2004) occupationally exposed (8-hr shift) 0.19 mg/m^3 ; Allergy-independent increased eosinophils, permeability (albumin index) and total protein in lavage: Pazdrak et al. (1993) acute (2 hr) 0.5 mg/m^3 ; increased eosinophils, leukocytes, and permeability (albumin index) in lavage: (Krakowiak et al., 1998) acute (2 hr) 0.5 mg/m^3 (reversible); indirect evidence of eosinophil infiltration (increased markers: lysozyme and eosinophil cationic protein), but not neutrophils, at very low levels (Norback et al., 2000): $<0.02 \text{ mg/m}^3$; unknown duration (likely months or more) in schools	Suggestive of cellular inflammation, particularly eosinophils, at 0.5 mg/m^3 and indirect markers of eosinophil recruitment at lower levels in humans, following <u>acute</u> exposure; neutrophil inflammation observed at $\geq 6 \text{ mg/m}^3$ in rats with <u>short-term</u> exposure	
<i>Animal</i> : Neutrophil inflammation: (Monteiro-Riviere and Popp, 1986) short-term $\geq 6 \text{ mg/m}^3$				
Altered URT Immunity	High or Medium	<i>Human</i> : Increased frequency and duration of URT infections in symptomatic workers; increased chronic URT inflammation (and decreased function of blood neutrophils, but N/C	Indirect evidence of decreased immune capacity in a single study of	Slight

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (<u>exposure duration</u>)	Conclusion
(inferred from URT infections)		in leukocyte counts) in exposed workers (Lyapina et al., 2004): chronic (years) 0.87 mg/m ³ [Note: recent URT infection was often an exclusion criterion in observational studies focusing on pulmonary function; see Section A.5.3]	chronic human exposure at 0.87 mg/m ³ (note: while altered immunity was observed in an mRNA study, these changes were not necessarily indicative of decreased immune response)	↑URT infection
		Animal: mRNA changes suggestive of altered immune response (Andersen et al., 2010): ≥12.3 mg/m ³ short-term (≥1 wk)		
	Low	Human: None Animal: None	No evidence to evaluate	

Synthesis on Mucociliary Function in the Respiratory Tract

Mucociliary function studies in animals, which primarily focused on quantifying mucus flow rate and qualitative descriptions of ciliary beat frequency and viscosity, were limited to a set of studies from one research group examining dissected nasal passages. Studies of exposed humans were similarly limited, with relevant endpoints being evaluated in a prevalence study and an acute, controlled exposure study. Data are sparse, but in general, mucus flow and/or ciliary beat were inhibited by formaldehyde exposure as a function of concentration and, at least in rats, exposure duration. Effects were most pronounced in the anterior nasal regions, with effects progressing towards posterior regions after extended exposure durations in rats (see Tables C-28 to C-29). These functional observations are consistent with histological changes observed in experimental animals, including decreased cilia content in rhesus monkeys after 1 or 6 weeks of exposure to 7.38 mg/m³ ([Monticello et al., 1989](#)) and blebbing of ciliary membranes at formaldehyde concentrations as low as 0.62 mg/m³, with more overt signs of damage at ≥7.38 mg/m³, in rats exposed for 1 or 4 days ([Monteiro-Riviere and Popp, 1986](#)).

In well-conducted experiments in F344 rats, mucociliary function was generally unaffected after exposure to 0.57 mg/m³ formaldehyde for <1 to 14 days ([Morgan et al., 1986a](#); [Morgan et al., 1986c](#)). Although sporadic, minor changes were notable at 2.46 mg/m³, including slight increases in mucus flow rate, inhibition of ciliary beat and mucus flow became clearly apparent at the next highest concentrations tested, ≥7.27 mg/m³. Initial increases in mucociliary activity at somewhat lower level formaldehyde concentrations were also apparent immediately after in vitro exposure, including increases in ciliary activity at 1.49 mg/m³ in ex vivo frog palates and at 1.0 mg/m³ in dissociated human nasal epithelial cells ([Wang et al., 2014b](#); [Morgan et al., 1984](#)), with observations of mucostasis and ciliastasis at ≥5.36 mg/m³ in frog palates and decreased cilia beating frequency in human epithelial cells at ≥3.46 mg/m³ ([Wang et al., 2014b](#); [Schafer et al., 1999](#); [Morgan et al., 1984](#)); however, these in vitro studies are interpreted with low confidence. Two studies in humans reported consistent effects, with decreased mucus flow at ≥0.3 mg/m³ after exposure for several hours, and pathological changes in mucociliary clearance in workers exposed to mean formaldehyde levels of 0.25–0.26 mg/m³ for several years ([Holmström and Wilhelmsson, 1988](#); [Andersen and Molhave, 1983](#)).

In rats, impaired function was most frequent in the dorsal and medial maxilloturbinate, the lateral wall, and portions of the nasoturbinate ([Morgan et al., 1986a](#); [Morgan et al., 1986c](#)). This is consistent with the locations of epithelial lesions, which correlate with areas of inhibited ciliary function ([Morgan et al., 1986c](#)). Similarly, mucus flow was inhibited in the anterior nose of exposed human volunteers ([Andersen and Molhave, 1983](#)). However, whereas mucociliary function was affected with increasing severity with increasing exposure duration over several days in rats ([Morgan et al., 1986c](#)), effects on mucus flow rate did not vary with exposure durations of up to several hours in human volunteers ([Andersen and Molhave, 1983](#)). Seemingly consistent with this finding, mucociliary function in rat nasal passages was reported to recover considerably within 1

hour after 90 minutes of exposure to 18.5 mg/m³ ([Morgan et al., 1986a](#)); however, less recovery occurred after exposure for 6 hours ([Morgan et al., 1986a](#)), and little or no recovery was observable 18 hours after exposure for multiple days at similar concentrations ([Morgan et al., 1986c](#)). These data suggest that the initial changes observed in response to exposure may vary somewhat from the functional changes induced by sustained formaldehyde exposure.

Overall, mucociliary function is affected in a concentration-dependent manner shortly after formaldehyde inhalation, and this impaired function can be persistent, at least when exposure exceeds several hours, as indicated by studies in F344 rats and exposed workers. In rats, impaired function worsens with increasing exposure duration, although durations longer than 2 weeks have not been tested.

Table C-28. Mucociliary function studies in experimental animals

Reference and study design	Results	
Rats		
High confidence		
Morgan et al. (1986a) Fischer 344 rats; male; 3–8/exposed groups and 9/control group. Exposure: Rats were exposed to FA in dynamic head-only chambers for 10, 20, 45, or 90 min or 6 hrs with or without a 1-hr recovery period. Test article: Paraformaldehyde. Actual concentrations were within 5% of nominal concentrations of 0, 2.5, or 18.5 mg/m³.^a Mucociliary function (i.e., mucus flow pattern, mucus flow rate, and ciliary activity) evaluated by using dissected nasal mucosa that included the nasal septum and lateral wall. Figure 2 from Morgan et al. (1986b) depicting areas of rat nasal passages used to determine flow rate on nasal septum and lateral wall. Main limitations: No major limitations	<i>Changes in mucociliary function</i>	
	Group	Observations
	Controls	Mean mucus flow rates for nasal septum were slower (0.91–1.2 mm/min) compared to rates on the lateral wall (3.61–8.15 mm/min); lateral wall mucus flow by region (slowest to fastest): anterior, midregions, posterior
	18.5 mg/m ³ (no recovery period)	Ciliastasis and mucostasis observed in specific regions of nose with discernible differences between recovery and nonrecovery groups; ciliastasis increased progressively with duration of exposure and was observed on anterior and ventral septum, antero-medial and dorsal maxilloturbinate, and lateral wall and lateral nasoturbinate; distribution of mucostasis exhibited greater variation within exposure groups compared to ciliastasis; mucostasis exhibited similar site specificity as ciliastasis but with greater coverage than ciliastasis (<1 to several mm posterior to regions of ciliastasis); mucus flow observed over areas of ciliastasis in antero-medial and antero-dorsal maxilloturbinate, anterior lateral wall, and anterior septum; mean mucus flow rates reduced in areas of nasal septum and lateral wall with intact mucociliary function
18.5 mg/m ³ (90-min or 6-hr exposure with 1-hr recovery period)	90-min group: recovery characterized to be almost complete, ciliastasis confined to small regions of antero-ventral septum, antero-medial maxilloturbinate, antero-lateral nasoturbinate, and adjacent lateral wall; extent of ciliastasis similar to 18.5 mg/m ³ , 20-min group 6-hr group: recovery characterized as considerable but incomplete, especially in posterior regions of	

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results	
Morgan et al. (1986c) (Fischer 344 rats; male; 6 exposed and 12 controls (n=6) morning, n=6 afternoon)/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 1, 2, 4, 9, or 14 d. Exposure was followed by an 18-hr recovery period for some groups. Test article: Paraformaldehyde. Actual concentrations were 0, 0.57 (0.5–0.6; range), 2.46 (2.4–2.7), 7.27 (7.0–7.5), and 17.7 (15.0–18.5) mg/m³.^a Mucociliary function and mucus flow rate evaluated by using dissected nasal mucosa within 20 min after death. Histopathologic evaluation of the respiratory tract included transverse sections of the nasal mucosa tissues used in the evaluation of mucociliary function. Figure 1 from Morgan et al. (1986b) depicting rat nasal passages opened near the midline. Septum was removed to reveal turbinates. Arrows indicate direction of mucus flow, and numbers represent areas assessed for mucus flow rate. Inset represents lateral aspect of nasoturbinate showing lateral scroll. Main limitations: No major limitations	2.5 mg/m ³	nose; reduced mucus flow rates compared to equivalent regions in control rats No evidence of impaired mucociliary function
	<i>Changes in mucociliary function</i>	
	Group	Observations (truncated from original article)
	Controls	Mucociliary apparatus functioned for 20–60 min after death; minimal inter-animal variation in mucus flow rate
	General observations for exposed groups	Concentration- and duration-related defects included cessation or severe slowing of mucus flow (mucostasis), loss of ciliary function (ciliastasis), or alterations in mucus flow patterns; minimal inter-animal variation; mucostasis observed to generally be more extensive than ciliastasis, mucus was found flowing over areas of inactivated cilia
	17.7 mg/m ³	Duration-dependent mucostasis most frequently observed on dorsal and medial aspects of maxilloturbinate, lateral aspect of nasoturbinate (especially lateral scroll), lateral ridge, and lateral wall; little or no recovery 18 hrs after exposure
	7.27 mg/m ³	Changes were much less extensive as those in 17.7 mg/m ³ group
	2.46 mg/m ³	Changes were characterized as minimal or absent; localized inhibition of ciliary activity for few animals was observed on ventral margin of nasoturbinates with 9 days of exposure
	0.57 mg/m ³	No inhibition of mucociliary function observed
	<i>Changes in mucus flow rate</i>	
	Group	Observations
	Controls	No significant differences observed between morning and afternoon groups, combined for statistical analysis with exposed groups
	General observations	Mucus flow rates found to be characteristic of specific regions of the nose and observed to be slowest on anteromedial naso- and maxilloturbinates and anterior margin of ethmoid turbinate, fastest on lateral wall, and intermediate on other regions
	17.7 mg/m ³	Reduction of mean mucus flow rate without histologic changes observed on ventromedial surface of nasoturbinate (area 1) after 1 d of exposure, with more pronounced and statistically significant reductions after 9 d of exposure even with 18 hrs of recovery
	7.27 mg/m ³	No consistent changes in mucus flow rate observed except in areas with mucostasis
	2.46 mg/m ³	No reduction in mucus flow rate observed; nonstatistically significant increases in mean mucus flow rates observed on posteromedial aspect of nasoturbinate (area 10)
	0.57 mg/m ³	No reductions in mucus flow rate observed; statistically significant increases in mean mucus flow rate observed in areas 6 and 9 after 4 d of exposure but not after 9 d of exposure

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results			
Frogs				
Low confidence				
Morgan et al. (1984) Leopard frogs; male; 6/group. Exposure: Frog palates were exposed to FA in an ex vivo chamber for up to 30 min after a 5-min equilibration period. Test article: Paraformaldehyde. Actual concentrations were within 20% of nominal values and are reported for each endpoint in the Results column.^a Mucociliary function (i.e., mucus flow and ciliary activity) evaluated by using dissected frog palates. Main Limitations: ex vivo, acute exposure; nonmamalian model	Group (± SE)	Initial response ^a to exposure ^b	Mucus stasis ^b (min ± SE)	Ciliastasis ^b (min ± SE)
	11.8 (±0.37) mg/m ³	6/6	6/6 (1.93±0.13)	6/6 (3.47±0.44)
	5.36 (±0.36) mg/m ³	6/6	4/6 (8.14±3.27) ^c	4/6 (13.6±5.18) ^c
	1.69 (±0.10) mg/m ³	6/6 ^d	0/6	0/6
	0.28 (±0.04) mg/m ³	0/6	0/6	0/6
	^a Response was increased ciliary activity in the presence or absence of increased mucus flow rate. ^b Number of cases in which change was observed/number of cases examined. ^c Values in parentheses indicate time to induce the effect for the four positive cases. ^d The response was variable and generally very slight in this group.			
Group mg/m ³ (± SE)	Observations for mucociliary function (truncated from original article)			
11.8 (±0.37)	Increased ciliary activity and mucus flow rate; peak mucus flow rate followed by rapid decline, cessation of flow, beating cilia, and changes to mucus flow; ciliastasis preceded by reduced beat frequency and amplitude			
5.36 (±0.36)	Considerable inter-animal variation observed			
1.69 (±0.10)	Inter-animal variation observed; initial response involved variable increase in mucus flow rate and increased ciliary activity or more frequent surges of increased activity			
0.28 (±0.04)	No apparent effect after 30-min exposure			
0	Very few ciliated cells observed to be actively beating; any ciliary beating occurred in individual or small groups of cells; basal mucus flow rate determined to be 0–4 mm/min			

As = anterior septum.

^aStudy authors originally reported FA concentrations in ppm. These values were converted based on 1 ppm = 1.23 mg/m³, assuming 25°C and 760 mm Hg.

Table C-29. Mucociliary function studies in humans

Study and design	Exposure	Results
Medium Confidence		
Andersen and Molhave (1983) Denmark <i>Controlled Human Exposure Study</i> Participants: 16 healthy students, 5 females and 11 males. Mean age: 23 years; range 20–33 years. 31% smokers with one heavy smoker having >20 cigarettes per day. None had past formaldehyde exposure and all had healthy upper airways.	A 5-hr exposure study. Subjects assigned to four groups, each group undergoing four different exposures over 4 consecutive days. Levels were 0.3, 0.5, 1.0 and 2.0 mg/m³ formaldehyde with order decided by latin square design. Each day began with 2 hr control period using clean air at 23 ± 0.5° C, 50 ± 5 % humidity, air velocity 10 ± 3cm/s and air supply	A statistically significant decrease in mucus flow rate occurred in the anterior two-thirds portion of the ciliated nose (slits 1–4). Mucus flow rate shown to decrease with increasing formaldehyde concentrations starting at 0.3 mg/m³ and then leveling off after 0.5 mg/m³. Flow rate decreases did not fluctuate with time of exposure.

Supplemental Information—Formaldehyde (Inhalation)

Study and design	Exposure	Results
All were habitually nasal breathers with no history of chronic or recent acute respiratory disease. Methods: Three identical sets of subject measurements taken each day, first during control period, second after 2–3 hrs of exposure and third after 4–5 hrs of exposure. Nasal mucociliary flow measurements in slits 1–2 are most anterior and slits 5–6 are most posterior part of the ciliated nose. ANOVA significance at 5%. Main limitations: short exposure duration; note: internal control	rate of 500 m³/h. Control air comprised of outdoor air filtered through absolute and charcoal filters. Following control period, formaldehyde was added to air, reaching steady state concentration after one hour. Formaldehyde generated by passing air through an 80°C oven containing paraformaldehyde. Variation monitored, ranging within ±20% from the target values.	
Low Confidence		
Holmström and Wilhelmsson (1988) <i>Sweden</i> <i>Prevalence Study</i> Population: Two exposed groups 170 total; 70 formaldehyde production workers, Mean age 36.9 years, 87% male, mean duration employment 10.4 year. 100 workers exposed to wood dust and formaldehyde at five furniture factories. Mean age 40.5 years, 93% male, mean duration employment 16.6 year. Referent: 36 persons from local government in the same village as the furniture workers, with no history of occupational exposure to formaldehyde or wood dust. Mean age 39.8 years, 56% male, mean duration employment 11.4 year. Methods: Pretesting questionnaire, Mucociliary activity tested using green dye spotted on both inferior turbinates 1 cm posterior to the anterior border of the turbinate. Measured transit time of spot to rhinopharynx. Chi-square tests or 2-tailed <i>t</i>-test for group comparisons. Main limitations: poor matching of referent group (i.e., different	Personal sampling in breathing zone for 1–2 hours in 1985. Total dust and respirable dust also measured. Previous measurements 1979-1984 in chemical company combined with 1985 values to estimate average annual values for each participant. Only 1985 values available for wood factories. Formaldehyde concentration: Chemical plant: 0.05–0.5 mg/m³, mean 0.26 [SD 0.17 mg/m³]. Furniture factory: 0.2–0.3 mg/m³, mean 0.25 [SD 0.05 mg/m³]. Referent mean 0.09 mg/m³ (based on 4 measurements in 4 seasons).	Mucociliary clearance is defined to be pathological if transit time is > 20 minutes for one or both spots. In formaldehyde only group, 20% of subjects (14/69, <i>p</i> <0.05 compared to referent) had clearance times > 20 minutes compared to 15% of the formaldehyde-dust group (14/95) and 3% of the referent group (1/36). Formaldehyde-only nasal specimens had higher mean score of 2.16 (range 0–4) (<i>p</i> <0.05) while formaldehyde-dust group had mean score 2.07 (range 0–6) (<i>p</i> >0.05). Referent group score was 1.56 (range 0–4). Combining formaldehyde-only and formaldehyde-dust group mean score 2.11 (<i>p</i> <0.05). No correlation observed between smoking habits and biopsy score, nor was a correlation found between the duration of exposure and any histological changes.

Study and design	Exposure	Results
occupation type; lower proportion of males); inclusion of only current workers and long duration of employment raises possibility of healthy worker effect due to irritation effects; crude measure.		

Synthesis on Cellular Proliferation in the Respiratory Tract

A number of quantitative cellular proliferation studies have been carried out in experimental animals, primarily in rats (see Figure C-22 and Tables C-30 and C-31). While these experiments provide more robust quantification of changes in cell number compared to histological determinations of tissue hyperplasia, the data provided by these approaches are limited to active proliferation and do not directly inform cumulative proliferative responses. For example, the most common approaches involve in vivo administration of either bromodeoxyuridine (BrdU, a thymidine analog) or tritiated thymidine ($[^3\text{H}]$ -thymidine), both of which label newly-synthesized DNA in dividing cells. When either of these are administered during the last 1–3 days of an exposure (nearly all of the studies followed a similar protocol), these experiments would only be able to measure the proliferation actively occurring during the 1–3 days at the end of the exposure; they would provide no information on proliferation induced earlier during the exposure period, or on adaptive changes to proliferative responses that might have resulted from those initial exposure effects. Despite this limitation, these studies still provide useful information on the magnitude of acute proliferation induced at different concentrations and following different durations of formaldehyde exposure. In addition, in some studies, histopathology was assessed along with cell proliferation, which may inform potential correlations between cellular proliferation and apical tissue pathology endpoints. The studies generally assessed cell proliferation in the anterior part of the nasal cavity, focusing on discrete regions (i.e., cross section levels) of the epithelium, with a few studies extending their investigation beyond the nasal cavity to include the trachea, larynx, and carina. There were notable differences in methodology across studies, including the use of different DNA synthesis-labeling agents (i.e., BrdU, $[^3\text{H}]$ thymidine, ^{14}C), different durations of labeling (i.e., 2 hours to 3 days), and different measures of proliferation (i.e., cell turnover; ^{14}C incorporation; labeling index [LI]: the ratio of labeled cells to total counted cells; unit length labeling index [ULLI]: the ratio of labeled cells per mm of basement membrane). While these methodological differences complicate direct comparisons across studies, increases in cell proliferation were in general consistently observed across several rat strains, with supportive findings in smaller databases of mice and monkey studies. Proliferation responses, at least in the anterior nasal cavity of exposed rats, were concentration-dependent, while in most studies the response magnitude remained relatively constant across exposure duration (i.e., acute proliferation responses were not notably larger after longer exposure at similar concentrations; see Figure C-22); the only study to test

proliferation beyond 13 weeks of exposure suggested that response magnitude may actually begin to decrease in most nasal regions after chronic exposure ([Monticello et al., 1996](#)).

As illustrated in Figure C-22, after ≤ 1 week, 1–6 weeks, or ≥ 12 weeks of exposure, proliferation in the nasal epithelium was increased in a concentration-dependent manner in F344 rats, and from a more limited set of studies, in Wistar rats. Proliferation was also shown to increase in single studies of rhesus monkeys (after exposure for either 1 or 6 weeks to 7.38 mg/m^3 formaldehyde; ([Monticello et al., 1989](#))) and B6C3F1 mice (after exposure for 1 to 5 days at approximately 18.45 mg/m^3 formaldehyde; ([Swenberg et al., 1983b](#); [Chang et al., 1983](#))). Interestingly, as with other respiratory tract effects, mice might be less sensitive to changes in cellular proliferation, although the data relevant to this interpretation are sparse. Specifically, proliferation in the epithelium lining nasal associated lymphoid tissue (NALT) was observed in F344 rats, but not in B6C3F1 mice, even at concentrations as high as 18.4 mg/m^3 ([Kuper et al., 2011](#)). This potential difference could reflect the differential sensitivity to reflex bradypnea across species (see Appendix C.2). In rats, although the data were variable across studies, particularly in Wistar rats exposed for ≤ 1 week ([Zwart et al., 1988](#); [Woutersen et al., 1987](#); [Wilmer et al., 1989](#); [Reuzel et al., 1990](#); [Cassee and Feron, 1994](#); [Cassee et al., 1996b](#)), the levels of cell proliferation in regions such as the anterior lateral meatus were typically 1.5- to 25-fold greater than control levels after exposure to $\geq \approx 12 \text{ mg/m}^3$ formaldehyde, regardless of exposure duration. While levels were similarly increased at $\approx 6\text{--}7.5 \text{ mg/m}^3$ after exposure durations ≤ 13 weeks, the only study to evaluate longer exposures observed less robust increases in proliferation after chronic exposure, as compared to proliferation levels after 3 months of exposure ([Monticello et al., 1996](#)). The results across studies were less consistent at formaldehyde concentrations below 4 mg/m^3 , with several studies at $2.5\text{--}3.67 \text{ mg/m}^3$ indicating that proliferation tended to increase in some nasal regions after ≥ 12 weeks ([Zwart et al., 1988](#); [Meng et al., 2010](#); [Andersen et al., 2010](#))¹⁶ and others suggesting elevations in proliferation at concentrations ranging from $1.24\text{--}3.69 \text{ mg/m}^3$ with exposure ≤ 1 week ([Zwart et al., 1988](#); [Roemer et al., 1993](#); [Reuzel et al., 1990](#)), although not all comparisons in all regions evaluated were statistically significant. Changes at these concentrations were not observed in several other studies of similar exposure duration, or in any studies examining 1–6 weeks of exposure. Increases in proliferation were typically not observed at formaldehyde concentrations below 1.23 mg/m^3 , although some weak induction was noted in a few studies.

Proliferation generally exhibited a decreasing anterior to posterior gradient and correlated with sites of respiratory tract pathology. For example, after adjusting for the number of animals with accurate tumor localization and including target cell population size in the comparison, increased cell proliferation was correlated ($R^2 = 0.88$) with the incidence of squamous cell carcinoma; however, cell proliferation alone (i.e., without considering target cell population size)

¹⁶These data from Meng et al. are revisited in the context of uncertainty and variability in the dose-response for cell replication in Appendix D.2.2.

was not as well correlated ([Monticello et al., 1996](#)), suggesting that some minimal cell population size may be important for tumor formation. Cell proliferation has also been shown to be correlated with hyperplasia and squamous metaplasia; nasal lesions indicative of cytotoxicity such as cell degeneration, necrosis, or erosion and/or inflammation ([Speit et al., 2011b](#); [Monticello et al., 1991](#); [Andersen et al., 2008](#); [Andersen et al., 2010](#)). Although most studies demonstrated proliferation in anterior regions of the nasal cavity, primarily examining sections at cross level 2 (variably including anterior and/or medial portions of structures such as the lateral meatus, maxilloturbinate, and nasoturbinate), some studies demonstrated formaldehyde-induced changes in more posterior regions, including regions outside of the URT. For example, exposure of groups ($n = 3$) of rhesus monkeys to 7.36 mg/m^3 for 1 or 6 weeks resulted in increased proliferation along with slight histological changes (e.g., inflammation, hyperplasia, and metaplasia) in both the nasal cavity and extranasal regions including the larynx, trachea, and carina, but not the bronchioles ([Monticello et al., 1989](#)). In F344 rats, increased proliferation was observed in the nasopharynx at $\geq 12.3 \text{ mg/m}^3$ (with slight increases at 2.48 mg/m^3) after 4 weeks of exposure ([Speit et al., 2011b](#)). Increased proliferation in the trachea and lung was observed in SD rats following 1 or 3 days of exposure to 24.6 mg/m^3 , with mixed findings at lower concentrations, including increased proliferation in the trachea at 2.5 mg/m^3 after 1 day of exposure, but decreased proliferation in the trachea with 3 days of exposure at $2.5\text{--}7.4 \text{ mg/m}^3$ ([Roemer et al., 1993](#)).

These latter data highlight the complicated nature of the association between formaldehyde exposure duration and cellular proliferation. While, generally, proliferation appears to be sustained at similar levels across exposure durations ranging from 1 day to 13 weeks (see Figure C-22), some studies reported differences in the magnitude of effects in specific regions of the respiratory tract tissue after different exposure durations. In studies of F344 and Wistar rats exposed to a wide range of formaldehyde concentrations ($0.37\text{--}18.5 \text{ mg/m}^3$), proliferation induced by formaldehyde exposure was typically not increased with longer exposure duration (in some instances, it was slightly decreased, but statistical comparisons were not performed) in various anterior nasal sections (approximately levels I–III), including comparisons of 3 days to 10 days ([Swenberg et al., 1983b](#); [Chang et al., 1983](#)), 5 days to 15 days ([Andersen et al., 2008](#)), and 4 days to 6 weeks ([Monticello et al., 1991](#)) in F344 rats (note: response magnitude increased from 1 to 4 days in the latter study) and comparisons of 3 days to 4 weeks ([Wilmer et al., 1987](#)) and 3 days to 13 weeks in Wistar rats ([Zwart et al., 1988](#)). In several of these studies, the data suggest that formaldehyde concentration had a much greater impact on proliferation than exposure duration, although the relative contributions of concentration versus duration could not be accurately defined ([Wilmer et al., 1987, 1989](#); [Swenberg et al., 1983b](#); [Chang et al., 1983](#)). Somewhat complicating this, an increasing magnitude of proliferation at the same formaldehyde concentration was observed in anterior nasal regions of F344 rats exposed to $7.4\text{--}18.5 \text{ mg/m}^3$ for 13 weeks, as compared to 1 or 4 weeks ([Andersen et al., 2010](#)), or for 5 days, as compared to 1 day ([Chang et al., 1983](#)), although an increase was not observed in B6C3F1 mice in the latter study. Similarly, in a study of rhesus

monkeys, there was a noted exposure duration-dependent increase in proliferation in more posterior regions (approximately nasal section levels III–V as well as regions posterior to the nasal cavity) at 7.4 mg/m³ from 1 to 6 weeks of exposure ([Monticello et al., 1989](#)). Interestingly, while duration-dependent increases in proliferation were observed in anterior nasal regions of F344 rats exposed to 0.86–18.5 mg/m³ for 1–13 weeks, cell proliferation was greatest at 4 weeks, as compared to 1 or 13 weeks, when examining central and posterior portions (levels 2–3) of the nasal cavity ([Meng et al., 2010](#)). Finally, as previously mentioned and of particular interest, are the results of Monticello et al. ([1996](#)) in F344 rats exposed to 0.85–18.4 mg/m³ formaldehyde. The authors observed decreases in proliferation when comparing 3 months of exposure with longer durations up to 18 months within most of the nasal regions examined, including the lateral meatus, the anterior and posterior mid-septum, and medial maxilloturbinate; however, the opposite finding (i.e., duration-dependent increases in proliferation) was observed in the anterior dorsal septum ([Monticello et al., 1996](#)). Overall, the pattern across studies is mixed but indicates region-specific differences in the impact of exposure duration on proliferation.

A large number of well-conducted studies have evaluated acute cellular proliferation after exposure to a wide range of formaldehyde concentrations for durations ranging from 1 day to 18 months. The data were variable across studies. This variability is assumed to result, at least in part, from methodological factors that include the selection and preparation of tissue for analysis, the composition and administration protocol of the labeling agent used to indicate proliferation, when the proliferation counts were made (e.g., age of the animal), and the units used to express proliferation data (e.g., LI versus ULLI) ([Monticello et al., 1993](#); [Monticello and Morgan, 1997](#); [Goldsworthy et al., 1991](#); [Goldsworthy et al., 1993](#)). Despite this methodological variability, cell proliferation was consistently increased in response to formaldehyde exposure in anterior portions of the rat, mouse, and monkey nasal cavity, with studies in rats demonstrating a prominent role for formaldehyde concentration. While some studies in rats and monkeys demonstrated a role for exposure duration in cell proliferation within specific regions of the respiratory tract, acute proliferation in most nasal regions generally remained constant regardless of exposure duration. The variability in the labeling index data in ([Monticello et al., 1991](#); [Monticello et al., 1996](#)) is extensively characterized in Section D.2.2 “Characterization of uncertainty-variability in cell replication rates.”

Supplemental Information—Formaldehyde (Inhalation)

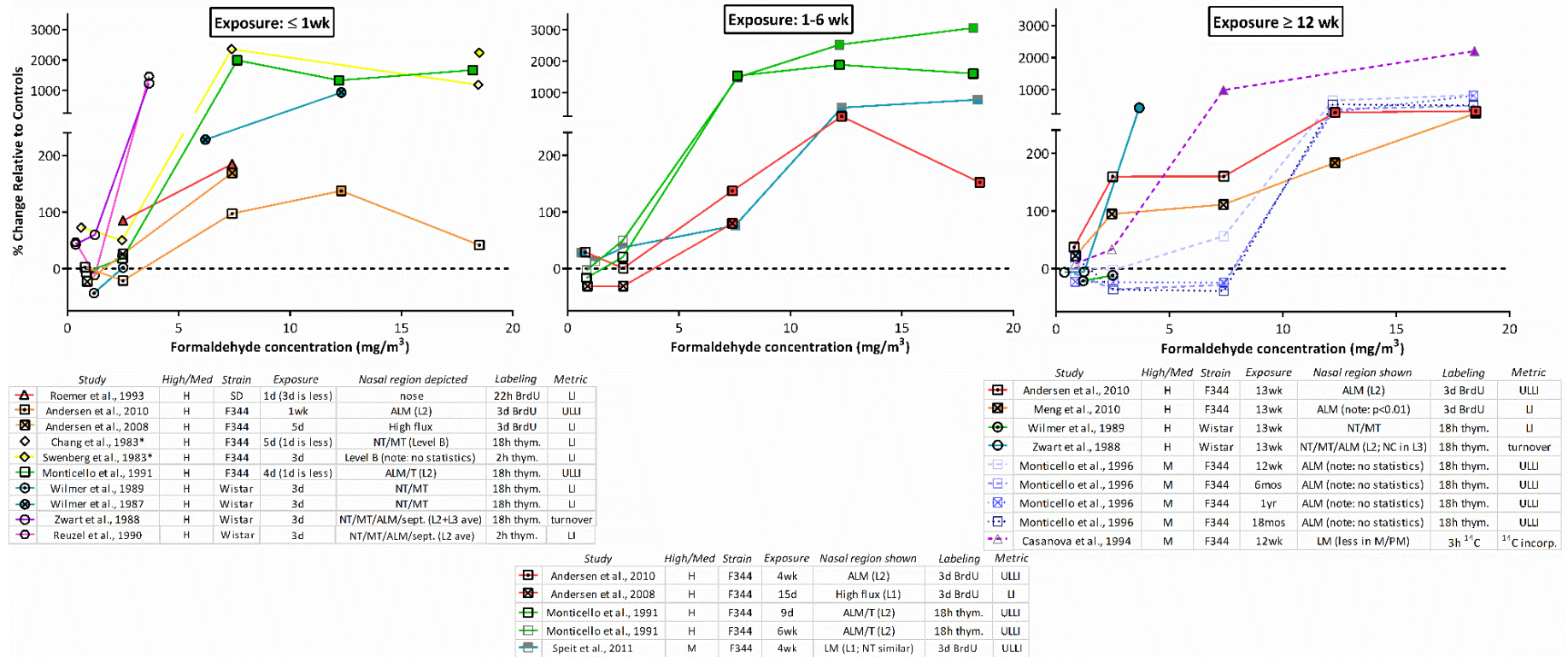


Figure C-22. Nasal cell proliferation in rats exposed to formaldehyde. Summary of rat studies of nasal cell proliferation (as % change relative to controls) following different durations of formaldehyde exposure, specifically ≤1 week (left panel), 1–6 weeks (center panel), or ≥ 12 weeks (right panel). The tables below each panel summarize the studies, study confidence determinations (only high and medium confidence studies are shown), exposure durations, nasal regions depicted, cell labeling methods used, and the method of data reporting for each corresponding panel. Note: solid symbols indicate statistical significance, as identified by the study authors. High confidence studies are indicated by bolder symbols and with solid, rather than dashed, connecting lines. Data at different timepoints from the same study are indicated by use of the same line colors and general symbol shapes. See Tables C-30 and C-31 for additional details.

Table C-30. Subchronic or chronic exposure cell proliferation studies in experimental animals

Reference and study design	Results				
Rats					
High confidence					
Andersen et al. (2010) Fisher 344; male; 8/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 1, 4, or 13 wks. Rats sacrificed immediately after last exposure. Test article: Paraformaldehyde. Actual concentrations reported in the Results column. Target concentrations were 0, 0.8, 2.5, 7.4, 12.3, and 18.5 mg/m³.¹ Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 days prior to sacrifice) and determining labeling index at levels I (highest FA flux near nose tip), II (anterior lateral meatus, anterior mid-septum, medial aspect of maxilloturbinate), and III (posterior lateral meatus, posterior mid-septum). Cell proliferation at each site reported as number of labeled cells per total cells (i.e., LI) and as the number of labeled cells per length (i.e., mm) of basement membrane (i.e., ULLI). Supplemental 4A from Andersen et al. (2010) depicting a schematic illustration of the nasal cavity levels used for cell proliferation studies.	<i>Nasal Epithelium ULLI</i>				
	Formaldehyde (mg/m ³)				
	Site	0	0.8	2.5	
	<i>High-flux region (HFR)</i>				
	1 week	12.8±3.5 ^a (7) ^b	15.0±12.5 (8)	13.8±7.0 (8)	
	4 weeks	20.3±4.1 (7)	17.8±3.8 (8)	18.5±4.6 (5)	
	13 weeks	21.9±20.3 (3)	12.2±10.3 (3)	29.1±32.7 (6)	
	<i>Anterior lateral meatus (ALM)</i>				
	1 week	31.9±26.3 (8)	32.6±30.2 (8)	25.1±26.1 (8)	
	4 weeks	26.6±17.1 (8)	34.3±21.3 (8)	26.7±7.9 (8)	
	13 weeks	21.7±15.1 (8)	29.7±24.6 (8)	56.3±33.3 (8)	
	^a Mean ULLI±SD; ^b Number of animals examined.				
	<i>Nasal Epithelium ULLI (continued)</i>				
	Formaldehyde (mg/m ³)				
	Site	0	7.4	12.3	18.5
	<i>High flux region (HFR)</i>				
	1 week	12.8±3.5 ^a (7) ^b	25.2±13.3 (8)	36.1±14.3 ^c (8)	25.3±17.5 (7)
4 weeks	20.3±4.1 (7)	40.9±24.9 (5)	69.2±17.7 ^c (6)	63.6±26.1 ^c (8)	
13 weeks	21.9±20.3 (3)	17.4 (1)	58.3±27.8 (5)	110.2±46.0 ^c (7)	
<i>Anterior lateral meatus (ALM)</i>					
1 week	31.9±26.3 (8)	62.9±50.3 (8)	75.7±31.1 ^d (8)	45.1±25.7 (8)	
4 weeks	26.6±17.1 (8)	63.1±21.6 ^c (8)	90.7±17.6 ^c (8)	67.0±10.5 ^c (8)	
13 weeks	21.7±15.1 (8)	56.4±17.2 (8)	83.3±33.3 ^c (8)	91.8±33.1 ^c (8)	
^a Mean ULLI±SD; ^b Number of animals examined; ^d p<0.01; ^e p<0.05.					
Meng et al. (2010) Fischer 344; males; 8/group. Exposure: Rats were exposed to FA in dynamic chambers (not otherwise specified) 6 hrs/d, 5 d/wk for 1, 4, or 13 wks. Test article: Paraformaldehyde. Actual concentrations were not reported. Target concentrations were 0, 0.86, 2.46, 7.38, 12.3, and 18.5 mg/m³.	Dose-dependent increases in cell proliferation of nasal epithelium at 1, 4, and 13 wks of exposure. Cell proliferation had a decreasing anterior to posterior gradient. Duration-dependent increases in cell proliferation at the anterior portion of nasal cavity. Cell proliferation greatest in the central and posterior regions of the nose following 4 weeks of exposure.				

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results			
Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 d prior to sacrifice) and determining labeling index in the anterior lateral meatus (lateral wall) for both sides of the nose. Cell proliferation data reported as percentage of BrdU-labeled cells among the total number of labeled and unlabeled cells.	FA (mg/m ³)	% BrdU-labeled cells after 13 wk		
	0	18		
	0.86	22		
	2.46	35		
	7.38	38		
	12.3	51 ^a		
	18.5	64 ^a		
^a <i>p</i> <0.01, compared to control group				
Wilmer et al. (1989) Wistar rats; male; 25/group. Exposure: Rats were exposed to FA in dynamic horizontally placed glass cylinders (with sampling ports at the inlet and outlet) either continuously for 8 hrs/d, 5 d/wk for 13 wks or intermittently 8 hrs/d (successive periods of 0.5 hr of exposure and 0.5 hr of nonexposure), 5 d/wk for 13 wks. Test article: Paraformaldehyde. Actual concentrations were not determined. Target concentrations were 0, 1.2, or 2.5 mg/m ³ for continuous exposures and 0, 2.5, or 4.9 mg/m ³ for intermittent exposures. ¹ Cell proliferation studies carried out after 3 d or 13 wks of FA exposure with [³ H]thymidine labeling (ip injection 18 hrs postexposure) and scoring of the cells lining the nasal (n=1,000) and maxillary (n=1,000) turbinates and the septum (n=3,000).	Percentage of [³ H]thymidine labeled cells in nasal epithelium			
		% labeled cells		
Exposure	Exposure x time	After 3 d	After 13 wk	
0 mg/m ³	0 mg/m ³ hr/d	0.60 (0.37) ^a	1.03 (0.26)	
1.2 mg/m ³ (continuous)	9.6 mg/m ³ hr/d	0.34 (0.10)	0.81 (0.54)	
2.5 mg/m ³ (continuous)	20 mg/m ³ hr/d	0.61 (0.28)	0.91 (0.59)	
2.5 mg/m ³ (intermittent)	10 mg/m ³ hr/d	0.29 (0.20)	1.16 (0.59)	
4.9 mg/m ³ (intermittent)	19.6 mg/m ³ hr/d	0.58 (0.32)	2.86 (1.80)	
^a SDs shown in parentheses.				
Zwart et al. (1988) Wistar rats; male and female; 50/group/sex. Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/day, 5 d/wk for 13 wks. Test article: Paraformaldehyde. Actual concentrations were 0, 0.37 (±0.02), 1.24 (±0.10), and 3.67 (±0.27) mg/m ³ . ¹ Cell proliferation studies carried out after 3 d or 13 wks of FA exposure with [³ H]thymidine labeling (i.p. injection 18 hrs postexposure) and scoring of the cells lining the nasal and maxillary turbinates (n=1,500), septum (n=2,000), and lateral wall (n=1,500) at Section III.	Cell proliferation (based on 5 rats/group/sex) 3 days: Section III – Exposure-related increase in cell turnover for combined data (males and female, <i>p</i> <0.001), with statistically significant differences between males and females (<i>p</i> <0.02). Section II – Cell turnover statistically significant (<i>p</i> <0.001) in 3.67 mg/m ³ group, no difference in 0.37 and 1.24 mg/m ³ groups compared to controls. 13 weeks: Section III – Statistically nonsignificant decrease in mean cell turnover for all groups. Section II – Cell turnover statistically significant (<i>p</i> <0.001) in 3.67 mg/m ³ group, no difference in 0.37 and 1.24 mg/m ³ groups compared to controls. Compared to Section II, cell turnover roughly 10 times greater at Section III.			

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results																									
Only cells lining the nasal septum were scored at Section II.	Data extracted using GrabIt software (mean+SEM converted from log scale): <table><tr><th>mg/m³</th><th>Level III (3 d)</th><th>Level III (13 wk)</th><th>Level II (3 d)</th><th>Level II (13 wk)</th></tr><tr><td>0</td><td>0.517 (0.043)</td><td>0.165 (0.029)</td><td>0.022 (0.005)</td><td>0.041 (0.014)</td></tr><tr><td>0.37</td><td>0.541 (0.045)</td><td>0.133 (0.021)</td><td>0.040 (0.008)</td><td>0.038 (0.010)</td></tr><tr><td>1.24</td><td>0.872 (0.104)*</td><td>0.141 (0.027)</td><td>0.034 (0.009)</td><td>0.038 (0.005)</td></tr><tr><td>3.67</td><td>3.71 (0.442)*</td><td>0.101 (0.027)</td><td>0.435 (0.147)*</td><td>0.214 (0.050)*</td></tr></table>	mg/m ³	Level III (3 d)	Level III (13 wk)	Level II (3 d)	Level II (13 wk)	0	0.517 (0.043)	0.165 (0.029)	0.022 (0.005)	0.041 (0.014)	0.37	0.541 (0.045)	0.133 (0.021)	0.040 (0.008)	0.038 (0.010)	1.24	0.872 (0.104)*	0.141 (0.027)	0.034 (0.009)	0.038 (0.005)	3.67	3.71 (0.442)*	0.101 (0.027)	0.435 (0.147)*	0.214 (0.050)*
mg/m ³	Level III (3 d)	Level III (13 wk)	Level II (3 d)	Level II (13 wk)																						
0	0.517 (0.043)	0.165 (0.029)	0.022 (0.005)	0.041 (0.014)																						
0.37	0.541 (0.045)	0.133 (0.021)	0.040 (0.008)	0.038 (0.010)																						
1.24	0.872 (0.104)*	0.141 (0.027)	0.034 (0.009)	0.038 (0.005)																						
3.67	3.71 (0.442)*	0.101 (0.027)	0.435 (0.147)*	0.214 (0.050)*																						
Medium confidence																										
Casanova et al. (1994) Fischer 344; male; 8/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 11 wks plus 4 d. On day 5 of week 12, rats were exposed to labeled FA (i.e., H¹⁴CHO) in nose-only chambers for 3 hrs. Test article: Paraformaldehyde. Actual concentrations were 0, 0.86 (±0.02), 2.52 (±0.05), 7.23 (±0.16), 12.35 (±0.23), 17.86 (±0.37) mg/m³ for whole body exposures and 0, 0.86 (±0.02), 2.53 (±0.04), 7.39 (±0.15), and 19.4 (±0.4) mg/m³ for nose-only exposures. Cell proliferation studies carried out by determining H¹⁴CHO incorporation into DNA (i.e., de novo DNA synthesis) via liquid scintillation counting.	Cell proliferation lateral meatus (LM) versus medial and posterior meatuses (M:PM)^a <table><tr><th>FA (mg/m³)^b</th><th>Observation</th></tr><tr><td>0</td><td>NA</td></tr><tr><td>0.86</td><td>No difference between LM and M:PM</td></tr><tr><td>2.53</td><td>No difference between LM and M:PM</td></tr><tr><td>7.39</td><td>Preexposed (PE) rats: significantly greater (p≤0.02) proliferation in LM than M:PM Naïve (N) rats: greater proliferation in M:PM than LM</td></tr><tr><td>19.4</td><td>PE rats: significantly greater (p≤0.02) proliferation in LM than M:PM N rats: greater proliferation in M:PM than LM</td></tr></table> ^aFor whole body exposures to unlabeled FA, rats exposed to 0 mg/m³ were considered N, whereas rats in the other exposure groups were considered PE; ^bConcentrations represent those used for nose-only exposures with H¹⁴CHO. Cell proliferation preexposed versus naïve rats^a <table><tr><th>FA (mg/m³)^b</th><th>Observation^c</th></tr><tr><td>0</td><td>NA</td></tr><tr><td>0.86</td><td>No difference between PE and N</td></tr><tr><td>2.53</td><td>No difference between PE and N</td></tr><tr><td>7.39</td><td>PE rats: greater (p <0.01) proliferation in LM than in N rats</td></tr><tr><td>19.4</td><td>PE rats: greater (p <0.01) proliferation in LM and M:PM than N rats</td></tr></table> ^aFor whole body exposures to unlabeled FA, rats exposed to 0 mg/m³ were considered N, whereas rats in the other exposure groups were considered PE; ^bConcentrations represent those used for nose-only exposures with H¹⁴CHO. ^cLateral meatus = L; medial and posterior meatuses = M:PM.	FA (mg/m ³) ^b	Observation	0	NA	0.86	No difference between LM and M:PM	2.53	No difference between LM and M:PM	7.39	Preexposed (PE) rats: significantly greater (p≤0.02) proliferation in LM than M:PM Naïve (N) rats: greater proliferation in M:PM than LM	19.4	PE rats: significantly greater (p≤0.02) proliferation in LM than M:PM N rats: greater proliferation in M:PM than LM	FA (mg/m ³) ^b	Observation ^c	0	NA	0.86	No difference between PE and N	2.53	No difference between PE and N	7.39	PE rats: greater (p <0.01) proliferation in LM than in N rats	19.4	PE rats: greater (p <0.01) proliferation in LM and M:PM than N rats	
FA (mg/m ³) ^b	Observation																									
0	NA																									
0.86	No difference between LM and M:PM																									
2.53	No difference between LM and M:PM																									
7.39	Preexposed (PE) rats: significantly greater (p≤0.02) proliferation in LM than M:PM Naïve (N) rats: greater proliferation in M:PM than LM																									
19.4	PE rats: significantly greater (p≤0.02) proliferation in LM than M:PM N rats: greater proliferation in M:PM than LM																									
FA (mg/m ³) ^b	Observation ^c																									
0	NA																									
0.86	No difference between PE and N																									
2.53	No difference between PE and N																									
7.39	PE rats: greater (p <0.01) proliferation in LM than in N rats																									
19.4	PE rats: greater (p <0.01) proliferation in LM and M:PM than N rats																									

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results						
	Data extracted using GrAbIt software (mean+SEM):						
	mg/m ³	<i>Lateral Meatus (3h)</i>	<i>Lateral Meatus (12 wk)</i>	<i>Med/Posterior Meatus (3d)</i>	<i>Med/Posterior Meatus (12 wk)</i>		
	0.861	69.16 (0.0001)	74.93 (5.76)	57.63 (5.76)	63.40 (5.76)		
	2.46	80.69 (5.76)	92.22 (5.76)	97.98 (0.0001)	109.5 (5.76)		
	7.38	115.3 (5.76)	749.3 (161.4)*	201.7 (23.05)	276.7 (23.05)		
	18.45	149.86 (11.53)	1591 (132.5)*	334.3 (23.05)	1002 (103.7)*		
*p<0.05 for 12 wk vs 3 hr exposure							
Monticello et al. (1996) F344 rats; male; 6/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers to FA 6 hrs/d, 5 d/wk for up to 24 mos with interim sacrifices at 3, 6, 12, and 18 mos. Test article: Paraformaldehyde. Actual FA concentrations were 0 (±0.0), 0.85 (±0.06), 2.52 (±0.18), 7.39 (±0.41), 12.2 (±0.54), or 18.4 (±0.98) mg/m ³ . ¹ Cell proliferation studies (6 rats/group) conducted with surgical implantation of [methyl- ³ H]thymidine-containing pumps (5 days prior to interim sacrifice) and determining labeling index at 7 locations in the nasal passages: anterior lateral meatus, posterior lateral meatus, anterior mid-septum, posterior mid-septum, anterior dorsal septum, medial maxilloturbinate, and maxillary sinus (excluding ostium). Cell proliferation data reported as the number of labeled cell profiles per mm of basement membrane (i.e., ULLI).	mg/m ³	Exposure (mos)	Anterior lateral meatus	Posterior lateral meatus	Anterior mid-septum	Posterior mid-septum	Anterior dorsal septum
0	3	10.11 ^a	7.69	6.58 ^a	11.94	2.14	
	6	11.14	11.92	5.73	27.31	3.61	
	12	8.28	7.67	3.25	31.31	8.63	
	18	5.74	8.99	4.80	19.86	3.80	
0.85	3	10.53	7.82	8.04	13.28	1.08	
	6	10.09	8.15	3.71	17.04	2.20	
	12	6.39	5.11	1.72	13.28	1.08	
	18	6.89	6.40	4.54	18.31	4.95	
2.52	3	9.83	11.24 ^b	12.74	13.11 ^b	3.38	
	6	7.14	9.15	4.78	12.07	2.06	
	12	6.35	6.19	2.14	10.35	0.92	
	18	3.66	5.24	3.02	7.20	1.93	
7.39	3	15.78	9.65	4.15	10.52	3.55	
	6	7.98	6.74	3.52	7.76	1.52	
	12	6.24	5.42	3.06	8.76	2.01	
	18	3.51	6.47	3.96	12.30	1.96	
12.2	3	76.79	15.29	39.01	21.43	5.28	
	6	53.57	17.97	28.22	15.81	2.64	
	12	32.42	5.60	10.29	6.79	2.20	
	18	36.28	19.45	11.92	24.44	3.22	
18.4	3	93.22	59.52	75.71	51.79	5.96	
	6	65.89	44.63	75.32	61.52	26.18	
	12	74.99	44.73	51.62	60.56	37.52	
	18	34.62	22.34	30.29	37.06	52.98	
^a n=5 or 6; ^b n=4							

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results							
	<i>Exposure (mos)</i>	<i>mg/m³</i>	<i>medial maxilla turbinate</i>	<i>maxillary sinus</i>	<i>mg/m³</i>	<i>medial maxilla turbinate</i>	<i>maxillary sinus</i>	
	3	0	7.84 ^a	8.10	7.39	9.23	ND	
	6		17.95	ND		10.18	ND	
	12		7.85	6.31		6.22	12.04	
	18		5.58	5.95		5.03	9.51	
	3	0.85	10.33	ND	12.2	89.20	ND	
	6		9.34	ND		57.83	ND	
	12		6.79	7.80		43.27	9.15	
	18		5.08	6.99		42.74	12.12	
	3	2.52	10.84	3.12	18.4	115.19	10.77 ^b	
	6		10.41	ND		101.97	13.13	
	12		5.98	7.73		66.64	17.06	
	18		3.42	8.52		63.11	13.16	
	^a n=5 or 6; ^b n=3							

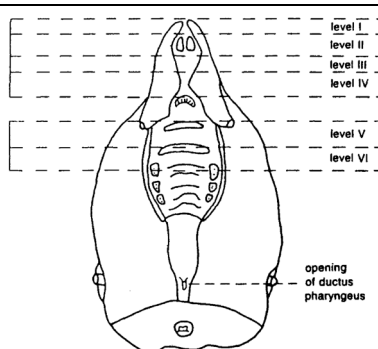
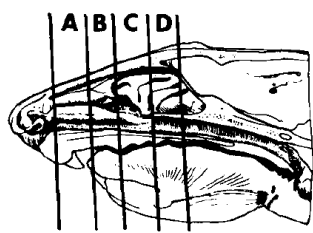
* $p < 0.05$ as reported by the study authors, unless otherwise indicated

Table C-31. Short-term exposure cell proliferation studies in experimental animals

Reference and study design	Results						
Rats							
High Confidence							
Andersen et al. (2008) Fischer 344 rats; male; 8/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for up to 3 wks. Rats sacrificed at end of single 6-hr exposure (Day 1), 18 hrs after single 6-hr exposure (Day 1 recovery), at end of 5 d of exposure (Day 5), at end of 6 d of exposure (Day 6), 18 hrs after 6 d of exposure (Day 6 recovery), and at end of 15 d of exposure (Day 15). Test article: Paraformaldehyde. Actual concentrations were determined on a daily basis and reported in the Results column. Target concentrations were 0, 0.9, 2.5, 7.4, and 18.5 mg/m³. This study also evaluated the effects of a single FA instillation (40 µL, 400 mM per nostril). Data presented here in the	Target concentration (mg/m ³)		Actual FA Concentrations ^a				
			Day 1 (mg/m ³)	Day 5 (mg/m ³)	Day 6 (mg/m ³)	Day 15 (mg/m ³)	
	0		0±0	0±0	0±0	0±0	
	0.9		0.74±0.23	0.79±0.15	0.75±0.16	0.7±0.11	
	2.5		2.08±0.46	2.14±0.43	2.26±0.49	2.2±0.31	
	7.4		5.83±1.73	6.43±0.76	6.00±1.25	6.14±0.97	
	18.5		17.7±5.7	NA	NA	NA	
	^a Daily means ± SD.						
	Cell proliferation in nasal epithelium ^a						
				Formaldehyde (mg/m ³)			
	Day	Level	Site	Control	0.9	2.5	7.4
	5	I	NA	38.6±8.5 ^b (13.2±4.6)	36.8±14.7 (10.2±2.8)	65.0±39.8 (16.6±6.0)	155.0±88.9 ^c (35.5±14.8) ^c
			Alm	6.0±2.5	7.5±1.1	7.3±1.7	29.0±21.9 ^c
II		As	5.6±3.0	6.0±1.6	6.6±3.5	14.2±10.3 ^c	
		Mam	6.5±2.1	6.8±3.1	9.7±3.8	35.1±22.0 ^c	
III		Plm	6.4±3.0	8.1±2.4	10.0±4.0	16.1±6.4 ^c	
		Ps	8.9±3.0	7.5±3.5	8.0±5.2	15.0±11.9 ^c	

Supplemental Information—Formaldehyde (Inhalation)

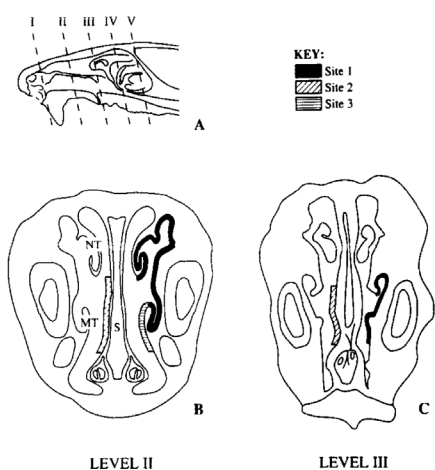
Reference and study design	Results																						
Results column are for inhalation exposures. Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 d prior to sacrifice) and determining labeling index at levels I (front of nose), II (anterior lateral meatus, anterior septum, medial aspect maxilloturbinate), and III (posterior lateral meatus, posterior septum). Cell proliferation determined only for days 5 and 15 and reported as the number of labeled cell profiles per mm of basement membrane (i.e., ULLI).	15	I	NA	78.9±54.7 (22.6±17.2)	55.8±37.3 (15.6±10.5)	50.8±44.2 (15.6±13.1)	119.1±38.0 (40.6±11) ^c																
		II	Alm	12.4±12.4	18.2±11.4	12.1±7.0	19.1±8.7																
			As	12.0±9.7	17.6±11.0	10.0±4.6	14.1±8.7																
			Mam	22.7±23.0	27.2±18.6	20.9±20.6	21.9±16.8																
		III	Plm	11.8±10.0	12.6±6.3	11.7±7.6	13.6±7.2																
			Ps	15.9±15.2	13.0±5.9	12.5±6.3	18.3±12.1																
^a Reported as mean±SD; ^b Data represent ULLI. Data in parenthesis represent LI: (labeled cells/total cells) × 100; ^c <i>p</i> <0.05.																							
Cassee et al. (1996b) Wistar rats; male; 5 to 6/group. Exposure: Rats were exposed to FA in dynamic nose-only chambers 6 hrs/d for 1 or 3 d. Rats sacrificed immediately after last exposure. Test article: Paraformaldehyde. Actual concentrations were 0, 1.2, 3.9, and 7.9 mg/m³. Cell proliferation studies carried out using deparaffinized standard cross sections of the nose and semi-quantitative proliferating cell nuclear antigen (PCNA) immunostaining. Cell proliferation studies were also conducted with surgical implantation of BrdU-containing pumps (20 hrs prior to sacrifice). Labeling index determined for the entire epithelium of both sides of anterior nasal cavity lining the nasoturbinate, maxilloturbinate, lateral wall, and septum. Cell proliferation at each site reported as number of positive-stained cells per length (i.e., mm) of basement membrane (i.e., ULLI).	1 d exposure: no treatment-related changes in cell proliferation <table><tr><th>FA (mg/m³)</th><th>Cell proliferation measured by PCNA after 3 days^a</th></tr><tr><td>1.2</td><td>Levels II and III: no increases in ULLIs</td></tr><tr><td>3.9</td><td>Level II: significant increase in ULLIs at maxilloturbinate (<i>p</i> <0.05) and nasal turbinate and lateral wall (<i>p</i> <0.01), compared to controls Level III: no increases in ULLIs</td></tr><tr><td>7.9</td><td>NR</td></tr></table> ^aBased on data from 3 to 5 rats per exposure group and 10 to 12 control rats. <table><tr><th>FA (mg/m³)</th><th>Cell proliferation measured by BrdU after 3 days^a</th></tr><tr><td>1.2</td><td>Levels II and III: no increases in ULLIs</td></tr><tr><td>3.9</td><td>Levels II and III: no increases in ULLIs</td></tr><tr><td>7.9</td><td>NR</td></tr></table> ^aBased on data from 3 to 5 rats per exposure group and 10 to 12 control rats. This study also evaluated the combined effects of FA, acetaldehyde, and acrolein on nasal epithelium. Data presented here are for formaldehyde-only exposed rats							FA (mg/m ³)	Cell proliferation measured by PCNA after 3 days ^a	1.2	Levels II and III: no increases in ULLIs	3.9	Level II: significant increase in ULLIs at maxilloturbinate (<i>p</i> <0.05) and nasal turbinate and lateral wall (<i>p</i> <0.01), compared to controls Level III: no increases in ULLIs	7.9	NR	FA (mg/m ³)	Cell proliferation measured by BrdU after 3 days ^a	1.2	Levels II and III: no increases in ULLIs	3.9	Levels II and III: no increases in ULLIs	7.9	NR
FA (mg/m ³)	Cell proliferation measured by PCNA after 3 days ^a																						
1.2	Levels II and III: no increases in ULLIs																						
3.9	Level II: significant increase in ULLIs at maxilloturbinate (<i>p</i> <0.05) and nasal turbinate and lateral wall (<i>p</i> <0.01), compared to controls Level III: no increases in ULLIs																						
7.9	NR																						
FA (mg/m ³)	Cell proliferation measured by BrdU after 3 days ^a																						
1.2	Levels II and III: no increases in ULLIs																						
3.9	Levels II and III: no increases in ULLIs																						
7.9	NR																						

Reference and study design	Results																																														
 Figure 1 from Cassee et al. (1996b) depicting cross levels of the rat nose evaluated for cell proliferation.																																															
Chang et al. (1983); [additional data from related Swenberg et al. (1983b) report] Fischer 344 rats; males; 4–5/exposure group, 9/control group. Exposure: Rats were exposed to FA in head-only chambers 6 hrs/d for 1, 3, 5, or 10 d. Test article: Paraformaldehyde. Actual concentrations were 0 and 18.5 (± 0.1) mg/m³. Target concentrations were 0, 0.62, 2.46, 3.69, 7.38, 14.76, or 18.45 mg/m³ in Swenberg et al. (1983b) report. Cell proliferation studies carried out after FA exposure with [³H]thymidine labeling (i.p. injection 2 or 18 hrs postexposure) and scoring of cells (n=9,000) lining the respiratory epithelium from the nasal and maxillary turbinates and lateral wall.  Levels A (with minimal mucociliary clearance) and B (with extensive mucociliary clearance) reported in Swenberg et al. (1983b)	<table><tr><th>Group (18.5 mg/m³)</th><th>Labeling index (%) in Level B</th></tr><tr><td>Control</td><td>0.43±0.05 (9)^a</td></tr><tr><td>1 day</td><td>5.51±0.35 (4)^b</td></tr><tr><td>5 days</td><td>10.05±0.27 (5)^{b, c}</td></tr></table> ^aNumber in parentheses represents number of animals studies; ^bSignificantly different from control, <i>p</i><0.05; ^cSignificantly different from 1-d exposed rats, <i>p</i><0.05. <i>% labeled respiratory epithelial cells in Level B (thymidine at 2 h postexposure)</i> <table><tr><th></th><th colspan="5">Formaldehyde Concentration (mg/m³)</th></tr><tr><th>Duration</th><th>0</th><th>0.62</th><th>2.46</th><th>7.38</th><th>18.45</th></tr><tr><td>3 days</td><td>0.22 (0.03)</td><td>0.38 (0.05)</td><td>0.33 (0.06)</td><td>5.4 (0.82)</td><td>2.83 (0.81)</td></tr></table> <i>% labeled respiratory epithelial cells (thymidine at 18 h postexposure)</i> <table><tr><th></th><th>3 d (Level B)</th><th>10 d (Level B)</th><th>3 d (Level A)</th></tr><tr><td>Control</td><td>0.54 (0.03)</td><td>0.26 (0.02)</td><td>3.0 (1.56)</td></tr><tr><td>3.69 mg/m³ × 12 hr/d</td><td>1.73 (0.63)</td><td>0.49 (0.19)</td><td>16.99 (1.5)</td></tr><tr><td>7.38 mg/m³ × 6 hr/d</td><td>3.07 (1.09)</td><td>0.53 (0.2)</td><td>15.46 (10.01)</td></tr><tr><td>14.76 mg/m³ × 3 hr/d</td><td>9.0 (0.88)</td><td>1.73 (0.65)</td><td>16.49 (2.07)</td></tr></table> Mean (SEM); Group sizes and statistical comparisons not reported in Swenberg et al. (1983b) Note: Pulse labeling with thymidine 18 hrs compared to 2 hrs postexposure resulted in ≈2-fold and ≈3-fold increase in labeling in control rats and at 7.38 mg/m³, respectively (Swenberg et al., 1983b).	Group (18.5 mg/m ³)	Labeling index (%) in Level B	Control	0.43±0.05 (9) ^a	1 day	5.51±0.35 (4) ^b	5 days	10.05±0.27 (5) ^{b, c}		Formaldehyde Concentration (mg/m ³)					Duration	0	0.62	2.46	7.38	18.45	3 days	0.22 (0.03)	0.38 (0.05)	0.33 (0.06)	5.4 (0.82)	2.83 (0.81)		3 d (Level B)	10 d (Level B)	3 d (Level A)	Control	0.54 (0.03)	0.26 (0.02)	3.0 (1.56)	3.69 mg/m ³ × 12 hr/d	1.73 (0.63)	0.49 (0.19)	16.99 (1.5)	7.38 mg/m ³ × 6 hr/d	3.07 (1.09)	0.53 (0.2)	15.46 (10.01)	14.76 mg/m ³ × 3 hr/d	9.0 (0.88)	1.73 (0.65)	16.49 (2.07)
Group (18.5 mg/m ³)	Labeling index (%) in Level B																																														
Control	0.43±0.05 (9) ^a																																														
1 day	5.51±0.35 (4) ^b																																														
5 days	10.05±0.27 (5) ^{b, c}																																														
	Formaldehyde Concentration (mg/m ³)																																														
Duration	0	0.62	2.46	7.38	18.45																																										
3 days	0.22 (0.03)	0.38 (0.05)	0.33 (0.06)	5.4 (0.82)	2.83 (0.81)																																										
	3 d (Level B)	10 d (Level B)	3 d (Level A)																																												
Control	0.54 (0.03)	0.26 (0.02)	3.0 (1.56)																																												
3.69 mg/m ³ × 12 hr/d	1.73 (0.63)	0.49 (0.19)	16.99 (1.5)																																												
7.38 mg/m ³ × 6 hr/d	3.07 (1.09)	0.53 (0.2)	15.46 (10.01)																																												
14.76 mg/m ³ × 3 hr/d	9.0 (0.88)	1.73 (0.65)	16.49 (2.07)																																												
Kuper et al. (2011) Fischer 344 rats; male; 8/group.	Lymph nodes: No FA-related effects on the number of BrdU-positive cells reported in the follicle and paracortex compartments and medulla																																														

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results						
Exposure: Mice were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 4 wks. Test article: Formalin (10.21% FA). Actual concentrations were 0, 0.63 (± 0.06), 1.23 (± 0.14), 2.48 (± 0.18), 7.53 (± 0.42), 12.3 (± 0.48), and 18.4 (± 0.06) mg/m³. Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 d prior to sacrifice) and determining labeling index of 2 sections of NALT and 1 section of an upper-respiratory tract-draining lymph node (i.e., posterior and superficial cervical lymph nodes). Cell proliferation data reported as BrdU-positive cells per length (i.e., mm) of epithelium.	<i>BrdU counts in section 1 of NALT</i>						
	<i>FA (mg/m³)</i>	<i>Interfollicular area</i>	<i>Interfollicular epithelium</i>	<i>Follicular area</i>	<i>Follicular epithelium</i>		
	0	61.9 $\pm$ 18.8 ^a	6.5 $\pm$ 3.2	73.0 $\pm$ 39.1	12.6 $\pm$ 17.5		
	0.63	57.3 $\pm$ 17.4	4.9 $\pm$ 2.2	53.5 $\pm$ 19.4	4.9 $\pm$ 3.8		
	1.23	55.7 $\pm$ 17.7	5.9 $\pm$ 3.4	52.2 $\pm$ 27.9	6.4 $\pm$ 6.5		
	2.48	53.5 $\pm$ 12.9	4.3 $\pm$ 2.7	49.8 $\pm$ 22.1	4.7 $\pm$ 3.2		
	7.53	51.1 $\pm$ 14.9	3.3 $\pm$ 2.4	47.6 $\pm$ 13.9	5.8 $\pm$ 5.3		
	12.3	55.5 $\pm$ 15.3	5.5 $\pm$ 3.5	51.2 $\pm$ 16.2	5.7 $\pm$ 2.9		
	18.4	54.4 $\pm$ 11.6	28.2 $\pm$ 11.1 ^b	41.4 $\pm$ 14.2	23.6 $\pm$ 13.6 ^c		
	^a Mean number of BrdU-positive cells $\pm$ SD; ^b <i>p</i> <0.001; ^c <i>p</i> <0.05.						
	<i>BrdU counts in section 2 of NALT</i>						
	<i>FA (mg/m³)</i>	<i>Interfollicular area</i>	<i>Interfollicular epithelium</i>	<i>Follicular area</i>	<i>Follicular epithelium</i>		
	0	48.3 $\pm$ 17.7 ^a	6.3 $\pm$ 2.2	62.3 $\pm$ 24.1	6.8 $\pm$ 1.5		
	0.63	51.0 $\pm$ 16.3	4.4 $\pm$ 2.7	58.0 $\pm$ 30.5	5.8 $\pm$ 5.6		
	1.23	53.9 $\pm$ 12.2	4.1 $\pm$ 2.9	47.0 $\pm$ 15.3	6.9 $\pm$ 3.8		
	2.48	53.4 $\pm$ 14.2	5.1 $\pm$ 2.4	52.2 $\pm$ 15.1	5.6 $\pm$ 4.0		
	7.53	48.2 $\pm$ 12.3	3.5 $\pm$ 2.3	47.2 $\pm$ 15.0	5.9 $\pm$ 2.8		
12.3	56.0 $\pm$ 16.3	6.4 $\pm$ 2.3	56.8 $\pm$ 17.4	6.2 $\pm$ 4.7			
18.4	49.9 $\pm$ 9.1	24.5 $\pm$ 12.6 ^b	40.1 $\pm$ 11.8	22.9 $\pm$ 10.5 ^b			
^a Mean number of BrdU-positive cells $\pm$ SD; ^b <i>p</i> <0.001.							
Monticello et al. (1991) Fischer 344 rats; males; 4–6/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 1, 4, or 9 d or 6 wks. Test article: Paraformaldehyde. Actual concentrations were 0, 0.85 (± 0.01), 2.48 (± 0.02), 7.63 (± 0.12), 12.2 (± 0.11), and 18.2 (± 0.28) mg/m³. Cell proliferation studies carried out after FA exposure with [³H]thymidine labeling (ip injection 18 hrs postexposure) and profiling nasal epithelial cells in serial sections of Levels II and III of the nose. Level II included the lateral meatus with the lateral aspect of the nasoturbinate, lateral wall, and lateral aspect of maxilloturbinate (Site 1); midseptum (Site 2); and medial aspect of maxilloturbinate (Site 3). Level III included the lateral wall (Site 1) and midventral septum (Site 2).	<i>Mean until length labeling indices^a</i>						
				<i>Exposure time</i>			
	<i>mg/m³</i>	<i>Level</i>	<i>Site</i>	<i>1 d</i>	<i>4 d</i>	<i>9 d</i>	<i>6 wks</i>
	0	II	1	2.16 ^b	1.46	1.44	0.91
			2	1.08	1.03	1.09	0.41
			3	2.49	1.36	1.38	1.02
		III	1	1.83	1.10	1.36 ^c	0.98
			2	3.02	2.81	1.68 ^c	2.18
	0.85	II	1	1.31 ^{c, e}	1.37	1.20	0.88 ^c
			2	1.01 ^c	0.97	0.80	0.24 ^c
			3	1.75 ^c	1.54	0.80	1.21 ^c
		III	1	1.72 ^c	1.27	1.40	0.91 ^c
			2	1.74 ^c	3.09	1.06	1.54 ^c
	2.48	II	1	2.36 ^c	1.72	1.73	1.36
			2	1.69 ^c	0.67	0.97	0.68
			3	2.81 ^c	1.09	1.48	1.11
		III	1	2.46 ^c	1.09 ^c	1.74	0.86
			2	2.39 ^c	1.43 ^c	1.43	2.57
	7.63	II	1	16.86 ^{c, f, g}	30.51 ^{f, g}	23.51 ^{f, g}	14.41 ^{f, g}
			2	3.85 ^c	10.00 ^f	10.85 ^f	2.10

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results																																									
 Figure 1 from Monticello et al. (1991). (A) Lateral view of the rat nose with Levels I–V of the nasal passage. (B) Level II and (C) Level III represent sites for cell proliferation studies.		3	18.15 ^{c, f}	25.03 ^f	22.54 ^f	16.32 ^f																																				
	III	1	7.53 ^f	8.77 ^{c, f}	7.35 ^f	2.08																																				
		2	4.20	9.22 ^{c, f}	9.50 ^f	2.58																																				
	12.2	II	1	11.17 ^{c, f}	20.91 ^f	28.59 ^f	23.87 ^{c, f}																																			
			2	17.90 ^{c, f}	26.12 ^{f, g}	19.62 ^f	21.44 ^{c, f, g}																																			
			3	5.87 ^c	20.26 ^f	20.95 ^f	26.07 ^{c, f}																																			
		III	1	14.48 ^f	20.01 ^{c, f}	30.59 ^f	24.21 ^f																																			
			2	24.44 ^f	18.70 ^{c, f}	28.60 ^f	13.98 ^f																																			
	18.2	II	1	12.68 ^f	25.78 ^f	24.57 ^{c, f}	28.74 ^{c, f}																																			
			2	16.72 ^f	29.10 ^f	29.09 ^{c, f}	25.95 ^{c, f}																																			
			3	5.31	19.39 ^f	28.71 ^{c, f}	25.10 ^{c, f}																																			
		III	1	16.35 ^{d, f}	30.80 ^{c, f}	40.36 ^f	34.78 ^{c, f}																																			
			2	19.26 ^{d, f}	34.43 ^{c, f}	32.53 ^f	27.47 ^{c, f}																																			
^a Unit length labeling index defined as the number of labeled cell profiles/mm basement membrane; ^b n=6, unless otherwise indicated; ^c n=5; ^d n=4; ^e Unless noted, not statistically different from control; ^f <i>p</i> <0.05 compared to control; ^g <i>p</i> <0.05 compared to level III.																																										
Reuzel et al. (1990) Wistar rats; male; 5/group. Exposure: Rats were exposed in dynamic whole-body chambers 22 hrs/d for 3 d to FA. Test article: Paraformaldehyde. Actual concentrations were 0, 0.37 (±0.01), 1.4 (±0.0), and 3.8 (±0.1) mg/m ³ FA. Cell proliferation studies carried out after FA exposure with [³ H]thymidine labeling (ip injection 2 hrs postexposure) and scoring of the cells lining the nasal (n=1,000) and maxillary (n=1,000) turbinates, lateral wall (n=1,000), and the septum (n=2,000). See diagram from Casseo et al. (1996b) (above) for cross levels of the rat nose evaluated for cell proliferation.	Data extracted using GrabIt software (mean from level 2, Figure 3, HCHO only): <table><tr><th>mg/m³</th><th>Maxilloturb.</th><th>Nasal Turb.</th><th>Lateral wall</th><th>septum</th></tr><tr><td>0</td><td>0.351855128</td><td>0.291340043</td><td>1.19765084</td><td>0.172349</td></tr><tr><td>0.369</td><td>0.287744031</td><td>0.842204054</td><td>1.04583032</td><td>0.221581</td></tr><tr><td>1.23</td><td>0.221580704</td><td>0.337503123</td><td>0.54215496</td><td>0.221581</td></tr><tr><td>3.69</td><td>4.456151692*</td><td>5.273729396*</td><td>5.8261316*</td><td>4.627466*</td></tr></table> Note: data were also presented for Level 3 (same regions). While slight increases became noticeable at 3.69 mg/m³, none reached statistical significance. This study also evaluated the combined effects of FA and ozone mixtures on nasal epithelium. Ozone co-exposure resulted in an increase in proliferation compared to formaldehyde exposure alone. Data are only presented herein for formaldehyde-only exposures.						mg/m ³	Maxilloturb.	Nasal Turb.	Lateral wall	septum	0	0.351855128	0.291340043	1.19765084	0.172349	0.369	0.287744031	0.842204054	1.04583032	0.221581	1.23	0.221580704	0.337503123	0.54215496	0.221581	3.69	4.456151692*	5.273729396*	5.8261316*	4.627466*											
mg/m ³	Maxilloturb.	Nasal Turb.	Lateral wall	septum																																						
0	0.351855128	0.291340043	1.19765084	0.172349																																						
0.369	0.287744031	0.842204054	1.04583032	0.221581																																						
1.23	0.221580704	0.337503123	0.54215496	0.221581																																						
3.69	4.456151692*	5.273729396*	5.8261316*	4.627466*																																						
Roemer et al. (1993) Sprague Dawley rats; male; 3 or 5/exposure group, 6 or 10/control group. Exposure: Rats were exposed to FA in dynamic head-only chambers 6 hrs/d for 1 or 3 d. Test article: Paraformaldehyde.	<i>Proportion of BrdU-labeled cells (%) after exposure</i> <table><tr><th colspan="2"></th><th colspan="4">Formaldehyde (mg/m³)</th></tr><tr><th>Cell origin and exposure frequency</th><th>Number of rats per group^a</th><th>0</th><th>2.5</th><th>7.4</th><th>24.6</th></tr><tr><td colspan="6">Nose</td></tr><tr><td>1 exposure</td><td>5</td><td>1.3 (0.1)^b</td><td>2.4 (0.6)^c</td><td>3.7 (0.5)^c</td><td>2.7 (0.8)^c</td></tr><tr><td>3 exposures</td><td>5</td><td>NR</td><td>1.4 (0.3)</td><td>2.5 (0.2)^c</td><td>2.3 (0.2)^c</td></tr><tr><td colspan="6">Trachea</td></tr></table>								Formaldehyde (mg/m ³)				Cell origin and exposure frequency	Number of rats per group ^a	0	2.5	7.4	24.6	Nose						1 exposure	5	1.3 (0.1) ^b	2.4 (0.6) ^c	3.7 (0.5) ^c	2.7 (0.8) ^c	3 exposures	5	NR	1.4 (0.3)	2.5 (0.2) ^c	2.3 (0.2) ^c	Trachea					
		Formaldehyde (mg/m ³)																																								
Cell origin and exposure frequency	Number of rats per group ^a	0	2.5	7.4	24.6																																					
Nose																																										
1 exposure	5	1.3 (0.1) ^b	2.4 (0.6) ^c	3.7 (0.5) ^c	2.7 (0.8) ^c																																					
3 exposures	5	NR	1.4 (0.3)	2.5 (0.2) ^c	2.3 (0.2) ^c																																					
Trachea																																										

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results																																	
Actual concentrations were within 10% of nominal concentrations of 0, 2.5, 7.4, or 24.6 mg/m ³ . ^a Cell proliferation studies carried out after FA exposure with BrdU labeling (i.p. injection 16–22 hrs postexposure) and flow cytometry analysis of 10,000 cells per measurement.	1 exposure	5	1.2 (0.1)	3.1 (0.6) ^c	2.1 (0.8)	2.8 (0.4) ^c																												
	3 exposures	5	NR	0.3 (0.1) ^c	0.6 (0.1) ^c	2.5 (0.2) ^c																												
	Lung																																	
	1 exposure	3	1.8 (0.3)	2.6 (0.6)	3.3 (0.4)	3.1 (0.7)																												
	3 exposures	3	NR	2.2 (0.0)	2.4 (0.7)	5.1 (1.5)																												
^a Twice the number of rats in control groups; ^b Standard error in parentheses; ^c Statistically significant at <i>p</i> ≤0.05, compared with controls.																																		
Wilmer et al. (1987) Wistar rats; male; 10/group. Exposure: Rats were exposed to FA (chamber type not reported) either continuously for 8 hrs/d, 5 d/wk for 4 wks or intermittently 8 hrs/d (successive periods of 0.5 hr of exposure and 0.5 hr of nonexposure), 5 d/wk for 3 d and 4 wks. Test article: Paraformaldehyde. Actual concentrations were not determined. Target concentrations were 0, 6.2, or 12.3 mg/m ³ for continuous exposures and 0, 12.3, or 24.6 mg/m ³ for intermittent exposures. Cell proliferation studies carried out after 3 d or 4 wks of FA exposure with [³ H]thymidine labeling (ip injection 18 hrs postexposure) and scoring of the cells (n=5,000) lining the nasal and maxillary turbinates, the septum, and the lateral wall.	<i>Percentage of [³H]thymidine labeled cells in nasal epithelium</i> <table><tr><td></td><td></td><td colspan="2">% labeled cells</td></tr><tr><td><i>Exposure</i></td><td><i>Exposure x time</i></td><td><i>After 3 d of exposure (n=3)</i></td><td><i>After 4 wks of exposure (n=3)</i></td></tr><tr><td>0 mg/m³</td><td>0 mg/m³ hr/d</td><td>0.86 (0.14)^a</td><td>0.68 (0.12)</td></tr><tr><td>6.2 mg/m³ (continuous)</td><td>49.6 mg/m³ hr/d</td><td>2.82 (0.47)^b</td><td>1.33 (0.75)</td></tr><tr><td>12.3 mg/m³ (continuous)</td><td>98.4 mg/m³ hr/d</td><td>8.87 (1.51)^b</td><td>8.85^c</td></tr><tr><td>12.3 mg/m³ (intermittent)</td><td>49.2 mg/m³ hr/d</td><td>9.80 (1.54)^d</td><td>3.41 (1.25)^e</td></tr><tr><td>24.6 mg/m³ (intermittent)</td><td>98.4 mg/m³ hr/d</td><td>19.77 (2.39)^d</td><td>13.87 (0.64)^d</td></tr></table> ^a SDs shown in parentheses; ^b <i>p</i> <0.01, compared to controls; ^c Data from one rat; ^d <i>p</i> <0.001, compared to controls; ^e <i>p</i> <0.05, compared to controls.								% labeled cells		<i>Exposure</i>	<i>Exposure x time</i>	<i>After 3 d of exposure (n=3)</i>	<i>After 4 wks of exposure (n=3)</i>	0 mg/m ³	0 mg/m ³ hr/d	0.86 (0.14) ^a	0.68 (0.12)	6.2 mg/m ³ (continuous)	49.6 mg/m ³ hr/d	2.82 (0.47) ^b	1.33 (0.75)	12.3 mg/m ³ (continuous)	98.4 mg/m ³ hr/d	8.87 (1.51) ^b	8.85 ^c	12.3 mg/m ³ (intermittent)	49.2 mg/m ³ hr/d	9.80 (1.54) ^d	3.41 (1.25) ^e	24.6 mg/m ³ (intermittent)	98.4 mg/m ³ hr/d	19.77 (2.39) ^d	13.87 (0.64) ^d
		% labeled cells																																
<i>Exposure</i>	<i>Exposure x time</i>	<i>After 3 d of exposure (n=3)</i>	<i>After 4 wks of exposure (n=3)</i>																															
0 mg/m ³	0 mg/m ³ hr/d	0.86 (0.14) ^a	0.68 (0.12)																															
6.2 mg/m ³ (continuous)	49.6 mg/m ³ hr/d	2.82 (0.47) ^b	1.33 (0.75)																															
12.3 mg/m ³ (continuous)	98.4 mg/m ³ hr/d	8.87 (1.51) ^b	8.85 ^c																															
12.3 mg/m ³ (intermittent)	49.2 mg/m ³ hr/d	9.80 (1.54) ^d	3.41 (1.25) ^e																															
24.6 mg/m ³ (intermittent)	98.4 mg/m ³ hr/d	19.77 (2.39) ^d	13.87 (0.64) ^d																															
Medium Confidence																																		
Cassee and Feron (1994) Wistar rats; male; 20/group. Exposure: Rats were exposed in dynamic nose-only chambers for 3 d (6 consecutive 12-hr periods of 8 hrs of exposure to FA followed by 4 hrs of nonexposure). Rats sacrificed immediately (i.e., within 30 min) after last exposure. Test article: Paraformaldehyde. Actual concentrations were 0 and 4.4 (SE ±0.1) mg/m ³ FA alone. Cell proliferation studies carried out using deparaffinized standard cross sections of the nose and semiquantitative proliferating cell nuclear antigen (PCNA) immunostaining.		Controls		FA alone ^a																														
	Site	II ^b	III ^b	II	III																													
	Nasoturbinates	+ ^c	+	+++	+++																													
	Maxilloturbinates	+	+	+++	+++																													
	Septum	+	+	+++	+++																													
	Lateral wall	+	+	+++	+++																													
^a Only nonnecrotic areas at cross level II showed severe PCNA expression; ^b Standard cross level II and III through the nose; ^c PCNA-expression scores: +, some nuclei stained; ++, a moderate number of nuclei stained; +++, many nuclei stained. In animals exposed to FA alone, no increased PCNA staining observed in olfactory epithelium. This study also evaluated the combined effects of FA and ozone mixtures on nasal epithelium. Ozone co-exposure resulted in an increase in																																		

Supplemental Information—Formaldehyde (Inhalation)

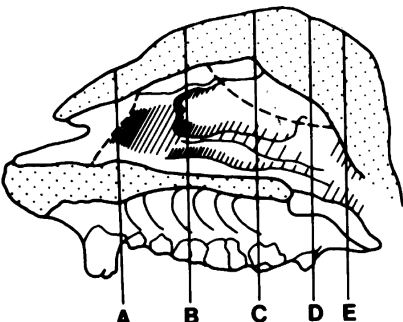
Reference and study design	Results																																																																																																																																																													
See diagram from Cassee et al. (1996b) (above) for cross sections of a rat nose examined for PCNA staining by Cassee and Feron (1994).	proliferation compared to formaldehyde exposure alone. Data are only presented herein for formaldehyde-only exposures.																																																																																																																																																													
Speit et al. (2011b) Fischer 344 rats; males; 6/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 4 wks. Test article: Formalin (methanol concentration NR). Actual concentrations were 0, 0.63 (±0.6), 1.23 (±0.14), 2.48 (±0.18), 7.53 (±0.42), 12.3 (±0.48), 18.4 (±0.06) mg/m ³ . Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 days prior to sacrifice) and determining labeling index of 3 levels of the nasal cavity: I (nasal septum, lateral meatus [wall], maxilloturbinate, nasoturbinate), II (nasal septum, lateral meatus [wall]), and IV (nasopharynx). Cell proliferation data reported as BrdU-labeled nuclei per mm of basal lamina (i.e., ULLI).	ULLI for level III not assessed due to author's expectation that this level was not a sensitive target tissue. <i>ULLI for nasal level I</i> <table><tr><th>mg/m³</th><th>Nasal septum</th><th>Lateral meatus</th><th>Maxillo-turbinate</th><th>Naso-turbinate</th></tr><tr><td>0</td><td>6.64±1.30^a</td><td>8.44±3.37</td><td>10.21±5.90</td><td>14.15±2.93</td></tr><tr><td>0.63</td><td>8.02±2.57</td><td>10.80±1.58^b</td><td>9.49±3.07</td><td>17.13±6.97</td></tr><tr><td>1.23</td><td>6.04±2.20</td><td>9.56±3.68</td><td>10.43±5.52</td><td>22.60±5.86^c</td></tr><tr><td>2.48</td><td>6.14±3.15</td><td>11.56±4.73</td><td>9.08±2.65</td><td>14.29±5.59</td></tr><tr><td>7.53</td><td>4.80±3.14</td><td>14.85±2.40^c</td><td>12.95±3.94</td><td>20.48±8.12^b</td></tr><tr><td>12.3</td><td>3.83±2.13</td><td>52.53±16.30^c</td><td>52.42±16.88^c</td><td>74.63±28.90^c</td></tr><tr><td>18.4</td><td>70.86±14.30^c</td><td>74.21±16.37^c</td><td>81.96±2.90^c</td><td>67.50±12.76^c</td></tr></table> ^a Group mean value±SD; ^b <i>p</i> <0.05; ^c <i>p</i> <0.01. <table><tr><th colspan="3"><i>ULLI for nasal level II</i></th><th colspan="2"><i>ULLI for nasal level IV</i></th></tr><tr><th>mg/m³</th><th>Nasal septum</th><th>Lateral meatus</th><th colspan="2">Naso-pharynx</th></tr><tr><td>0</td><td>14.59±6.37^a</td><td>9.33±4.22</td><td colspan="2">17.81±2.18^a</td></tr><tr><td>0.63</td><td>19.93±7.66</td><td>7.58±2.32</td><td colspan="2">21.23±5.19</td></tr><tr><td>1.23</td><td>22.36±7.04^b</td><td>8.04±2.92</td><td colspan="2">21.56±3.17</td></tr><tr><td>2.48</td><td>21.79±5.28^b</td><td>9.47±3.31</td><td colspan="2">21.33±3.55^b</td></tr><tr><td>7.53</td><td>19.07±6.43</td><td>9.28±3.54</td><td colspan="2">20.93±4.13</td></tr><tr><td>12.3</td><td>26.66±11.31</td><td>37.13±5.22^c</td><td colspan="2">29.23±4.25^c</td></tr><tr><td>18.4</td><td>62.36±12.30^c</td><td>55.21±10.99^c</td><td colspan="2">73.29±15.87^c</td></tr></table> ^a Group mean value±SD; ^b <i>p</i> <0.05; ^c <i>p</i> <0.01. <i>Relative change (% control) in ULLI in metaplastic/ degenerative (M) and nonmetaplastic (O) epithelia</i> <table><tr><th></th><th colspan="2">Nasal septum</th><th colspan="2">Lateral meatus</th><th colspan="2">Maxillo-turbinate</th><th colspan="2">Naso-turbinate</th></tr><tr><th>mg/m³</th><th>M</th><th>O</th><th>M</th><th>O</th><th>M</th><th>O</th><th>M</th><th>O</th></tr><tr><td colspan="9">Level I</td></tr><tr><td>12.3</td><td>58</td><td>61</td><td>622^{a,b}</td><td>1195^a</td><td>513^{a,c}</td><td>262^a</td><td>527^{a,c}</td><td>139</td></tr><tr><td>18.4</td><td>1066^a</td><td>1386^a</td><td>879^{a,c}</td><td>1399^a</td><td>802^a</td><td>735^a</td><td>477^{a,b}</td><td>280^d</td></tr><tr><td colspan="9">Level II</td></tr><tr><td>12.3</td><td>183</td><td>161</td><td>398^{a,c}</td><td>110</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td></tr><tr><td>18.4</td><td>428^{a,c}</td><td>1188^a</td><td>592^{a,c}</td><td>195^a</td><td>NA</td><td>NA</td><td>NA</td><td>NA</td></tr></table> ^a <i>p</i> <0.01, compared to corresponding untreated control; ^b <i>p</i> <0.05, comparison between metaplastic and nonmetaplastic tissues; ^c <i>p</i> <0.01,	mg/m ³	Nasal septum	Lateral meatus	Maxillo-turbinate	Naso-turbinate	0	6.64±1.30 ^a	8.44±3.37	10.21±5.90	14.15±2.93	0.63	8.02±2.57	10.80±1.58 ^b	9.49±3.07	17.13±6.97	1.23	6.04±2.20	9.56±3.68	10.43±5.52	22.60±5.86 ^c	2.48	6.14±3.15	11.56±4.73	9.08±2.65	14.29±5.59	7.53	4.80±3.14	14.85±2.40 ^c	12.95±3.94	20.48±8.12 ^b	12.3	3.83±2.13	52.53±16.30 ^c	52.42±16.88 ^c	74.63±28.90 ^c	18.4	70.86±14.30 ^c	74.21±16.37 ^c	81.96±2.90 ^c	67.50±12.76 ^c	<i>ULLI for nasal level II</i>			<i>ULLI for nasal level IV</i>		mg/m ³	Nasal septum	Lateral meatus	Naso-pharynx		0	14.59±6.37 ^a	9.33±4.22	17.81±2.18 ^a		0.63	19.93±7.66	7.58±2.32	21.23±5.19		1.23	22.36±7.04 ^b	8.04±2.92	21.56±3.17		2.48	21.79±5.28 ^b	9.47±3.31	21.33±3.55 ^b		7.53	19.07±6.43	9.28±3.54	20.93±4.13		12.3	26.66±11.31	37.13±5.22 ^c	29.23±4.25 ^c		18.4	62.36±12.30 ^c	55.21±10.99 ^c	73.29±15.87 ^c			Nasal septum		Lateral meatus		Maxillo-turbinate		Naso-turbinate		mg/m ³	M	O	M	O	M	O	M	O	Level I									12.3	58	61	622 ^{a,b}	1195 ^a	513 ^{a,c}	262 ^a	527 ^{a,c}	139	18.4	1066 ^a	1386 ^a	879 ^{a,c}	1399 ^a	802 ^a	735 ^a	477 ^{a,b}	280 ^d	Level II									12.3	183	161	398 ^{a,c}	110	NA	NA	NA	NA	18.4	428 ^{a,c}	1188 ^a	592 ^{a,c}	195 ^a	NA	NA	NA	NA
mg/m ³	Nasal septum	Lateral meatus	Maxillo-turbinate	Naso-turbinate																																																																																																																																																										
0	6.64±1.30 ^a	8.44±3.37	10.21±5.90	14.15±2.93																																																																																																																																																										
0.63	8.02±2.57	10.80±1.58 ^b	9.49±3.07	17.13±6.97																																																																																																																																																										
1.23	6.04±2.20	9.56±3.68	10.43±5.52	22.60±5.86 ^c																																																																																																																																																										
2.48	6.14±3.15	11.56±4.73	9.08±2.65	14.29±5.59																																																																																																																																																										
7.53	4.80±3.14	14.85±2.40 ^c	12.95±3.94	20.48±8.12 ^b																																																																																																																																																										
12.3	3.83±2.13	52.53±16.30 ^c	52.42±16.88 ^c	74.63±28.90 ^c																																																																																																																																																										
18.4	70.86±14.30 ^c	74.21±16.37 ^c	81.96±2.90 ^c	67.50±12.76 ^c																																																																																																																																																										
<i>ULLI for nasal level II</i>			<i>ULLI for nasal level IV</i>																																																																																																																																																											
mg/m ³	Nasal septum	Lateral meatus	Naso-pharynx																																																																																																																																																											
0	14.59±6.37 ^a	9.33±4.22	17.81±2.18 ^a																																																																																																																																																											
0.63	19.93±7.66	7.58±2.32	21.23±5.19																																																																																																																																																											
1.23	22.36±7.04 ^b	8.04±2.92	21.56±3.17																																																																																																																																																											
2.48	21.79±5.28 ^b	9.47±3.31	21.33±3.55 ^b																																																																																																																																																											
7.53	19.07±6.43	9.28±3.54	20.93±4.13																																																																																																																																																											
12.3	26.66±11.31	37.13±5.22 ^c	29.23±4.25 ^c																																																																																																																																																											
18.4	62.36±12.30 ^c	55.21±10.99 ^c	73.29±15.87 ^c																																																																																																																																																											
	Nasal septum		Lateral meatus		Maxillo-turbinate		Naso-turbinate																																																																																																																																																							
mg/m ³	M	O	M	O	M	O	M	O																																																																																																																																																						
Level I																																																																																																																																																														
12.3	58	61	622 ^{a,b}	1195 ^a	513 ^{a,c}	262 ^a	527 ^{a,c}	139																																																																																																																																																						
18.4	1066 ^a	1386 ^a	879 ^{a,c}	1399 ^a	802 ^a	735 ^a	477 ^{a,b}	280 ^d																																																																																																																																																						
Level II																																																																																																																																																														
12.3	183	161	398 ^{a,c}	110	NA	NA	NA	NA																																																																																																																																																						
18.4	428 ^{a,c}	1188 ^a	592 ^{a,c}	195 ^a	NA	NA	NA	NA																																																																																																																																																						

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results																																																				
	comparison between metaplastic and nonmetaplastic tissues; ^d <i>p</i> <0.05, compared to corresponding untreated control.																																																				
Woutersen et al. (1987) Wistar rats; male and female; 10/sex/group. Exposure: Rats were exposed to FA in dynamic whole-body chambers for 6 hrs/d, 5 d/wk for 3 d. Test article: Paraformaldehyde. Actual concentrations were 0, 1.2 (±0.00), 11.9 (±0.15), and 24.4 (±0.09) mg/m ³ . Cell proliferation studies carried out after 3 d of FA exposure with [³ H]thymidine labeling of dissected nasoturbينات (18 hrs postexposure) and scoring of the cells (n=1,000) of the respiratory epithelium.	<i>Percentage of [³H]thymidine labeled cells in nasal epithelium (males, n=2/group)</i> <table><tr><td></td><td colspan="5">% labeled cells</td></tr><tr><td>mg/m³</td><td colspan="3">Visibly unaffected epithelium</td><td colspan="2">Metaplastic epithelium</td></tr><tr><td>0</td><td colspan="3">1.6 (1.2–2.0)^a</td><td colspan="2">NR</td></tr><tr><td>1.2</td><td colspan="3">1.2 (0.8–1.5)</td><td colspan="2">NR</td></tr><tr><td>11.9</td><td colspan="3">2.6 (1.4–3.8)</td><td colspan="2">31.4 (29.5–33.2)</td></tr><tr><td>24.4</td><td colspan="3">2.8^b</td><td colspan="2">37.6 (32.6–42.5)</td></tr></table> ^a Range in parentheses; ^b Value based on one rat since most respiratory epithelium was metaplastic.							% labeled cells					mg/m ³	Visibly unaffected epithelium			Metaplastic epithelium		0	1.6 (1.2–2.0) ^a			NR		1.2	1.2 (0.8–1.5)			NR		11.9	2.6 (1.4–3.8)			31.4 (29.5–33.2)		24.4	2.8 ^b			37.6 (32.6–42.5)												
	% labeled cells																																																				
mg/m ³	Visibly unaffected epithelium			Metaplastic epithelium																																																	
0	1.6 (1.2–2.0) ^a			NR																																																	
1.2	1.2 (0.8–1.5)			NR																																																	
11.9	2.6 (1.4–3.8)			31.4 (29.5–33.2)																																																	
24.4	2.8 ^b			37.6 (32.6–42.5)																																																	
Mice																																																					
High Confidence																																																					
Chang et al. (1983) [additional data from related Swenberg et al. (1983b) report] B6C3F1 mice; males; 4–5/exposure group, 10/control group. Exposure: Mice were exposed to FA in head-only chambers 6 hr/d for either 1, 3, 5 or 10 d. Test article: Paraformaldehyde. Actual concentrations were 0 and 18.5 (±0.1) mg/m ³ . Target concentrations were 0, 0.62, 2.46, 3.69, 7.38, 14.76 or 18.45 mg/m ³ in Swenberg et al. (1983b) report. Cell proliferation studies carried out after FA exposure with [³ H]thymidine labeling (ip injection 2 or 18 hrs postexposure) and scoring of cells (n=4,000) lining the respiratory epithelium from the nasal and maxillary turbinates and lateral wall. See diagram from Swenberg et al. (1983b) for rats (above) for locations of Levels A (with minimal mucociliary clearance) and B (with extensive mucociliary clearance)	<i>Group (18.5 mg/m³)</i> <table><tr><td>Control</td><td colspan="5">0.27±0.04 (10)^a</td></tr><tr><td>1 day</td><td colspan="5">2.14±0.56 (5)^b</td></tr><tr><td>5 days</td><td colspan="5">3.42±0.84 (4)^b</td></tr></table> ^a Number in parentheses represents number of animals studies. ^b Significantly different from control, <i>p</i> <0.05. <i>% labeled respiratory epithelial cells in Level B (thymidine at 2 hr postexposure)</i> <table><tr><td></td><td colspan="5">Formaldehyde Concentration (mg/m³)</td></tr><tr><td></td><td>0</td><td>0.62</td><td>2.46</td><td>7.38</td><td>18.45</td></tr><tr><td>3 days</td><td>0.12 (0.02)</td><td>0.09 (0.04)</td><td>0.08 (0.04)</td><td>0.15 (0.06)</td><td>0.97 (0.04)</td></tr></table>		Control	0.27±0.04 (10) ^a					1 day	2.14±0.56 (5) ^b					5 days	3.42±0.84 (4) ^b						Formaldehyde Concentration (mg/m ³)						0	0.62	2.46	7.38	18.45	3 days	0.12 (0.02)	0.09 (0.04)	0.08 (0.04)	0.15 (0.06)	0.97 (0.04)	<i>% labeled respiratory epithelial cells in Level A (thymidine at 18 hr postexposure)</i> <table><tr><td>Control</td><td colspan="2">1.24 (0.57)</td></tr><tr><td>3.69 mg/m³ × 12 hr/d for 10 d</td><td colspan="2">10.14 (3.20)</td></tr><tr><td>7.38 mg/m³ × 6 hr/d for 10 d</td><td colspan="2">4.72 (1.61)</td></tr><tr><td>14.76 mg/m³ × 3 hr/d for 10 d</td><td colspan="2">1.76 (0.49)</td></tr></table> Mean (SEM); Group sizes and statistical comparisons not reported in Swenberg et al. (1983b)			Control	1.24 (0.57)		3.69 mg/m ³ × 12 hr/d for 10 d	10.14 (3.20)		7.38 mg/m ³ × 6 hr/d for 10 d	4.72 (1.61)		14.76 mg/m ³ × 3 hr/d for 10 d	1.76 (0.49)	
Control	0.27±0.04 (10) ^a																																																				
1 day	2.14±0.56 (5) ^b																																																				
5 days	3.42±0.84 (4) ^b																																																				
	Formaldehyde Concentration (mg/m ³)																																																				
	0	0.62	2.46	7.38	18.45																																																
3 days	0.12 (0.02)	0.09 (0.04)	0.08 (0.04)	0.15 (0.06)	0.97 (0.04)																																																
Control	1.24 (0.57)																																																				
3.69 mg/m ³ × 12 hr/d for 10 d	10.14 (3.20)																																																				
7.38 mg/m ³ × 6 hr/d for 10 d	4.72 (1.61)																																																				
14.76 mg/m ³ × 3 hr/d for 10 d	1.76 (0.49)																																																				
Kuper et al. (2011) B6C3F1 mice; females; 6/group.																																																					

Supplemental Information—Formaldehyde (Inhalation)

Reference and study design	Results	
Exposure: Mice were exposed to FA in dynamic whole-body chambers 6 hr/d, 5 d/wk for 4 wk. Test article: Formalin (10.21% FA). Actual concentrations were 0, 0.63 (± 0.06), 1.23 (± 0.14), 2.48 (± 0.18), 7.53 (± 0.42), 12.3 (± 0.48), and 18.4 (± 0.06) mg/m³. Cell proliferation studies conducted with surgical implantation of BrdU-containing pumps (3 d prior to sacrifice) and determining labeling index of 2 sections of NALT and 1 section of an upper-respiratory tract-draining lymph node (i.e., posterior and superficial cervical lymph nodes). Cell proliferation data reported as BrdU-positive cells per length (i.e., mm) of epithelium.	NALT: No FA-related effects on the number of BrdU-positive cells reported in the follicular and interfollicular compartments and epithelium Lymph nodes: No FA-related effects on the number of BrdU-positive cells reported in the follicle and paracortex compartments and medulla	
Monkeys		
Medium Confidence		
Monticello et al. (1989) Rhesus monkeys; male; 3/group. Exposure: Monkeys were exposed to FA in dynamic whole-body chambers 6 hrs/d, 5 d/wk for 1 or 6 wks. Test article: Paraformaldehyde. Actual concentrations were not determined. Target concentration was 7.4 mg/m³. Controls were sham exposed to biologically filtered air for 6 wks. Cell proliferation studies carried out after FA exposure with [³H]thymidine labeling (iv injection 18 hrs postexposure) and scoring of respiratory epithelial cells. For nasal passages (transitional, respiratory, and olfactory epithelia), larynx, trachea, and carina, LIs defined as the number of labeled cells per mm of basal lamina.	Exposure	Observations between nasal passage epithelia
	Controls (6 wk)	Highest LIs in transitional epithelium compared to respiratory and olfactory epithelia
	7.4 mg/m ³ (1 wk)	Transitional and respiratory epithelia elevated compared to controls ($p \leq 0.05$)
	7.4 mg/m ³ (6 wk)	Transitional epithelium LIs slightly elevated over controls and had decreased from 1-wk group; olfactory epithelium LIs had mild increase over controls ($p \leq 0.05$); respiratory epithelium LIs elevated compared to controls ($p \leq 0.05$)
	Exposure	Observations between levels of nasal passages
	Controls (6 wk)	LIs for Levels B–E significantly increased over controls ($p \leq 0.05$), antero-posterior gradient (i.e., greatest to lowest) in cell proliferation rates
	7.4 mg/m ³ (1 wk)	LIs for Levels B–E significantly increased over controls ($p \leq 0.05$)
	7.4 mg/m ³ (6 wk)	Levels C–E significantly elevated over 1-wk group ($p \leq 0.05$)
	Group	Observations within levels of nasal passages
	Level A	NR

Reference and study design	Results			
 Figure 4 from (Monticello et al., 1989) depicting the nasal passage levels selected for cell proliferation studies. A, nasal atrium; B, anterior aspect of the middle and ventral turbinates; C, mid-region of the maxillary sinuses; D, posterior nasal cavity; and E, nasopharynx.	Level B	LIs for 1- and 6-wk groups elevated over controls ($p \leq 0.05$) for septum, inferior meatus, inferior turbinate, lateral wall, and middle turbinate		
	Level C	LIs for 1- and 6-wk groups elevated over controls ($p \leq 0.05$) for septum, inferior meatus, inferior turbinate, lateral wall, and middle turbinate; no increase in LIs for 1- and 6-wk groups over controls for maxillary sinuses		
	Level D	LIs for 1-wk group elevated over controls ($p \leq 0.05$) for septum, inferior meatus, inferior turbinate, and lateral wall; LIs for 6-wk group elevated over controls ($p \leq 0.05$) for inferior meatus and inferior turbinate		
	Level E	LIs for 1-wk group elevated over controls ($p \leq 0.05$) for floor and lateral and dorsal walls; LIs for 6-wk group elevated over controls ($p \leq 0.05$) for septum, floor, and lateral and dorsal walls		
	Group	Observations for nonnasal tissues		
	Larynx	LIs for 1- and 6-wk groups elevated over controls; LIs increased with duration of exposure		
	Trachea	Significant elevation in LIs for 1-wk ($p \leq 0.05$) but not 6-week group over controls; LIs increased with duration of exposure		
	Carina	Significant elevation in LIs for 1-wk ($p \leq 0.05$) but not 6-wk group over controls; LIs increased with duration of exposure		
	Interanimal variation in LIs for trachea and carina			
	Exposure	Animal #	Trachea LI	Carina LI
Controls (6 wk)	1	0.29	0.42	
	2	0.46	0.37	
	3	0.91	0.50	
	ave	0.55±0.19 ^a	0.43±0.04 ^a	
7.4 mg/m ³ (1 wk)	4	1.34	1.09	
	5	0.90	1.95	
	6	1.19	0.99	
	ave	1.14±0.13 ^a	1.34±0.31 ^a	
7.4 mg/m ³ (6 wk)	7	8.00	3.86	
	8	2.30	6.49	
	9	0.88	0.45	
	ave	3.73±2.18 ^a	3.60±1.75 ^a	
^a Represents Mean±SEM.				
Exposure	LI in respiratory bronchioles ^a			
Controls (6 wk)	0.01±0.001			
7.4 mg/m ³ (1 wk)	0.01±0.003			

Reference and study design	Results	
	7.4 mg/m ³ (6 wk)	0.01±0.001
	^a LIs expressed as percent labeled cells per total cell count from ≥500 respiratory bronchiolar nucleated epithelial cells per animal.	

C.7.2. Synthesis of Changes in the Lower Respiratory Tract (LRT)

Although the URT and the LRT are physically and functionally connected, this analysis delineates findings across these two tissue compartments. This was done due to the distribution of the overwhelming majority of inhaled formaldehyde to the URT (noting that some data suggest that oronasal breathing in humans, as compared to nose-only breathing in rodents, might result in slight differences in the distribution of inhaled formaldehyde, including a possible increase in the portion reaching proximal regions of the LRT such as the trachea; see additional discussion on toxicokinetics in Appendix C.1). Thus, evidence related to studies of BAL (bronchoalveolar lavage) fluid and airway function, both of which may involve some contribution from URT-related changes but are largely driven by effects on the lung, are described in this section. The specific studies and summary findings supporting the synthesis below are described in Table C-32 and are considered as part of the broader integration of MOA information in Figures C-23 and C-24. In general, compared to effects on the URT, the methodological approaches for evaluating LRT changes are more commonly applied to studies of exposed humans, so this section considers a wider range of evidence. A greater level of concern exists for the erroneous attribution of changes in the LRT (and other, non-URT, compartments in subsequent sections) to inhaled formaldehyde when studies used methanol-containing formalin; thus, findings from some studies using exposure paradigms similar to those described in the previous section are interpreted with comparably less confidence.

As previously mentioned, formaldehyde-induced stimulation of TRPA1 receptors on trigeminal nerve endings distributed within the epithelial cell layer in the URT appears to cause a localized release of neuropeptides, including substance P, which can cause local inflammatory changes. Consistent with this, ex vivo models of LRT tissues and *low confidence* studies of in vivo exposure suggest that indirect activation of sensory nerve endings in the LRT, presumably of the vagus nerve, occurs after formaldehyde inhalation exposure. In the URT, this activation is expected to occur via direct interaction of formaldehyde with receptors. However, while these direct interactions might occur in upper portions of the LRT during certain, very rare human exposure scenarios (e.g., in the trachea at high exposure levels), they would be unexpected in the lungs or during typical exposure scenarios; thus, this is not considered a plausible initial effect of typical exposure. Notwithstanding this assumption, the available evidence indicates that formaldehyde exposure likely causes downstream sequelae in the lung that could be attributed to sensory nerve activation in the LRT, predominantly related to substance P-related pathways (see below). However, the mechanistic event(s) critical to understanding this potential relationship remain

unknown: namely, how sensory nerve endings in the LRT would be stimulated without distribution of inhaled formaldehyde to the LRT. The most likely explanations involve a secondary response to TRP channel-activating stimuli increased via other mechanisms, such as increased LRT oxidative stress and/or inflammatory mediators released from activated immune cells or damaged epithelial cells in the LRT. It could also be explained by a central trigeminal-to-vagal neural reflex response to irritation of the URT (i.e., a “nasobronchial” reflex¹⁷); however, the existence of this reflex in humans is debated and a clear scientific consensus does not exist ([Togias, 1999, 2004](#); [Sahin-Yilmaz and Naclerio, 2011](#); [Giavina-Bianchi et al., 2016](#)). No studies specifically designed to assess any of these potential linkages after formaldehyde exposure were identified.

Studies in several species provide moderate evidence that formaldehyde exposure results in increased LRT neuropeptides, including substance P (see “Changes in the URT” Section above), as well as a rapid activation of the primary receptor for substance P, the neurokinin receptor (NK₁R), typically at formaldehyde concentrations ≥ 2.5 mg/m³. Further, the activation of this pathway has been experimentally linked to both formaldehyde-induced leakage of the LRT microvasculature (which has been observed in rodents at ≥ 1.23 mg/m³) as well as airway hyperresponsiveness (which has been observed in animals and humans at < 0.5 mg/m³). In addition to facilitating the recruitment of inflammatory cells, NK₁R activation can promote immune cell survival and activation through the release of cytokines and chemokines ([Tuluc et al., 2009](#)). The substance P-NK₁R pathway has been implicated in mast cell degranulation, which can lead to bronchoconstriction ([Bienenstock and Mcdermott, 2005](#)); however, while inhibiting mast cell activation prevented microvascular leakage in a *low confidence* rat study after acute exposure to high levels of formaldehyde ([Kimura et al., 2010](#)), an acute *medium* or *high confidence* study of a cohort of guinea pigs failed to observe any changes in mast cells ([Swiecichowski et al., 1993](#); [Leikauf, 1992](#)). Importantly, an understanding of potential changes to substance P and NK₁R-dependent effects (e.g., due to desensitization) with long-term formaldehyde exposure remains unclear. While a transient depletion of neuropeptides from sensory nerve terminals after acute exposure seems plausible ([Leikauf, 1992](#)). Importantly, an understanding of potential changes to substance P and NK₁R-dependent effects (e.g., due to desensitization) with long-term formaldehyde exposure remains unclear. While a transient depletion of neuropeptides from sensory nerve terminals after acute exposure seems plausible ([Kimura et al., 2010](#)), substance P is still elevated, at least in the blood, after subchronic exposure ([Fujimaki et al., 2004b](#)). Overall, the activation characteristics of this pathway in the LRT across various formaldehyde exposure scenarios have not been established.

Microvascular leakage can lead to inflammatory structural changes observable by histology, which are supported by *moderate* evidence in formaldehyde-exposed rodents, particularly those

¹⁷ Note: neural reflexes involving afferent and efferent activity of the vagus nerve (e.g., across different LRT regions), some of which may involve C fibers and TRP channels, are better established ([Mazzone and Undem, 2016](#)).

sensitized with the allergen, ovalbumin (OVA). The available studies indicate changes including airway edema (swelling) or thickening of airway walls, with general support for inflammatory changes in airway bronchi, but not necessarily alveoli. In addition, the pattern of structural changes varied across studies, with a study in guinea pigs observing airway swelling without signs of inflammation at low formaldehyde (<0.5 mg/m³) levels ([Riedel et al., 1996](#)), while studies in rats and mice generally observed mild inflammatory-related structural changes at higher levels (i.e., ≥ 3.0 mg/m³) that only became pronounced with allergen sensitization. It is important to note that animal models vary in their ability to mimic some features of human airways. Airway responses in guinea pigs often differ from those in rats and mice, and while no animal model fully recapitulates human airway function, in many ways the sensitivity of guinea pig airways may be more relevant than other small mammals (e.g., similar structure of the lung to humans; responsiveness to stimuli that induce sensitivity in humans) ([Shin et al., 2009](#); [Ricciardolo et al., 2008](#)). Alongside airway inflammation and structural changes, including edema, which could narrow or obstruct airways, an increased permeability to bronchoconstrictors such as histamine would be expected to influence airway function, possibly linking these changes to observations of hyperresponsiveness or decreased pulmonary function.

A *moderate* association between formaldehyde exposure and increases in LRT eosinophils was identified, including amplification of the response of these cells in rodents previously exposed to allergens (see Table C-33). Taken together with similar findings in the URT, a general increase in airway eosinophils as a result of formaldehyde exposure is supported by robust evidence. As in the URT, this finding has been reported in the LRT following exposure for several weeks at effective concentrations above 0.5 mg/m³. The only study of longer-term exposure available ([Fujimaki et al., 2004b](#)) indicated that formaldehyde exposure at 2.46 mg/m³, but not ≈ 0.5 mg/m³, for 3 months caused increased eosinophils in mice sensitized to OVA, but not in unsensitized mice. While the data are not conclusive, it appears that eosinophil recruitment does not occur immediately after acute exposure, as this increase was not observed in the available studies of acute exposure (see Table C-33). Although it has not been mechanistically demonstrated based on increased eosinophils and other immune cells after acute tachykinin release ([Barnes, 1992, 1998](#)), repeated release of neuropeptides could plausibly lead to sustained airway inflammation and, depending on the phenotype of the recruited cells, this could result in airway hyperresponsiveness. In both the URT and LRT, recruitment of eosinophils might also be related to changes in markers of oxidative stress observed across formaldehyde exposure paradigms. However, whereas oxidative stress in the URT may be related to damage to the local epithelial cells, most studies indicate that formaldehyde exposure does not result in overt damage to the LRT airway epithelium (*slight* evidence, at relatively high formaldehyde levels: >5 mg/m³), making this potential linkage less plausible. It is considered more likely that increases in oxidative stress are the result of changes in inflammatory factors and immune cells in the LRT, rather than LRT epithelial damage.

The evidence for LRT immunological changes other than those seen in eosinophils is mixed and generally only suggestive of potential effects. As shown in Figure C-24, *slight* evidence exists to suggest that formaldehyde exposure amplifies recruitment of innate immune cells such as neutrophils and monocytes to the LRT; notably, this finding has only been observed when animals exposed to >2 mg/m³ were previously sensitized to an allergen. Importantly, few studies examined lymphocyte subsets, and no studies reported on the response of lymphocytes in animals sensitized to allergens or at exposure levels below 5 mg/m³, highlighting important gaps in the literature. Two studies suggest that CD8⁺, but not CD4⁺, T cells may be increased with formaldehyde exposure above 7 mg/m³ ([Sandikci et al., 2007b](#); [Jung et al., 2007](#)). The only study meeting the inclusion criteria that evaluated lymphocyte changes in both immature and adult animals only observed changes in animals exposed as adults ([Sandikci et al., 2007b](#)), which could suggest that a functionally mature immune system is necessary for these alterations (the immune system is not considered to be fully mature in rodents until around six weeks of age ([Burns-Naas et al., 2008](#))). While these findings should be interpreted with substantial caution, there may be a role for CD8⁺ T cells in promoting the recruitment and survival of airway eosinophils, as well as a requirement of these cells for the development of airway hyperresponsiveness (e.g., to allergen or infection) ([Schwarze et al., 1999](#); [Hamelmann et al., 1997](#)). CD8⁺ T cells make up a heterogeneous population of lymphocytes which migrate by recruitment to sites of inflammation, proliferate in response to antigen stimulation, and help to mediate long-term cellular immunity against foreign pathogens, particularly viruses. The conventional role for IFN γ -producing CD8⁺ T cells is to inhibit eosinophil function; however, some emerging evidence suggests that certain CD8⁺ T cell subpopulations may induce eosinophil recruitment ([Huber and Lohoff, 2015](#)). No data are available to evaluate the potential for effects of formaldehyde exposure on different subpopulations of LRT CD8⁺ T cells.

Studies of markers of immune cell activation in the LRT after formaldehyde exposure generally provide mixed results, making it difficult to draw inferences (see Table C-33). Most cytokine-related changes reported in the LRT occur at high formaldehyde levels (>5 mg/m³) after short-term exposure and include *slight* evidence to support an increase in eosinophil chemotactic factors, and a decrease in markers and counts of natural killer (NK) cells. NK cells respond rapidly to infection and appear to have a role in regulating chronic inflammation and infection of the airways ([FJ, 2009](#)). Thus, this change, were it to be experimentally verified, could be associated with the *moderate* evidence of an increased propensity for LRT infections, similar to the *slight* evidence of altered URT immune responses (see previous section); however, definitive studies relevant to long-term exposure have not been identified and additional data are necessary to interpret these alterations in respiratory immune responses as consistent with immune suppression. A number of consistent studies in exposed rodents do suggest an increase in T helper type 2 (Th2)-related cytokines, most notably IL-4, with short term exposure at ≥ 0.5 mg/m³ and particularly in animals sensitized to an allergen. The *slight* evidence supporting increased IL-5, a Th2 cytokine that can be both synthesized by and act upon airway mast cells and eosinophils and which is believed to be

integral to the development of airway eosinophilia and airway hyperresponsiveness ([Schwarze et al., 1999](#); [Greenfeder et al., 2001](#)), is considered to be inconclusive (i.e., two *low confidence* studies testing exposure levels >5 mg/m³). Along with IL-5 and IL-13, IL-4 is recognized for its established role in chronic respiratory disorders ([Maes et al., 2012](#)), and this change may be relevant to other LRT-specific changes. IL-4, which can stimulate T cell receptors on CD4⁺ and CD8⁺ T cells ([Serre et al., 2010](#)), can influence the activation and development of antigen-specific CD8⁺ T cell immunity by shifting the phenotype of these cells from IFN- γ production to IL-4 production ([Erb and Le Gros, 1996](#)).

The cytokine changes could be related to the *moderate* evidence for increased LRT infections and the *slight* evidence suggesting reduced NK cell numbers (see Tables C-32 and C-33), as Th2 cytokines have been shown to reduce pulmonary bacterial immunity ([Beisswenger et al., 2006](#)) and NK cells have a role in regulating chronic inflammation and infection of the airways ([Fl. 2009](#)). A key limitation of the data is that the few formaldehyde-specific studies have not demonstrated consistent increases in CD4⁺ Th2 cells in the airways of exposed individuals. Similarly, interactions between airway innate and adaptive immune responses, and between CD4⁺ and CD8⁺ T cells, topics of current interest ([Koya et al., 2007](#); [Gasteiger and Rudensky, 2014](#)), have not been well studied following formaldehyde exposure. Experiments focused on these types of endpoints would help to integrate the currently available data.

The consistent evidence of amplified airway responses to immunogenic stimuli (e.g., to allergens such as OVA) following formaldehyde exposure is of particular interest. As described above, multiple LRT parameters are affected or exacerbated by the combination of formaldehyde exposure and sensitization to allergenic materials. At concentrations ranging from 0.31–3 mg/m³ over durations of several days to several weeks, formaldehyde exposure in combination with allergen sensitization exacerbates immune-related changes, such as: recruitment of eosinophils and possible increases in IL-4; airway structural changes, including edema; and airway functional changes, including exaggerated responses to muscarinic receptor agonists. These observations may be relevant to the associations between human formaldehyde exposure at much lower concentrations (<0.05 mg/m³) and conditions that may reflect an enhanced response to allergens (e.g., rhinoconjunctivitis; asthma).

The formaldehyde exposure-induced effects associated with allergen sensitization varied depending on the specific mechanistic effect and the experimental animal model. This variability may reflect a lack of consistency in the methods used for sensitization and challenge, or other experimental design differences across studies. Alternatively, these differences might reflect variability in susceptibility to these types of effects across different populations or groups of individuals (e.g., animals of different species, strains, sex, or age). This variable sensitivity of subsets of the population to formaldehyde-induced effects would be consistent with observations of substantial interindividual human variability for several potential health effects. Further, these data suggest that vulnerability to some formaldehyde-induced health effects might be influenced by

the exposure history of the individuals, including exposure to known allergens. The mechanism for this amplified response to allergens (and, possibly, nonallergenic antigens) due to formaldehyde exposure, including what airway component(s) formaldehyde may interact with to initiate this particular alteration, remains unknown. Possible explanations include formaldehyde acting as an antigen (capable of directly eliciting an antibody response) or as a hapten (capable of eliciting an antibody response when bound to a larger molecule such as a protein), or formaldehyde-induced chronic inflammation acting as an adjuvant (enhancing immune responses to antigens); however, these speculations have not been examined by directed testing following inhalation exposure. While changes in airway responsiveness could be dependent on stimulation of sensory nerve endings, observations in isolated tracheae by Swiecichowski et al. ([1993](#)) and Leikauf ([1992](#)) suggest that the amplified response to stimuli is at least partly mediated by interactions with local immuno-modulatory factors. As airway hyperreactivity and other indicators of immunologic sensitization are known to be related to markers (e.g., antibodies) in the blood, some evidence related to these responses are discussed in the subsequent section. Overall, the essential airway immunologic target(s) of inhaled formaldehyde has not yet been identified and verified, thereby presenting a key uncertainty.

Table C-32. Summary of changes in the lower respiratory tract (LRT) as a result of formaldehyde exposure

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (exposure duration)	Conclusion
Structural Modification of the Lower Airways				
Microvascular Leakage	High or Medium	Human: None	Demonstrated increased leakage from acute exposure ≥6.15 mg/m³ in 1 study, which might be mediated by substance P	Moderate ↑ only examined in acute studies
		Animal: Increased in rats (Ito et al., 1996): acute at ≥6.15 mg/m³; note: inhibited at 18.45 mg/m³ by NK1 receptor antagonist (note: substance P binds NK1R), but not histamine or bradykinin antagonists		
	Low	Human: None	One study suggests acute exposure as low as 1.23 mg/m³ induces microvascular leakage, although continued exposure appeared to (at least in the near-term) result in less leakage	
		Animal: Transiently increased in rats (Kimura et al., 2010): acute at ≥1.23 mg/m³ (duration-independent); Note: leakage blocked by inhibiting mast cells, but not blocking cyclooxygenases; potential additional mechanistic understanding by injection of formalin into the trachea causing leakage that appeared to be dependent on substance P release after stimulation of C-fiber afferents (Lundberg and Saria, 1983)		
Airway Edema and/or Other Inflammatory Structural Change	High or Medium	Human: None	Bronchial edema in 1 short-term study at 0.31 mg/m³	Moderate ↑ may require higher exposure levels and/or allergen sensitization for pronounced changes
		Animal: Increased edema in lung bronchi, but not alveoli, without signs of inflammation in lower airways in guinea pigs (Riedel et al., 1996): 5 d at 0.31 mg/m³, not 0.16 mg/m³		
	Low	Human: None	Airway structural changes with allergen sensitization in 2 species (and, to a lesser extent, without sensitization) with short-term exposure at ≥3 mg/m³	
		Animal: Airway structural changes consistent with inflammation (e.g., wall thickening; cell infiltration) in mice (Jung et al., 2007); (Wu et al., 2013 ; Liu et al., 2011) and in mice and rats sensitized with OVA (Wu et al., 2013 ; Qiao et al., 2009 ; Liu et al., 2011), but not in nonsensitized rats (Qiao et al., 2009): all 2–3 wk at ≥3 mg/m³ [Note: most studies indicated assessment of bronchial airways]		
Airway/Airway Epithelial Cell Damage	High or Medium	Human: None	N/C in a single mouse subchronic study with i.p. sensitization and up to 2.46 mg/m³ exposure, nor in a guinea pig study at 4.18 mg/m³	Slight at higher formaldehyde levels
		Animal: N/C (histology for mouse epithelial cell damage) (Fujimaki et al., 2004b): 12 wk at up to 2.46 mg/m³ N/C in histology in guinea pigs (Swiecichowski et al., 1993 ; Leikauf, 1992): acute at 4.18 mg/m³		
	Low	Human: None		

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Animal: Increased in mice (Jung et al., 2007): 2 wk at ≥6.15 mg/m ³ and in rats (Aydin et al., 2014): 4 wk at ≥6.15 mg/m ³ ; indirect evidence of damage in rats ((Kimura et al., 2010) and (Dallas et al., 1987) and (Sandikci et al., 2009)): 20 hr after acute at 6.15 mg/m ³ and 1 wk at ≥0. 62 mg/m ³ (effect magnitude decreased with longer exposures) and 6 wk at 7.38 mg/m ³ (in adults, not young), and in mice (Abreu et al., 2016): 6–8 hr after acute at 3.7 mg/m ³ , but N/C in rats in another study (Dinsdale et al., 1993): 4 d at 12.3 mg/m ³	A single short-term study in mice and another in rats, and indirect evidence from several studies in rats, suggests damage at higher formaldehyde levels (e.g., around 4 mg/m ³); however, another similar study did not observe effects at 12.3 mg/m ³	
LRT Sensory Nerve Activation	High or Medium	Human: None	No evidence to evaluate	Slight levels required for potential activation unknown (note: may involve TRPA1 binding)
		Animal: None		
	Low	Human: None	A single acute rat study and indirect evidence from potentially related exposures suggest that lower airway sensory nerve afferents may be activated, but the inhaled formaldehyde levels required for such potential activation have not been experimentally demonstrated	
		Animal: With acute exposure, dose-dependent increase in nerve currents and Cl [−] release in intact rat trachea (Luo et al., 2013), with supporting evidence of substance P and NK Receptor involvement. Indirectly, increased substance P and CGRP were observed in mouse lung tissue, both were amplified with OVA, and both were dependent on TRP activation (Wu et al., 2013): short term at 3 mg/m ³ . Note: the potential involvement of tracheobronchial reflexes in the pulmonary effects of cigarette smoke constituents, such as nicotine and formalin, may add indirect support		
Immune and Inflammation-Related Changes				
[[See Table C-33 for Cellular and Cytokine Response in BAL and LRT tissues]]				
Oxidative Stress	High or Medium	Human: Increased exhaled nitric oxide, a noninvasive marker of lower airway inflammation and oxidative stress, in healthy or asthmatic children (Franklin et al., 2000 ; Flamant-Hulin et al., 2010): unknown duration (likely months to years: classrooms or homes) at 0.04–0.06 mg/m ³ , but not in elderly nursing home patients at lower levels (Bentayeb et al., 2015): unknown duration (likely months to years) at 0.005–0.01 mg/m ³	Increased biomarkers (indirect evidence) of oxidative stress in children at ≥0.04 mg/m ³ , but not in elderly individuals at ≤0.01 mg/m ³ with prolonged (months–years) exposure	Moderate ↑ in children at ≈0.04 mg/m ³
		Animal: None		
	Low	Human: None		

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Animal: in mice: NO and NOS activity increased with 3 d at 3 mg/m ³ (Yan et al., 2005), GSH levels decreased with 3 wk at ≥0.5 mg/m ³ (Ye et al., 2013), and increased ROS and/or lipid peroxidation markers with 3 wk at ≥1 mg/m ³ (Ye et al., 2013) or 2 wk at ≥6.15 mg/m ³ (Jung et al., 2007), but decreased with acute exposure in 1 study (Matsuoka et al., 2010): 24 hr at 0.12 mg/m ³ in rats: at ≥12.3 mg/m ³ increased total oxidant levels and decreased total antioxidant level (Aydin et al., 2014): 4 wk, increased lipid peroxidation markers and protein oxidation markers (Sul et al., 2007): 2 wk, and decreased gamma-glutamyl transpeptidase- indirect evidence (Dinsdale et al., 1993): 4 d	Multiple studies in two species suggest elevated oxidative stress at ≥1 mg/m ³ with short-term exposure	
Sustained Inflammation	High or Medium	Human: Increased exhaled nitric oxide, a noninvasive marker of lower airway inflammation and oxidative stress, in healthy or asthmatic children (Franklin et al., 2000 ; Flamant-Hulin et al., 2010): unknown duration (likely months to years: classrooms or homes) at 0.04–0.06 mg/m ³	Immune cell counts are continually elevated in a subchronic mouse study with allergen stimulation at 2.46 mg/m ³ ; increased biomarkers (indirect evidence) of lower airway inflammation are observed in children with prolonged exposure.	Moderate may require allergen sensitization in some cases
		Animal: Eosinophils and monocyte counts remain elevated with continued exposure for subchronic duration with allergen (OVA) sensitization (Fujimaki et al., 2004b): 12 wks at 2.46 mg/m ³		
	Low	Human: None Animal: Immune cell counts were increased with short term exposure in several studies at ≥0.5 mg/m ³ (see Table C-33); histological evidence of inflammation without epithelial damage was noted in short-term studies, typically at higher concentrations, which were amplified by allergen (e.g., ≥3 mg/m ³ , (Wu et al., 2013 ; Kimura et al., 2010)).	BAL cell counts and histologic evidence suggest that inflammation persists for several weeks with short-term exposure, and these effects are amplified by allergen	
Immune Function (inferred from LRT infections)	High or Medium	Human: Increased LRT infections in infants (Roda et al., 2011): 32–41% increase in incidence per 0.0124 mg/m ³ increase in formaldehyde (LOD: 0.008 mg/m ³); ≈1-year exposure at 0.020 mg/m ³ (median)	Indirect evidence in a single study of infants exposed to a median of 0.020 mg/m ³ that observed an association between exposure and increased infections. One acute mouse study also provided indirect support for an increased likelihood of respiratory infections.	Moderate supports an increased propensity for LRT infections, particularly during development
		Animal: Decreased antibacterial activity in mice (Jakab, 1992): acute at 1.23 mg/m ³ , noting that this finding appeared to be particularly sensitive to the pattern of formaldehyde exposure		

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (exposure duration)	Conclusion
	Low	Human: Increased emergency room visits for episodes including LRT infections (Rumchev et al., 2002): children aged 6–36 mos with mean levels of 0.028–0.030 (maximum 0.12–0.22) mg/m ³ Animal: Decreased expression of immune-related genes in rat lung (Sul et al., 2007), specifically HSP701a (may be involved in antigen presentation), complement 4 binding protein (may bind necrotic or apoptotic cells for cleanup), and Fc portion of IgGiii (may be involved in leukocyte activation): 2 wk at ≥6.15 mg/m ³	Direct and indirect evidence of impaired LRT immune function in children and in a short-term rat study, respectively	
Changes in pulmonary function with challenge (e.g., with bronchoconstrictors and/or allergens) (Note: unprovoked responses are not included)	High or Medium	Human: None Animal: [allergen challenge]: With ovalbumin [OVA] sensitization, increased airway obstruction in guinea pigs (Riedel et al., 1996): short-term at 0.31 mg/m ³ and increased reactivity in mice (Larsen et al., 2013): acute at ≈5–7 mg/m ³ in humid or dry environments; [acetylcholine challenge]: Increased airway resistance and reactivity in guinea pigs (Swiecichowski et al., 1993 ; Leikauf, 1992): acute at 1.23 mg/m ³	Acute and short-term studies in two animal species demonstrate that formaldehyde increases responsiveness to allergens and bronchoconstrictors, particularly with prior sensitization, at levels as low as 0.31 mg/m ³	Robust ↑ Hyperresponsive airways ^a (↑ effects with allergen)
	Low	Human: [histamine challenge]: Hyperreactive airways with prolonged exposure (Górski and Krakowiak, 1991): ≥1 year at ≤0.5 mg/m ³ , but N/C after acute exposure (Krakowiak et al., 1998): 2 hr at 0.5 mg/m ³ ; [allergen challenge]: hypersensitivity with acute exposure when exposure was restricted to mouth breathing in allergic asthmatics with a large allergen (mite) (Casset et al., 2006): ≤1 hr at 0.1 mg/m ³ , but N/C after acute oronasal (normal) exposure in allergic asthmatics using a different allergen (pollen), including a test of methacholine (MCh) responsiveness 8 hr after allergen exposure (Ezratty et al., 2007): 1 hr at 0.5 mg/m ³	Suggestive evidence of increases with prolonged exposure, and possibly acute mouth-breathing exposure when challenged with specific allergens, but not acute exposure alone, to ≤0.5 mg/m ³ in human adults; also, increased at ≥3 mg/m ³ in short-term or acute studies across three species, particularly with prior sensitization.	
		Animal: [MCh challenge]: Hyperresponsive airways (increased reactivity and sensitivity) noted with FA alone in mice and rats (Wu et al., 2013 ; Qiao et al., 2009 ; Liu et al., 2011): short-term at ≥3 mg/m ³ , and in monkeys (Biagini et al., 1989): acute at 3.1 mg/m ³ ; in mice and rats, this response was amplified with OVA sensitization; Note: TRP antagonists reduced the hyperresponsiveness in mice (Wu et al., 2013)		

^aAs the challenge stimuli used in the formaldehyde studies included allergens as well as nonimmunological stimuli, and because most experiments did not attempt to delineate the specifics of the functional changes, “airway hyperresponsiveness” or “hyperresponsive airways” encompasses any of a range of possible airway features: hyperreactivity (exaggerated response), hypersensitivity (lower dose to elicit response), altered ventilatory parameters (e.g., maximal response; resistance), recovery (longevity of response), or others.

Table C-33. Summary of changes in LRT cell counts and immune factors as a result of formaldehyde exposure

Endpoint(s)		No changes observed (high or medium confidence experiments are bolded)		Significant ^a increases or decreases (high or medium confidence experiments are bolded)		Summary conclusion Clarifying notes and exposure duration
		Duration (species)	Concentration(s) [allergen stimulus] (study)	Duration (species)	Concentration(s) [allergen stimulus] (study)	
White blood cells (WBCs)	Total WBCs (or Total Inflammatory Cells)	<i>Acute (g pigs)</i> <i>Acute (humans)</i> <i>Acute (mice)</i> <i>Acute (mice)</i>	0.13–0.31 mg/m³ [–OVA] (Riedel et al., 1996) 0.5 mg/m³ [+ pollen] (Ezratty et al., 2007) 0.5–6.2 mg/m³ [–OVA] (Larsen et al., 2013) 0.25–3.7 mg/m³ [–OVA] (Abreu et al., 2016)	<i>Subchronic (mice)</i> <i>Short term (mice)</i> <i>Short term (mice)</i> <i>Short term (mice)</i> <i>Short term (rats)</i>	↑ 2.5 mg/m³ [+ OVA] (Fujimaki et al., 2004b) ↑ 12.3 mg/m³ [–OVA] (Kim et al., 2013a); total BAL cells ↑ 12.3 mg/m³ [–OVA] (Jung et al., 2007) ↑ 3 mg/m³ [± OVA] (Wu et al., 2013) ↑ 0.5–3.1 mg/m³ [+ OVA] (Qiao et al., 2009)	Moderate ↑ short-term ≥0.5 mg/m³; amplifies allergen effect
	Neutrophils	<i>Subchronic (mice)</i> <i>Acute (g pigs)</i> <i>Short term (mice)</i> <i>Acute (humans)</i>	0.1–2.5 mg/m³ [± OVA] (Fujimaki et al., 2004b) 4.2 mg/m³ [–OVA] (Swiecichowski et al., 1993) 6.2–12.3 mg/m³ [–OVA] (Jung et al., 2007) 0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)	<i>Short term (mice)</i> <i>Acute (rats)</i>	↑ 3 mg/m³ [+ OVA] (Wu et al., 2013) ↑ 6.2 mg/m³ [–OVA] (Kimura et al., 2010)	Slight ↑ amplifies allergen response at >3 mg/m³ (short-term)
	Eosinophils	<i>Acute (humans)</i> <i>Acute (humans)</i> <i>Acute (rats)</i>	(trend ↑) 0.1 mg/m³ [+ Der^b f] (Casset et al., 2007) 0.5 mg/m³ [+ pollen] (Ezratty et al., 2007) 6.2 mg/m³ [–OVA] (Kimura et al., 2010)	<i>Subchronic (mice)</i> <i>Short term (mice)</i> <i>Short term (mice)</i> <i>Short term (mice)</i> <i>Short term (mice)</i> <i>Short term (rats)</i>	↑ 2.5 mg/m³ [+ OVA] (Fujimaki et al., 2004b) ↑ 12.3 mg/m³ [–OVA] (Jung et al., 2007) ↑ 0.5–3 mg/m³ [± OVA] (Liu et al., 2011) ↑ 3 mg/m³ [± OVA] (Wu et al., 2013) ↑ infer¹ >12.3 mg/m³ [+ Der f] (Sadakane et al., 2002) ↑ 0.5–3.1 mg/m³ [+ OVA] (Qiao et al., 2009)	Moderate ↑ short-term ≥0.5 mg/m³; amplifies allergen effect
	All	<i>Subchronic (mice)</i> <i>Short term (mice)</i> <i>Short term (mice)</i> <i>Acute (humans)</i>	0.1–2.5 mg/m³ [± OVA] (Fujimaki et al., 2004b) 6.2–12.3 mg/m³ [–OVA] (Kim et al., 2013a) 12.3 mg/m³ [–OVA] (Jung et al., 2007) 0.5 mg/m³ [+ pollen] (Ezratty et al., 2007)	<i>Short term (mice)</i>	↑ 3 [–OVA] mg/m³ (Wu et al., 2013)	Indeterminate suggests total number unchanged

Supplemental Information—Formaldehyde (Inhalation)

Endpoint(s)		No changes observed (high or medium confidence experiments are bolded)		Significant ^a increases or decreases (high or medium confidence experiments are bolded)		Summary conclusion Clarifying notes and exposure duration
		Duration (species)	Concentration(s) [allergen stimulus] (study)	Duration (species)	Concentration(s) [allergen stimulus] (study)	
	B Cells	<i>Acute (g pigs)</i> <i>Short term (mice)</i> <i>Short term (mice)</i>	4.2 mg/m³ [-OVA] (Swiecichowski et al., 1993) 6.2–12.3 mg/m ³ [-OVA] (Kim et al., 2013a) (trend ↓) 6.2–12.3 mg/m ³ [-OVA] (Jung et al., 2007)			Indeterminate allergen stimulus unstudied
	T Cells (CD4 ⁺)	<i>Short term (mice)</i> <i>Short term (mice)</i>	6.2–12.3 mg/m ³ [-OVA] (Kim et al., 2013a) (trend ↑) 6.2–12.3 mg/m ³ [-OVA] (Jung et al., 2007)	<i>Short term (rats)</i>	↑ (adults) 7.4 mg/m ³ [-OVA] (Sandikci et al., 2007b)	Indeterminate allergen stimulus unstudied
	T Cells (CD8 ⁺)	<i>Short term (mice)</i>	6.2–12.3 mg/m ³ [-OVA] (Kim et al., 2013a)	<i>Short term (rats)</i> <i>Short term (mice)</i>	↑ (adults) 7.4 mg/m ³ [-OVA] (Sandikci et al., 2007b) ↑ (slight) 12.3 mg/m ³ [-OVA] (Jung et al., 2007)	Slight ↑ short-term >7 mg/m ³ , allergen stimulus unstudied
	NK Cells			<i>Short term (mice)</i>	↓ 12.3 mg/m ³ [-OVA] (Kim et al., 2013a)	Indeterminate
	Monocytes	<i>Acute (g pigs)</i> <i>Acute (humans)</i> <i>Acute (rats)</i>	4.2 mg/m³ [-OVA] (Swiecichowski et al., 1993) 0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007) 6.2 mg/m ³ [-OVA] (Kimura et al., 2010)	<i>Subchronic (mice)</i>	↑ 2.5 mg/m ³ [+ OVA] (Fujimaki et al., 2004b)	Slight ↑ long-term ≥2.5 mg/m ³ amplifies allergen effect
Mast Cells		<i>Acute (g pigs)</i>	4.2 mg/m³ [-OVA] (Swiecichowski et al., 1993)			Indeterminate
Secreted factors and immune markers	Primarily Th1-related	TNF-α and GM-CSF	<i>Subchronic (mice)</i> 0.1–2.5 mg/m³ [± OVA] (Fujimaki et al., 2004b) <i>Acute (humans)</i> <i>Acute (mice)</i> 0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007) 0.25–3.7 mg/m ³ [-OVA] (Abreu et al., 2016)			Indeterminate suggests unchanged or highly variable
		IFN-γ	<i>Short term (mice)</i> 0.5–3 mg/m ³ [± OVA] (Liu et al., 2011) <i>Short term (mice)</i> 3 mg/m ³ [± OVA] (Wu et al., 2013) <i>Acute (humans)</i> 0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007)	<i>Short term (mice)</i> <i>Short term (rats)</i>	↓ 6.2–12.3 mg/m ³ [-OVA] (Kim et al., 2013a) ↑ 3.1 mg/m ³ [-OVA] (Qiao et al., 2009)	

Supplemental Information—Formaldehyde (Inhalation)

Endpoint(s)		No changes observed (high or medium confidence experiments are bolded)		Significant ^a increases or decreases (high or medium confidence experiments are bolded)		Summary conclusion Clarifying notes and exposure duration
		Duration (species)	Concentration(s) [allergen stimulus] (study)	Duration (species)	Concentration(s) [allergen stimulus] (study)	
	IL-1 (IL-1 β in animals)	<i>Acute (humans)</i> <i>Acute (mice)</i>	0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007) 0.25–3.7 mg/m ³ [–OVA] (Abreu et al., 2016)	<i>Subchronic (mice)</i> <i>Short term (mice)</i> <i>Short term (mice)</i>	↓ 2.5 mg/m ³ [+ OVA] (Fujimaki et al., 2004b) ↑ 3 mg/m ³ [–OVA] (Wu et al., 2013) ↑ 6.2–12.3 mg/m ³ [–OVA] (Jung et al., 2007)	
Primarily Th2-related	IL-4	<i>Short term (mice)</i> <i>Acute (humans)</i>	infer ^a >12.3 mg/m ³ [± Der f] (Sadakane et al., 2002) 0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007)	<i>Short term (mice)</i> <i>Short term (mice)</i> <i>Short term (mice)</i> <i>Short term (mice)</i> <i>Short term (rats)</i>	↑ 1–3 mg/m ³ [–OVA] (Lu et al., 2005) ↑ 6.2–12.3 mg/m ³ [–OVA] (Jung et al., 2007) ↑ 0.5–3 [+ OVA] or 3 [–OVA] mg/m ³ (Liu et al., 2011) ↑ 3 mg/m ³ [± OVA] (Wu et al., 2013) ↑ 0.5–3.1 mg/m ³ [+ OVA]; ↓ 3.1 mg/m ³ [–OVA] (Qiao et al., 2009)	Slight ↑ IL-4 at ≥0.5 mg/m ³ and IL-5 at ≥6.15 mg/m ³ , short-term and likely amplifying allergen effects
	IL-5	<i>Acute (humans)</i>	0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007)	<i>Short term (mice)</i> <i>Short term (mice)</i>	↑ 6.2–12.3 mg/m ³ [–OVA] (Jung et al., 2007) ↑ infer ^a >12.3 mg/m ³ [+ Der f] (Sadakane et al., 2002)	
	IL-10	<i>Acute (humans)</i>	0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007)			
	IL-6	<i>Subchronic (mice)</i> <i>Acute (mice)</i>	0.1–2.5 mg/m ³ [± OVA] (Fujimaki et al., 2004b) 0.25–3.7 mg/m ³ [–OVA] (Abreu et al., 2016)	<i>Short term (mice)</i>	↑ 0.5–3 [+ OVA] or 3 [–OVA] mg/m ³ (Liu et al., 2011)	Indeterminate
	IL-13	<i>Short term (mice)</i>	6.2–12.3 mg/m ³ [–OVA] (Jung et al., 2007)			
NK cell factors	IL-2R			<i>Short term (mice)</i>	↓ 6.2–12.3 mg/m ³ (Kim et al., 2013a)	Indeterminate
	Perforin					
Eosinophil	RANTES			<i>Short term (mice)</i>	↑ infer ^a >12.3 mg/m ³ [± Der f] (Sadakane et al., 2002)	Slight ↑

Supplemental Information—Formaldehyde (Inhalation)

Endpoint(s)		No changes observed (high or medium confidence experiments are bolded)		Significant ^a increases or decreases (high or medium confidence experiments are bolded)		Summary conclusion Clarifying notes and exposure duration
		Duration (species)	Concentration(s) [allergen stimulus] (study)	Duration (species)	Concentration(s) [allergen stimulus] (study)	
Other	ICAM and CCR3			Short term (mice)	↑ (indirect ^b) 12.3 mg/m ³ [–OVA] (Jung et al., 2007)	chemoattractants relevant to eosinophil recruitment with short-term exposure
	Eotaxin	<i>Subchronic (mice)</i> 0.1–2.5 mg/m ³ [± OVA] (Fujimaki et al., 2004b) ³ <i>Acute (humans)</i> 0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007)		Short term (mice)	↑ (indirect ^b) 12.3 mg/m ³ [–OVA] (Jung et al., 2007)	
	ECP	<i>Acute (humans)</i> 0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007)		<i>Acute (humans)</i>	↑ 0.1 mg/m ³ [+ Der f] (Casset et al., 2007)	
	MIP-1α	<i>Subchronic (mice)</i> 0.1–2.5 mg/m ³ [± OVA] (Fujimaki et al., 2004b) ³				
	IL-8	<i>Acute (humans)</i> 0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007)		<i>Acute (in vitro)</i>	↑ 1.23 mg/m ³ (Rager et al., 2011)	Indeterminate
	MCP-1	<i>Subchronic (mice)</i> 0.1–2.5 mg/m ³ [± OVA] (Fujimaki et al., 2004b) ³ <i>Acute (humans)</i> 0.5 mg/m ³ [+ pollen] (Ezratty et al., 2007)				Indeterminate

Der f: *Dermatophagoides farina* (house dust mite); OVA: ovalbumin (major protein of chicken egg whites); both are immunogenic materials used to stimulate an allergic response.

Gray box = no data meeting the inclusion criteria were available.

Notes: Two studies with evidence that may inform the potential for formaldehyde exposure-induced inflammatory changes in the LRT are not captured in these tables, specifically a proteomics analysis of the BAL fluid after short-term exposure at ≥2.46 mg/m³ ([Ahn et al., 2010](#)) and an miRNA microarray study of gaseous paraformaldehyde exposure in a human lung cancer cell line with acute exposure to 1.23 mg/m³ ([Rager et al., 2011](#)). Swiecichowski et al. (1993) may include information from an earlier study interpreted to have been conducted in the same cohort of guinea pigs ([Leikauf, 1992](#)).

^aPrimarily, this reflects reporting of a statistically significant change; in rare instances where a *p* value was not given, changes are indicated if the authors discussed the change as a significant effect.

^bReported as 0.5% formaldehyde solution; concentration assumed to be >12.3 mg/m³ ([Sadakane et al., 2002](#)).

^cGene expression levels.

^dThese factors were not present at detectable levels regardless of treatment.

C.7.3. Synthesis of Changes in the Blood and Lymphoid Organs

Although this mechanistic evaluation is focused on mechanisms underlying respiratory health effects, these effects can be influenced by changes in nonrespiratory tissue compartments, most notably the blood and lymphoid organs. The direction, magnitude and type of immune responses observed in the blood should not be assumed to represent immunological changes occurring in the airways, as responses can differ. The nonrespiratory changes most likely relevant to respiratory system health effects are immune-related changes because these could induce extrapulmonary signals (e.g., cellular; secreted factors) to travel through the blood to perfused regions of the respiratory tract. This section emphasizes changes in exposed humans, unlike the emphasis on experimental animal studies in the URT and LRT sections, because blood sampling in humans is more convenient than sampling from respiratory tissue compartments; thus, more human data are available for changes in the blood (see Tables C-34 and C-35).

A number of studies, across different human and animal populations, spanning an array of formaldehyde exposure scenarios, have reported changes in blood cell counts and other secreted factors (see Tables C-34 and C-35). Although some of the specific changes vary across studies, taken together, the data provide *robust* evidence of an association between formaldehyde exposure and hematological effects. Although additional studies clarifying inconsistencies across the studies would be informative, several tentative patterns could be discerned. Interestingly, looking at the picture as a whole (see Figures C-23 and C-24), the direction of some changes noted in the blood of individuals exposed to formaldehyde are contrary to the cellular changes noted in the respiratory tract. For example, data suggest (*slight*) or support (*moderate*) that total cells, neutrophils, and CD8⁺ T cells are increased in the respiratory tract by formaldehyde exposure, while these same cells appear to be decreased in the blood. One potential explanation for this difference could involve recruitment of particular subsets of immuno-responsive cells from the circulation to the irritated and inflamed respiratory tract, as is observed with viral infections of the respiratory system ([Levandowski et al., 1986](#)); however, none of the identified human studies report data from both tissue compartments, and the animal data do not address such a hypothesis. It is plausible that this pattern could reflect species differences (i.e., LRT data are mostly from animal studies), but this possibility is considered unlikely given the blood data. As with investigations of the airways, very few studies tested mechanistic hypotheses for how formaldehyde exposure could affect blood immune cell counts. Despite this lack of information and variability in responses, the available data support a conclusion that formaldehyde exposure can modify immune system function in the blood across a range of concentrations and exposure durations.

One of the most consistent cellular changes observed across studies was a decrease in the total number of white blood cells (WBCs). This is a nonspecific finding, as WBCs encompass a spectrum of functional phenotypes, and this change may be driven by decreases in only one or several subpopulations. When looking more specifically at the WBCs, *moderate* evidence of CD8⁺ T cell decreases following formaldehyde exposure is provided by several studies, together with a

corresponding increase in the ratio of CD4⁺/CD8⁺ T cells (see Table C-35). As mentioned previously, CD8⁺ T cells comprise a heterogeneous cell population, which complicates interpretations regarding the potential impact of decreased numbers in peripheral blood. Depending on the specific stimuli, stimulated CD8⁺ T cells can produce interferon- γ (IFN- γ) and inhibit production of IL-4 and immunoglobulin (i.e., IgE) responses ([Holmes et al., 1997](#)), or their phenotype can be driven towards production of excess IL-4, a situation hypothesized to be associated with atopic asthma ([Lourenço et al., 2016](#)). *Moderate* evidence provides support for increases in blood IL-4 (which was similarly increased in the LRT) and decreases in IFN- γ after formaldehyde exposure. A more complete understanding of the phenotype of the depleted CD8⁺ T cells would be informative to ascertain whether these changes are related to the profile of secreted factors observed in the blood after formaldehyde exposure.¹⁸

Moderate evidence also indicates that formaldehyde exposure alters the number or percentage of B cells in the circulation. These cells produce antibodies upon stimulation with antigen (e.g., allergens) and contribute to airway hyperresponsiveness ([Hamelmann et al., 1997](#)). While this finding, along with *slight* evidence of increased antigenic markers, suggests potential for alteration of the adaptive immune response as a result of formaldehyde exposure, this observation alone is insufficient to indicate functional changes such as exposure-induced differences in clonal expansion and differentiation to antibody-producing cells, evidence of which would support a more convincing biological relationship.

Slight evidence suggests that neutrophils are also decreased in the blood by formaldehyde exposure. This could plausibly be explained by the suggestive (*slight*) findings of decreased lymphocyte and neutrophil chemoattractants in the blood and increased levels in the airways (possibly attracting blood neutrophils), suggesting that a gradient of these factors across tissue compartments may be induced and maintained as a result of formaldehyde exposure and, perhaps, sustained inflammation.

Finally, although variable across studies, several lines of evidence suggest a pattern of immune cell effects related to formaldehyde concentration, with stimulation at lower formaldehyde exposure levels and decreases at higher levels. This included changes in total T cells, NK cells, and IL-10 (and, perhaps, TNF- α). A complex relationship exists between IL-10, NK cells, and subsets of CD4⁺ T cells (e.g., Th1 and Th2 cells), which direct the type of antibody responses; however, the specifics of this suggestive (*slight*) association with formaldehyde exposure remain to be

¹⁸ Several studies examining the lineage and maturity of immune and non-immune cells in the bone marrow and other systemic tissues (e.g., blood; spleen) are not discussed in this section. Although it is possible that differences in the maturation phenotype of cells could indirectly contribute to the immune changes of interest to this section, such alterations would be expected to cause functional or other detectable changes in more apical mechanistic events relevant to immune responses in the respiratory system. Thus, this discussion focuses on those mechanistic events considered more directly relevant to these POE outcomes. Please see Section 3.3.3 of the Toxicological Review for a discussion of these cell lineage and maturation markers in the context of lymphohematopoietic cancer MOA.

elucidated. Many of these observations would benefit from additional, more specific studies on WBCs.

Red blood cell (RBC) counts were decreased in both human and animal studies (*moderate* evidence), generally at formaldehyde concentrations above 0.5 mg/m³. *Slight* data exist to suggest that platelets may also be decreased, which could plausibly be related to the single, low confidence animal study that reported increased megakaryocytes (cells that produce platelets) in the bone marrow ([Zhang et al., 2013](#)). The relevance of these changes to respiratory system health effects is unknown. It is plausible that sustained increases in oxidative stress (which has been observed in the blood and, to a lesser extent, other lymphoid tissues) and/or other soluble factors in the blood resulting from airway inflammation could affect the viability of circulating erythrocytes and immune cells or the circulating precursors for these cells; however, no evidence exists to substantiate this hypothesis. An increased level of the circulating stress hormone, corticosterone (the major animal glucocorticoid; in humans, it is cortisol), with short-term, but not acute, formaldehyde exposure is also suggested by *slight* data. Persistent increases in circulating glucocorticoids can also negatively impact the function and health of circulating immune cells, causing immunosuppression of most cell types ([O'Connor et al., 2000](#)). However, these potential linkages have not been examined.

As with findings for WBC changes, antibody, or immunoglobulin (Ig), responses resulting from formaldehyde exposure are consistently altered, although the specific changes observed across studies provide a mixed picture (see Table C-34). Much of the *moderate* evidence is based on animal sensitization models using the protein allergen ovalbumin, although the human data also indicate changes after exposure. In general, the variable evidence of formaldehyde-induced modification of humoral immunity in humans demonstrates different patterns of results depending on the population (e.g., children vs. adults), the duration of exposure, and the specific Ig measure (e.g., Ig isotype) across studies. The animal studies consistently report amplified responses with allergen stimulation and/or sensitization, although the pattern and magnitude of these effects appears to vary depending on the type of allergen and the sensitization protocol used. The Igs most relevant to the blood and respiratory tract are IgA (IgA1 and IgA2), IgE, IgM, and IgG (IgG1, IgG2, and IgG3; also, IgG4 in humans). No changes of note in IgA or IgM were identified across the available studies. *Slight* data suggest that formaldehyde exposure may cause elevated levels of IgE antibodies in certain exposure scenarios, including in exposed children; however, this finding should be interpreted with caution, as comparable studies did not observe effects, and explanations for this inconsistency are not available. IgEs are implicated in allergic hypersensitivity responses of the airways ([Hamelmann et al., 1999](#)), although they may not be essential for all hypersensitivity-related responses (e.g., intrinsic [nonallergic] asthma occurs in one-third of all adult patients; ([Knudsen et al., 2009](#))). Despite the variability in models, several of the available studies consistently identified changes in antibodies of the IgG class (*moderate* evidence), including increases in IgGs specific to formaldehyde or antigens (e.g., allergens) to which the subjects had previously been

exposed. IgGs are the most prevalent Ig in the serum of humans, and they are the only Ig that can be transferred to neonatal/infant circulation (i.e., by crossing the placenta; through breast milk in animals) to influence immunity in offspring ([Van de Perre, 2003](#)). None of the included studies examined antibody titers or transferred immunity with developmental formaldehyde exposure (note: *not informative* studies from one lab: ([Silva Ibrahim et al., 2015](#); [Maiellaro et al., 2014](#)) reported immune-related effects of gestational formaldehyde exposure). While IgEs are most commonly associated with sensitization-related airway hyperresponsiveness to allergens, subclasses of IgGs also contribute to allergic responses; however, their exact role in the pathophysiology of airway disorders remains unclear [([Williams et al., 2012](#); [Hofmaier et al., 2014](#); [Bogaert et al., 2009](#))]. Overall, although a body of evidence indicates changes in antibody-mediated responses after formaldehyde exposure, particularly in regard to IgGs, an explanation for the variable pattern of changes in Igs (e.g., to formaldehyde alone or with coexposure to different types of antigens by specific Ig subclasses) does not exist, and the likely consequences of these changes are unknown.

Table C-34. Summary of changes in the blood and lymphoid organs as a result of formaldehyde exposure

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (exposure duration)	Conclusion
Formaldehyde-Induced Antibody Response in the Blood				
Total IgE	High or Medium	Human: None	No changes in a <u>subchronic</u> mouse study at ≤ 2.46 mg/m ³	Moderate Altered antibody responses (basis below) <u>Total</u> <i>Moderate</i> ↓: IgG [naïve subjects] <i>Slight</i> ↑: IgE [3 mg/m ³] IgA [6 mg/m ³] <i>Indeterminate</i> : IgM [mixed] <u>FA-specific</u> <i>Moderate</i> ↑: IgG [long-term] <i>Slight</i> ↑: IgE [children; long-term] <i>Indeterminate</i> : IgM or IgA <u>Antigen-specific</u> <i>Moderate</i> ↑: IgG [inhaled antigen] <i>Slight</i> ↑: IgE [certain scenarios] <i>Indeterminate</i> : IgM or IgA
		Animal: No evidence suggesting changes (Fujimaki et al., 2004b): subchronic ≤ 2.46 mg/m ³		
	Low	Human: No evidence suggesting changes (Wantke et al., 1996b ; Wantke et al., 2000 ; Palczynski et al., 1999 ; Ohmichi et al., 2006 ; Erdei et al., 2003): short-term ≤ 1.8 mg/m ³ (duration in Erdei unknown)	Suggestive evidence of increased IgE in 2 <u>short-term</u> formalin studies in mice at ≥ 3 mg/m ³ , but no evidence for changes in mice or humans at < 2 mg/m ³	
		Animal: Evidence of increases in mice, which were increased further by OVA (Wu et al., 2013 ; Jung et al., 2007): short-term ≥ 3 mg/m ³ ; evidence of no changes in mice by FA alone (Kim et al., 2013a ; Gu et al., 2008), although FA exacerbated HDM-induced IgE (Kim et al., 2013a): short-term 0.12–1.2 mg/m ³		
Formaldehyde (FA)-Specific IgE	High or Medium	Human: Elevated in one study of children (Wantke et al., 1996a): years (assumed) at ≈ 0.06 compared to ≈ 0.03 mg/m ³ (unrelated to symptoms); N/C in adults (Kim et al., 1999): 4 years at 3.74 mg/m ³	Increased in a single <u>long-term</u> study of children at < 0.1 mg/m ³ ; N/C in a single long-term study of adults at 3.74 mg/m ³	
		Animal: None		
	Low	Human: No evidence of changes across multiple studies in adults (Zhou et al., 2005 ; Wantke et al., 1996b ; Thrasher et al., 1987 ; Ohmichi et al., 2006 ; Kim et al., 1999 ; Górski and Krakowiak, 1991): short-term (weeks) or long-term (years) at ≈ 0.1 –3.74 mg/m ³ ; unclear in 2 long-term adult studies in which a small proportion of subjects did have FA-IgE (Thrasher et al., 1990 ; Dykewicz et al., 1991); one study noted slight increases with longer exposure (Wantke et al., 2000): 10 wk, not 5 wk, at 0.265 mg/m ³	No clear evidence of changes across multiple <u>short-term and long-term</u> studies in adults at ≤ 3.74 mg/m ³ ; no studies in children	
		Animal: Isotype unspecified- no change in guinea pigs with acute challenge (Lee et al., 1984) at 2.5 or 4.9 mg/m ³ after short term exposure to 7.4 or 12.3 mg/m ³ (note: no measures without formaldehyde)		
	High	Human: None		

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (exposure duration)	Conclusion
Antigen-Specific IgE (does not include FA-specific Ig)		Animal: N/C in OVA-IgE (Fujimaki et al., 2004b): 12 wks at 0.1–2.46 mg/m ³ (OVA i.p.)	N/C in a single subchronic study with i.p. sensitization	
	Low	Human: None	Two mouse studies suggest formaldehyde can increase IgE specific to antigen at ≥1 mg/m ³ , but this appears to be highly situational (e.g., dependent on duration and periodicity of formaldehyde exposure, and antigen type and administration route)	
		Animal: Increased OVA-specific IgE in mice in 2 studies—(Tarkowski and Gorski, 1995; Gu et al., 2008): 10 d at 2 mg/m ³ (but not 1 d/wk for 7 wk, or when OVA sensitization i.p.) and 5 wk at 0.98 mg/m ³ with i.p. OVA (but not ≤4 wk), respectively; however, N/C in mice in 3 studies: (Wu et al., 2013): 4 wk at 3 mg/m ³ (s.c. OVA sensitization), (Kim et al., 2013b): 0.2–1.23 mg/m ³ for 4 wk (dermal house dust mite, HDM, sensitization), and (Sadakane et al., 2002): 4 wk at 0.5% (i.p. Der f sensitization)		
Total IgG	High or Medium	Human: Decreased in a single study of exposed workers (Aydin et al., 2013): 7 year at 0.264 mg/m ³	A single study in adult workers and another in male rats showed decreased IgG at 0.264 or ≥6.15 mg/m ³ with long-term or short-term exposure, but subclass not examined	
		Animal: Decreased total IgG in rats (Sapmaz et al., 2015): short-term at ≥6.15 mg/m ³		
	Low	Human: N/C in children at ≈0.007–0.07 mg/m ³ (Erdei et al., 2003): unknown duration (likely months-years)	Suggestive evidence based on increased IgG1 in 2 short-term mouse studies, but a third mouse study and a human study did not observe effects at <1 mg/m ³	
		Animal: IgG1 (N/C in IgG2a) increased by FA alone, whereas FA exacerbated IgG2a (N/C in IgG1) in atopic-prone mice (Kim et al., 2013b): short-term 0.25, not 1.2 mg/m ³ ; increased IgG1 and IgG3, but decreased IgG2a and 2b, in C57 mice (Jung et al., 2007) short-term ≥6.15 mg/m ³ ; N/C in IgG Balb/c mice (Gu et al., 2008): short-term <1 mg/m ³		
FA-Specific IgG	High or Medium	Human: Slight (<10%) increase in a single study of adults (Kim et al., 1999): years at 3.74 mg/m ³	Slightly increased in a single long-term study of adults at 3.74 mg/m ³ ; no studies in children	
		Animal: None		
	Low	Human: Increased in two studies (Thrasher et al., 1987; Thrasher et al., 1990) and unclear in 1 study in which 5/55 subjects did have FA-IgG (Dykewicz et al., 1991): [all 3 studies] years at <0.1–<1.0 mg/m ³ ; N/C in one study (Wantke et al., 2000): short-term at 0.265 mg/m ³	Suggestive of slight increases in adults with long-term exposure at <1 mg/m ³ , but not with short-term exposure at higher levels; no studies in children	
		Animal: Isotype unspecified—no change in guinea pigs with acute challenge (Lee et al., 1984) at 2.5 or 4.9 mg/m ³ after short term exposure to 7.4 or 12.3 mg/m ³ (note: no measures without formaldehyde)		

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (exposure duration)	Conclusion
Antigen-Specific IgG (does not include FA-specific Ig)	High or Medium	Human: None	Increased OVA-IgG1 in 1 <u>short-term</u> study in guinea pigs at 0.31 mg/m ³ with inhaled allergen, but not a longer mouse study using injected allergen	
		Animal: Increased OVA-specific IgG1 in guinea pigs (Riedel et al., 1996): 5 d at 0.31 mg/m ³ (inhaled OVA); questionable decrease (no dose-response) in OVA-IgG1 and OVA-IgG3 in mice (Fujimaki et al., 2004b): 12 wks at 0.49, but not 2.46 mg/m ³ (OVA i.p.; N/C in OVA-IgG2)		
	Low	Human: Increased IgG against 2 bacterial pathogens by linear regression in 3 rd grade children with respiratory complaints (Erdei et al., 2003): <0.1 mg/m ³ , unknown duration (likely years, home measures)	1 <u>long-term</u> study suggests increased IgG sensitization to an airway antigen by FA in children; multiple studies in mice and rats suggest that IgG sensitization does not occur when antigen sensitization occurs by injection	
		Animal: N/C in OVA-IgG or Der f-IgG1 in mice (Wu et al., 2013 ; Sadakane et al., 2002 ; Gu et al., 2008): up to 5 wk at 0.123–3 mg/m ³ or higher; N/C in IgG specific to vaccine antigens in rats (Holmstrom, 1989): 22 months at 15.5 mg/m ³ . In all cases, s.c. or i.p. sensitization		
Total IgM or IgA	High or Medium	Human: Decreased IgM, N/C in IgA, in a study of exposed workers (Aydin et al., 2013): 7 year at 0.26 mg/m ³	IgM, but not IgA, decreased in a single study in adult workers at 0.26 mg/m ³ with <u>long-term</u> exposure	
		Animal: Increased total IgM and IgA in rats (Sapmaz et al., 2015): short-term at ≥6.15 mg/m ³		
	Low	Human: No evidence of IgA or IgM changes (Erdei et al., 2003): duration unknown ≤0.1 mg/m ³	IgA increased in 1 <u>short-term</u> study at >6 mg/m ³ ; N/C in IgM in 2 studies	
		Animal: Increased IgA and N/C in IgM in C57 mice (Jung et al., 2007): short-term ≥6.15 mg/m ³		
FA-Specific IgM or IgA	High or Medium	Human: None	No evidence to evaluate	
		Animal: None		
	Low	Human: Unclear evidence in 1 long-term study in which a small proportion of subjects appear to have elevated FA-specific IgM (Thrasher et al., 1990): months–years at ≈0.1–1 mg/m ³	Evidence could not be interpreted	
		Animal: Isotype unspecified- no change in guinea pigs with acute challenge (Lee et al., 1984) at 2.5 or 4.9 mg/m ³ after short term exposure to 7.4 or 12.3 mg/m ³ (note: no measures without formaldehyde)		
Antigen-Specific IgM or IgA (does not include FA-specific Ig)	High or Medium	Human: None	No evidence to evaluate	
		Animal: None		
	Low	Human: N/C in airway pathogen bacteria-specific IgM or IgA in one study in children (Erdei et al., 2003): unknown duration (likely months to years) at <0.1 mg/m ³		

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Animal: N/C in IgM specific to vaccine antigens in rats (Holmstrom et al., 1989a): 22 mos at 15.5 mg/m ³ (s.c. injection)	The minimal data available suggest that formaldehyde does not alter these parameters	
Immune and Inflammation-Related Changes in the Blood				
[[See Table A-81 for Cellular and Cytokine Response in Blood]]				
Oxidative Stress	High or Medium	Human: Increased marker of lipid peroxidation in adult serum lymphocytes (Bono et al., 2010): likely months to years (assumed) at ≥0.066 mg/m ³ ; Increased F2-Isoprostanes (suggested as the best in vivo biomarker of lipid peroxidation) in urine (Romanazzi et al., 2013): 0.21 mg/m ³ chronic occupational (indirect), although smoking and formaldehyde were not additive, both were independently associated with ROS—Note: serum and urine IsoP measures are correlated (Rodrigo et al., 2007), suggesting that urine levels may reflect similar serum changes	Two studies in adults indicate elevated oxidative stress markers in blood at ≥0.066 mg/m ³ with <u>long-term</u> exposure. Given the uncertainty with concluding urine levels exhibit the same pattern of association as blood, 1 study contributes as indirect evidence	Moderate ↑
		Animal: None		
	Low	Human: Increased oxidative stress biomarkers (F2-Isoprostanes; malondialdehyde) in urine (Bellisario et al., 2016): ≈0.034 mg/m ³ work shift occupational (indirect; responses likely reflect short-term exposure)	Several studies in three species suggest increases in markers of oxidative stress with <u>acute</u> or <u>short-term</u> exposure, even at formaldehyde levels ≤1 mg/m ³ ; it is not clear whether and to what extent this persists with long-term exposure	
		Animal: Increased oxidative stress markers in mice (Ye et al., 2013 ; Matsuoka et al., 2010): acute or short-term as low as 0.12 mg/m ³ ; increased markers and protein indicators in rats (Im et al., 2006 ; Aydin et al., 2014): short term at 6.48–12.3 mg/m ³ , although 1 study with longer exposure observed a decrease in MDA, but decreased SDH in lymphocytes (Katsnelson et al., 2013): 10 wk at 12.8 mg/m ³ ; other indicators including decreased GSH (Ye et al., 2013 ; Katsnelson et al., 2013) and increased NO and SOD (Matsuoka et al., 2010) at ≥1 mg/m ³		
Circulating Stress Hormones	High or Medium	Human: None	Increased stress hormone at 3 mg/m ³ formaldehyde in a single rodent study with <u>short-term</u> , but not acute, exposure	Slight ↑
		Animal: Increased corticosterone in rats with short-term, but not acute, exposure (Sorg et al., 2001a): ≈3 mg/m ³		
	Low	Human: None	No evidence to evaluate	
		Animal: None		
	High	Human: None	No evidence to evaluate	Indeterminate

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (exposure duration)	Conclusion
Altered Immune Function		Animal: None		
	Low	Human: Increased autoantibodies in adults (Thrasher et al., 1990): long-term at 0.06–0.95 mg/m³	1 study in adults suggests that autoantibodies are elevated with low level, long-term exposure; somewhat in contrast, 1 mouse study suggests short-term high level exposure improves host response to bacteria	
		Animal: Improved cell-mediated immune response to bacteria challenge, but N/C against tumor challenge or delayed-type hypersensitivity response in mice (Dean et al., 1984): 3 wk at 18.5 mg/m³; however, N/C in vitro measures of immune cell function.		
Changes in Other Immune-related tissues				
Cell counts in immune tissues (not including bone marrow)	High or Medium	Human: None	Suppression of CD8+ T cells in immune tissues (e.g., spleen) is indicated in one 8-wk mouse study, with indirect support from a second short-term mouse study, at around 2 mg/m³; effects on CD4+/CD8+ ratio were mixed across 2 subchronic mouse studies	Moderate (for ↓ CD8+ T cell response in spleen and thymus) Slight
		Animal: Decreased CD8+ T cells and increased CD4+/CD8+ ratio in both thymus (immature immune cells) and spleen (mature immune cells) in male mice (Ma et al., 2020): Eight weeks of exposure at 2 mg/m³; No change in splenic CD4+/CD8+ ratio in female mice (Fujimaki et al., 2004b): 12 wk at up to 2.46 mg/m³; Increased splenic regulatory T cells (subset of CD4+) and indirect markers for suppression of effector T cell (CD8+) activity in female mice (Park et al., 2020): short-term exposure at ≥1.38 mg/m³		
	Low	Human: None	Multiple short-term mouse studies suggest that overall splenic cell T and B cells are unchanged; however, 2 studies suggest that NK cells may be affected (1 study showed NK cells were stimulated at low formaldehyde levels, and another that high levels are inhibitory/toxic)	NK cells (in spleen: ↑ at low level; ↓ at high level) Indeterminate for other cell counts
		Animal: N/C in tissue weight, total cellularity or T or B cell counts in mice (Kim et al., 2013a; Gu et al., 2008; Dean et al., 1984); altered NK cell number and function was noted in mice, with one study showing decreases (Kim et al., 2013a): 2–3 wk at 12.3 mg/m³, and another showing increases (Gu et al., 2008): 5 wk at up to 0.12 mg/m³, and a third showing N/C in lymphocyte proliferation, functional parameters, IgM production, or NK cytotoxicity (Dean et al., 1984): 3 wk at 18.5 mg/m³		
Splenic and Lymph Cytokines and other Markers	High or Medium	Human: None	No evidence to evaluate	Slight ↑ oxidative stress and cytokine production, especially in response to antigen
		Animal: None		
	Low	Human: None	1 short-term mouse study suggests increased oxidative stress at ≥1 mg/m³, and another ↓ IL-13 at 0.25–1.23 mg/m³, and 3 others suggest that the response (splenic or lymph) to antigen stimulation (and 1	
		Animal: Spleen: ↑ oxidative stress markers in mice (Ye et al., 2013): 7 d at ≥1 mg/m³; exaggerated IFNγ response (at 2.46 mg/m³) of lymphocytes to LPS and ↑ MCP-1 response to OVA in mice (Fujimaki et al., 2004b): 12 wk at ≥0.49 mg/m³; ↓ IL-13 (Kim et al., 2013a): short-term at 0.25–1.23 mg/m³; with allergen (HDM), exacerbated ↑ in IL-4, IL-5, IL-13, and IL-17a, but ↓ IFNγ (Kim et al., 2013a): short-term at 0.25 or 1.23 mg/m³;		

Supplemental Information—Formaldehyde (Inhalation)

Endpoint	Study-specific findings from “high or medium” or “low” confidence experiments		Summary of evidence (exposure duration)	Conclusion
		Lymph Nodes: ↑ IL-4 and IL-10 (and IL-12, slightly), but N/C in IFN γ in mice with sensitization (De Jong et al., 2009): 4 wk at 3.6 mg/m ³ ; thymus: ↑ IL-4 and IL-1B in mice (Jung et al., 2007): short-term (2 wk) at ≥0.5 mg/m ³	study without stimulation), most notably increased IL-4, is exacerbated at ≥0.25 mg/m ³ formaldehyde	
Bone Marrow Cell Counts and Function	High or Medium	Human: None Animal: ↑ bone marrow hyperplasia in rats (Kerns et al., 1983): 24 mos at 17.6 mg/m ³	No evidence to evaluate	Indeterminate
	Low	Human: None Animal: In mice: N/C in cell counts or functional properties in mice (Dean et al., 1984): 3 wk at 18.5 mg/m ³ [Note: thymus measures also unchanged]; Bone marrow toxicity, impaired function, and decreased cell counts at excessive levels (Yu et al., 2014b ; Yu et al., 2015a): short-term at ≥40 mg/m ³ ; increased megakaryocytes (Zhang et al., 2013): short-term at ≥0.5 mg/m ³	1 mouse study suggests BM megakaryocytes may be increased with short-term exposure at ≥0.5 mg/m ³ ; Total cell counts are unchanged with short-term exposure at ≤20 mg/m ³ in 2 mouse studies, while excessive levels appear to cause toxicity	
Bone Marrow Cytokines and other Markers	High or Medium	Human: None Animal: N/C in BM mRNAs or miRNAs in rats (Rager et al., 2014): short term at 2.46 mg/m ³	Indirect evidence suggests no changes at ≤2.46 mg/m ³	Slight ↑ oxidative stress and inflammation
	Low	Human: Animal: ↑ indicators of oxidative stress in mice (Zhang et al., 2013 ; Yu et al., 2014b ; Yu et al., 2015a ; Ye et al., 2013): short-term at ≥0.5 mg/m ³ ; increased markers of cell death (caspase-3) and inflammation (↑ NFkB, TNF α , IL-1 β) in mice (Zhang et al., 2013 ; Yu et al., 2015a): short-term at 3 and 20 mg/m ³ , respectively; N/C in DNA or RNA measures of proliferation and health in rats (Dallas et al., 1987): subchronic at 0.62–18.5 mg/m ³	3 mouse studies suggest that oxidative stress is increased with short-term exposure, even at 0.5 mg/m ³ . 1 short-term mouse study suggests the BM is damaged and inflamed, while 1 longer-term rat study suggests there is no damage	

Table C-35. Summary of changes in blood cell counts and immune factors as a result of formaldehyde exposure

Endpoint(s)		No changes observed (<i>high or medium confidence</i> experiments are bolded)		Significant ^a increases or decreases (<i>high or medium confidence</i> experiments are bolded)		Summary conclusion Clarifying notes
		<i>Duration^b</i> (<i>species</i>)	<i>Concentration(s) [notes] (study)</i>	<i>Duration</i> (<i>species</i>) ^b	<i>Concentration(s) [notes] (study)</i>	
White blood cells (WBCs)	Total WBCs	<i>Years (humans)</i> <i>Years (humans)</i> <i>Short term (mice)</i> <i>Years (children)</i>	0.87 mg/m³ (Lyapina et al., 2004) 0.25 mg/m³ (Aydin et al., 2013) ≥9.23 mg/m³ (Morgan et al., 2017) ≈0.02 mg/m ³ [year assumed] 90767	<i>Years (humans)</i> <i>Short term (rats)</i> <i>Years (humans)</i> <i>Unclear^c (humans)</i> <i>Short term (mice)</i>	↓ 1.6 mg/m ³ (Zhang et al., 2010 ; Hosgood et al., 2013 ; Bassig et al., 2016) ≥2.46 mg/m ³ (Rager et al., 2014); [indirect] ↓ ≤0.29 mg/m ³ [mean levels] (Kuo et al., 1997) ↓ N/A ^h (≤1 mg/m ³) [years, not months] (Thrasher et al., 1990) ↓ 0.5–3 mg/m ³ (Zhang et al., 2013)	Moderate ↓ ⁴ Possibly concentration- and/or duration-dependent, but this dependence is unclear
	All	<i>Short term (mice)</i>	18.5 mg/m ³ [WBC differentials ^d] (Dean et al., 1984)	<i>Years (humans)</i>	↓ 1.6 mg/m ³ (Zhang et al., 2010 ; Hosgood et al., 2013 ; Bassig et al., 2016)	Slight ↓ most likely neutrophils at higher concentrations with short-term or longer exposure
	Neutrophils	<i>Years (humans)</i> <i>Short term (mice)</i> <i>Years (children)</i> <i>Years (humans)</i> <i>Short term (mice)</i>	0.25 mg/m³ (Aydin et al., 2013) ≥9.23 mg/m³ (Morgan et al., 2017) ≈0.02 mg/m ³ [year assumed] (Erdei et al., 2003) ≤0.29 mg/m ³ [mean levels] (Kuo et al., 1997) 0.5–3 mg/m ³ (Zhang et al., 2013)	<i>Years (humans)</i> <i>Short term (rats)</i>	↓ 0.87 mg/m ³ [note: function, not counts, in workers with URT dysfunction] (Lyapina et al., 2004) ↓ 13 mg/m ³ (Katsnelson et al., 2013)	
	Eosinophils	<i>Short term (mice)</i> <i>Years (children)</i> <i>Years (humans)</i>	≥9.23 mg/m³ (Morgan et al., 2017) ≈0.02 mg/m ³ [year assumed] (Erdei et al., 2003) ≤0.29 mg/m ³ [mean levels] (Kuo et al., 1997)			
	Basophils	<i>Years (humans)</i>	≤0.29 mg/m ³ [mean levels] (Kuo et al., 1997)			

Supplemental Information—Formaldehyde (Inhalation)

Endpoint(s)			No changes observed (<i>high or medium confidence</i> experiments are bolded)	Significant ^a increases or decreases (<i>high or medium confidence</i> experiments are bolded)	Summary conclusion Clarifying notes
			<i>Duration^b</i> (<i>species</i>) <i>Concentration(s) [notes] (study)</i>	<i>Duration</i> (<i>species</i>) ^b <i>Concentration(s) [notes] (study)</i>	
Lymphocytes	All		Months (humans) 0.2–0.8 mg/m ³ (Jia et al., 2014) Short term (mice) ≥9.23 mg/m ³ (Morgan et al., 2017) <i>Years (children)</i> ≈0.02 mg/m ³ [year assumed] (Erdei et al., 2003) <i>Years (humans)</i> ≤0.29 mg/m ³ [mean levels] (Kuo et al., 1997) <i>Weeks (humans)</i> 0.51 mg/m ³ (Ying et al., 1999) <i>Unclear^c (humans)</i> N/A ^h (≤1 mg/m ³) [years vs. months] (Thrasher et al., 1990) <i>Short term (mice)</i> 18.5 mg/m ³ [WBC differentials ^e] (Dean et al., 1984)	Years (humans) ↓ 1.6 mg/m ³ (Zhang et al., 2010 ; Hosgood et al., 2013 ; Bassig et al., 2016) Years (humans) ↑ 0.25 mg/m ³ (Aydin et al., 2013) <i>Short term (mice)</i> ↓ 0.5–3 mg/m ³ (Zhang et al., 2013) <i>Short term (rats)</i> ↑ 13 mg/m ³ (Katsnelson et al., 2013)	Indeterminate multiple changes noted, but pattern is indiscernible
	B Cells		Years (humans) 1.6 mg/m ³ (Zhang et al., 2010 ; Hosgood et al., 2013 ; Bassig et al., 2016) Years (humans) 0.25 mg/m ³ (Aydin et al., 2013) <i>Years (humans)</i> 0.09–0.68 mg/m ³ (Thrasher et al., 1987)	Years (humans) ↓ 0.36 [up to 0.69 peaks] mg/m ³ (Costa et al., 2013) Months (humans) ↑ 0.99 [up to 1.69 peaks] mg/m ³ (Ye et al., 2005) Months (humans) ↑ 0.2 and 0.8 mg/m ³ (Jia et al., 2014) Years (humans) ↓ 0.47 [up to 3.94 peaks] mg/m ³ (Costa et al., 2019) <i>Unclear^c (humans)</i> ↑ N/A ^h (≤1 mg/m ³) [years, not months] (Thrasher et al., 1990) <i>Weeks (humans)</i> ↑ 0.51 mg/m ³ (Ying et al., 1999)	Moderate For altered number of B cells (direction of change may differ by exposure levels or duration)

Supplemental Information—Formaldehyde (Inhalation)

Endpoint(s)		No changes observed (<i>high or medium confidence</i> experiments are bolded)		Significant ^a increases or decreases (<i>high or medium confidence</i> experiments are bolded)		Summary conclusion Clarifying notes
		<i>Duration^b</i> (<i>species</i>)	<i>Concentration(s) [notes] (study)</i>	<i>Duration</i> (<i>species</i>) ^b	<i>Concentration(s) [notes] (study)</i>	
	T Cells (Total)	Months (humans) 0.2–0.8 mg/m ³ (Jia et al., 2014) <i>Unclear^c (humans)</i> N/A ^h (≤1 mg/m ³) [years vs. months] (Thrasher et al., 1990)		Years (humans) ↓ 1.6 mg/m ³ (Zhang et al., 2010 ; Hosgood et al., 2013 ; Bassig et al., 2016) Months (humans) ↓ 0.99 [up to 1.69 peaks] mg/m ³ (Ye et al., 2005) Years (humans) ↑ 0.36 [up to 0.69 peaks] mg/m ³ (Costa et al., 2013) Years (humans) ↑ 0.25 mg/m ³ (Aydin et al., 2013) <i>Years (humans)</i> ↓ 0.09–0.68 mg/m ³ (Thrasher et al., 1987) <i>Years (humans)</i> ↓ 0.9 mg/m ³ [indirect: apoptosis] (Jakab et al., 2010) <i>Weeks (humans)</i> ↓ 0.51 mg/m ³ (Ying et al., 1999) <i>Short term (rats)</i> ↑ 7.4 mg/m ³ (Sandikci et al., 2007a, b)		Slight mixed results suggests concentration-dependence, with ↓ at higher levels (possibly ↑ at low levels) with <u>months–years</u> exposure
	T Cells (CD4 ⁺)	Years (humans) 1.6 mg/m ³ [↓ T _{reg}] (Zhang et al., 2010 ; Hosgood et al., 2013 ; Bassig et al., 2016) Months (humans) 0.99 [up to 1.69 peaks] mg/m ³ (Ye et al., 2005) Years (humans) 0.47 [up to 3.94 peaks] mg/m ³ (Costa et al., 2019) Years (humans) 0.25 mg/m ³ (Aydin et al., 2013) Months (humans) 0.2–0.8 mg/m ³ (Jia et al., 2014)		Years (humans) ↑ 0.36 [up to 0.69 peaks] mg/m ³ (Costa et al., 2013) <i>Weeks (humans)</i> ↓ 0.51 mg/m ³ (Ying et al., 1999)		Indeterminate data suggest N/C, but variable, considering also studies of spleen above, suggests effects may exist at CD4 subset level
	T Cells (CD8 ⁺)	Years (humans) 0.25 mg/m ³ (Aydin et al., 2013) Years (humans) 0.36 [up to 0.69 peaks] mg/m ³ (Costa et al., 2013) Months (humans) 0.2–0.8 mg/m ³ (Jia et al., 2014) [N/C CD4/CD8 ratio in 3 studies and (Thrasher et al., 1990)]		Years (humans) ↓ 1.6 mg/m ³ (Zhang et al., 2010 ; Hosgood et al., 2013) Months (humans) ↓ 0.99 [up to 1.69 peaks] mg/m ³ (Ye et al., 2005) Years (humans) ↑ 0.47 [up to 3.94 peaks] mg/m ³ (Costa et al., 2019) <i>Weeks (humans)</i> ↓ 0.51 mg/m ³ (Ying et al., 1999) [↑ CD4/CD8 ratio in all but one of these studies]		Moderate ↓ CD8 and ↑ CD4/CD8 ratio likely related to concentration

Supplemental Information—Formaldehyde (Inhalation)

Endpoint(s)			No changes observed (<i>high or medium confidence</i> experiments are bolded)		Significant ^a increases or decreases (<i>high or medium confidence</i> experiments are bolded)		Summary conclusion Clarifying notes
			<i>Duration^b</i> (<i>species</i>)	<i>Concentration(s) [notes] (study)</i>	<i>Duration</i> (<i>species</i>) ^b	<i>Concentration(s) [notes] (study)</i>	
		NK Cells			<i>Years (humans)</i> <i>Years (humans)</i> <i>Years (humans)</i> <i>Months (humans)</i>	↓ 0.36 [up to 0.69 peaks] mg/m ³ (Costa et al., 2013) ↓ 1.6 mg/m ³ (Zhang et al., 2010 ; Hosgood et al., 2013 ; Bassig et al., 2016) ↑ 0.25 mg/m ³ (Aydin et al., 2013) ↑ 0.2, but not at 0.8 mg/m ³ (Jia et al., 2014)	Slight mixed results suggest role of concentration similar to total T cell findings
		Monocytes	<i>Years (humans)</i> <i>Years (humans)</i> <i>Short term (mice)</i>	1.6 mg/m ³ (Zhang et al., 2010 ; Hosgood et al., 2013 ; Bassig et al., 2016) 0.25 mg/m ³ (Aydin et al., 2013) ≥9.23 mg/m ³ (Morgan et al., 2017)	<i>Years (children)</i> <i>Short term (mice)</i> <i>Short term (mice)</i>	↑ ≈0.02 mg/m ³ [year assumed] (Erdei et al., 2003) ↓ 0.5, but not 3, mg/m ³ (Zhang et al., 2013) ↓ 18.5 mg/m ³ (Dean et al., 1984)	Indeterminate data suggest N/C, at least in human adults
Red Blood Cells			<i>Years (humans)</i> <i>Short term (mice)</i> <i>Years (children)</i> <i>Years (humans)</i>	0.25 mg/m ³ (Aydin et al., 2013) ≥9.23 mg/m ³ (Morgan et al., 2017) ≈0.02 mg/m ³ [year assumed] (Erdei et al., 2003) ≤0.29 mg/m ³ [mean levels] (Kuo et al., 1997)	<i>Years (humans)</i> <i>Years (humans)</i> <i>Short term (mice)</i>	↓ 0.87 mg/m ³ [note: duration] (Lyapina et al., 2004) ↓ 1.6 mg/m ³ (Zhang et al., 2010 ; Hosgood et al., 2013) ↓ 0.5–3 mg/m ³ (Zhang et al., 2013)	Moderate ↓ ⁶ suggests combined role of concentration and duration
Platelets			<i>Years (humans)</i> <i>Short term (mice)</i> <i>Years (children)</i> <i>Years (humans)</i>	0.87 mg/m ³ (Lyapina et al., 2004) ≥9.23 mg/m ³ (Morgan et al., 2017) ≈0.02 mg/m ³ [year assumed] (Erdei et al., 2003) ≤0.29 mg/m ³ [mean levels] (Kuo et al., 1997)	<i>Years (humans)</i> <i>Short term (mice)</i>	↓ 1.6 mg/m ³ (Zhang et al., 2010 ; Hosgood et al., 2013 ; Bassig et al., 2016) ↑ 0.5–3 mg/m ³ (Zhang et al., 2013)	Slight ↓ ⁷ possible concentration dependence similar to above
Secreted factors and immune Primarily Th1-related	t	TNF-α	<i>Years (humans)</i> <i>Months (humans)</i>	1.8 [up to 6.9 peaks] mg/m ³ (Seow et al., 2015) 0.2–0.8 mg/m ³ (Jia et al., 2014)	<i>Years (humans)</i>	↑ 0.25 mg/m ³ (Aydin et al., 2013)	Slight ↑ TNF-α and C3
		Complement	<i>Years (humans)</i>	(C3, C4) 0.25 mg/m ³ (Aydin et al., 2013)	<i>Short term (rats)</i>	↑ (C3) 6.15 mg/m ³ (Sapmaz et al., 2015)	

Supplemental Information—Formaldehyde (Inhalation)

Endpoint(s)			No changes observed (<i>high or medium confidence</i> experiments are bolded)	Significant ^a increases or decreases (<i>high or medium confidence</i> experiments are bolded)	Summary conclusion Clarifying notes
			<i>Duration^b</i> (<i>species</i>) <i>Concentration(s) [notes] (study)</i>	<i>Duration</i> (<i>species</i>) ^b <i>Concentration(s) [notes] (study)</i>	
	Primarily Th2-related	IFN-γ		<i>Months (humans)</i> ↓ 0.8, but not 0.2, mg/m ³ (Jia et al., 2014) <i>Short term (rats)</i> ↓ 6.2–12.3 mg/m ³ (Im et al., 2006)	Moderate ↓ IFN-γ
		IL-4		<i>Months (humans)</i> ↑ 0.8, but not 0.2, mg/m ³ (Jia et al., 2014) <i>Short term (rats)</i> ↑ 6.2–12.3 mg/m ³ (Im et al., 2006)	Moderate ↑ IL-4
		IL-10		<i>Years (humans)</i> ↓ 1.8 mg/m ³ [less strict 20% FDR] (Seow et al., 2015) <i>Months (humans)</i> ↑ 0.2–0.8 mg/m ³ (Jia et al., 2014)	Slight IL-10 <i>Suggestive</i> of concentration role similar to total T and NK cell findings
		IL-6	<i>Acute (mice)</i> 0.12 mg/m ³ (Matsuoka et al., 2010)		Inadequate IL-6
	Chemo-attractants	CXCL11 (IFNγ-related)		<i>Years (humans)</i> ↓ 1.8 mg/m ³ [stringent 10% FDR] (Seow et al., 2015)	Slight ↓ chemoattractants (attracting neutrophils-IL-8, and lymphocytes-Cxcl11, Ccl17)
		CCL17 (Th2-related)			
		IL-8 (neutrophils)		<i>Months (humans)</i> ↓ 0.2–0.8 mg/m ³ (Jia et al., 2014)	
	Other	Ta1		<i>Unclear³ (humans)</i> ↑ N/A ^h (≤1 mg/m ³) [years, not months, change in antigen reactivity markers] (Thrasher et al., 1990)	Indeterminate (data suggest N/C in B cell activation markers)
		IL-2R			
		CD27 and CD30	<i>Years (humans)</i> 1.6 mg/m ³ (Bassig et al., 2016)		

Der f: *Dermatophagoides farina* (house dust mite); OVA: ovalbumin (major protein of chicken egg whites); both are immunogenic materials used to stimulate an allergy-like response.

Gray box = no data meeting the inclusion criteria were available.

Note: one study observing increased substance P and related changes in the serum ([Fujimaki et al., 2004b](#)) is primarily discussed in the context of changes in the URT and LRT.

^aPrimarily, this reflects reporting of a statistically significant change; in rare instances where a *p* value was not given, changes are indicated if the authors discussed the change as a significant effect.

^bHuman study exposure durations are indicated as “years,” “months,” “weeks,” or “acute” and defined based on the anticipated exposure duration for the majority of the exposed population(s); these durations are interpreted to approximate animal study exposure durations of chronic (>1 year), subchronic (several months), short term (<30 days), and acute (1 day or less).

^cThe comparison presented by Thrasher et al. ([1990](#)) reflects differences in exposure duration (years compared to weeks or months), but there appeared to be minimal difference in concentration.

^dThis finding (decreased total WBCs) is supported by 3 studies in humans evaluated by the NRC ([2014](#)) ([Tong et al., 2007](#); [Tang and Zhang, 2003](#); [Cheng et al., 2004](#)), but not evaluated in this analysis; additionally, this finding is supported by a study in mice ([Yu et al., 2014b](#)) and a study in rats ([Brondeau et al., 1990](#)), which are not included as they only tested formaldehyde levels ≥ 20 mg/m³.

^eAuthors indicated no changes in “WBC differentials” other than decreased monocytes, but further details NR ([Dean et al., 1984](#)). This test was assumed to include basic granulocyte and lymphocyte counts.

^fThis finding (decreased erythrocytes) is supported by 1 study in humans evaluated by the NRC ([2014](#)) ([Yang, 2007](#)), but not evaluated in this analysis.

^gThis finding (decreased platelets) is supported by 2 studies in humans evaluated by the ([2014](#)) ([Yang, 2007](#); [Tong et al., 2007](#)), but not evaluated in this analysis, and a mouse study testing excessive formaldehyde levels ([Yu et al., 2014b](#)).

^hThe exposure level is, in general, considered not applicable (N/A), as the comparison presented by Thrasher et al. ([1990](#)) reflected differences in exposure duration (i.e., years of exposure [Yr], as compared to weeks or months [Mo] of exposure), but there appeared to be minimal differences in concentration from the controls.

C.7.4. Integrated Mechanistic Evidence Synthesis by Tissue Compartment

The most likely initial effects of formaldehyde exposure include evidence of direct interactions of formaldehyde with biological macromolecules (e.g., DNA; receptors; redox proteins) in the upper respiratory tract (URT). These direct interactions would typically not be expected to occur in other tissue compartments given the lack of substantial distribution of inhaled formaldehyde to distal sites (see Appendix C.1). While stress hormone increases likely involve prior modification of the hypothalamic-pituitary-adrenal (HPA) axis, *slight* evidence of this change is indicated as a plausible initial effect of exposure due to a general lack of knowledge of the specific type of stressor(s) (e.g., direct responses due to subtle changes in fear or anxiety; indirect effects via sustained inflammation) and the nature of the interactions with the HPA axis that might result from formaldehyde inhalation. The *slight* evidence of indirect evidence for sensory nerve stimulation in the LRT is not indicated as a plausible initial effect of exposure because inhaled formaldehyde is unlikely to reach the LRT in appreciable amounts and it is expected that LRT sensory nerve activation would be reliant on a secondary response to TRP channel-activating stimuli such as increased LRT oxidative stress or inflammatory mediators; although, certain exposure scenarios (e.g., after exposure to high levels of formaldehyde or mouth breathing during exercise, perhaps only in susceptible individuals) might, in rare scenarios, result in distribution of minimal amounts of formaldehyde to upper regions of the LRT (see Appendix C.1) that may be sufficient to induce such receptor-mediated events. Although it is difficult to disentangle the multiple mechanistic events manifested soon after formaldehyde inhalation, it appears that formaldehyde can initiate overlapping events in the URT, including effects at the level of the respiratory epithelial cells and overlying mucociliary layer, as well as at trigeminal nerve endings. While uncertainties remain¹⁹, the effects in the lower respiratory tract (LRT), blood, and other organs are likely secondary to the changes observed in the URT. Figures C-23 and C-24 illustrate the potential relationships between the mechanistic events reported from formaldehyde exposure, based on the more reliable evidence (see Figure C-23) or including evidence that should be interpreted with greater caution (see Figure C-24). These figures are based on evidence summarized in the tables in the prior sections (Appendix C.7.1 to C.7.3), and they are discussed according to tissue compartment below.

Figures C-23 and C-24 (on the following pages) present network summaries of mechanistic data related to potential noncancer respiratory health effects of formaldehyde. These figures present an integrated picture of the mechanistic events identified from studies of formaldehyde exposure. The figures are organized by tissue type or region (i.e., upper respiratory tract, “URT”;

¹⁹Controlled human exposure studies observed pulmonary function deficits when a longer exercise component (15 minutes) was included. These deficits were not observed by other studies with shorter periods or no exercise ([Green et al., 1987](#); [Green et al., 1989](#)), and another study observed airway hyperresponsiveness with an exposure protocol using nose clips requiring mouth-only breathing ([Casset et al., 2006](#)).

lower respiratory tract, “LRT”; “blood”; and other tissues related to immunological responses, “other”), the data for which are summarized in the following subsections. Figure C-23 presents events interpreted with greater confidence (i.e., *robust*, or *moderate* evidence), while Figure C-24 includes events based on *slight* evidence. In both figures, the mechanistic events and the relationships between events are characterized. Lines with arrows on both ends indicate events for which the association appears to be bidirectional. The figures also identify events that are “plausibly an initial effect of exposure,” and each event is related to one or more “key features of a potential hazard” (see explanations in Section 2.5.1 of the Toxicological Review). Note: Some events and relationships are not shown for clarity, but nearly all mechanistic events discussed in Appendix C.7.1 to C.7.3 for which at least *slight* evidentiary support was concluded are presented. Note that “decreased pulmonary function” encompasses a range of possible contributing effects including, but not limited to, increased bronchoconstriction, flow limitation, and decreased bronchodilation.

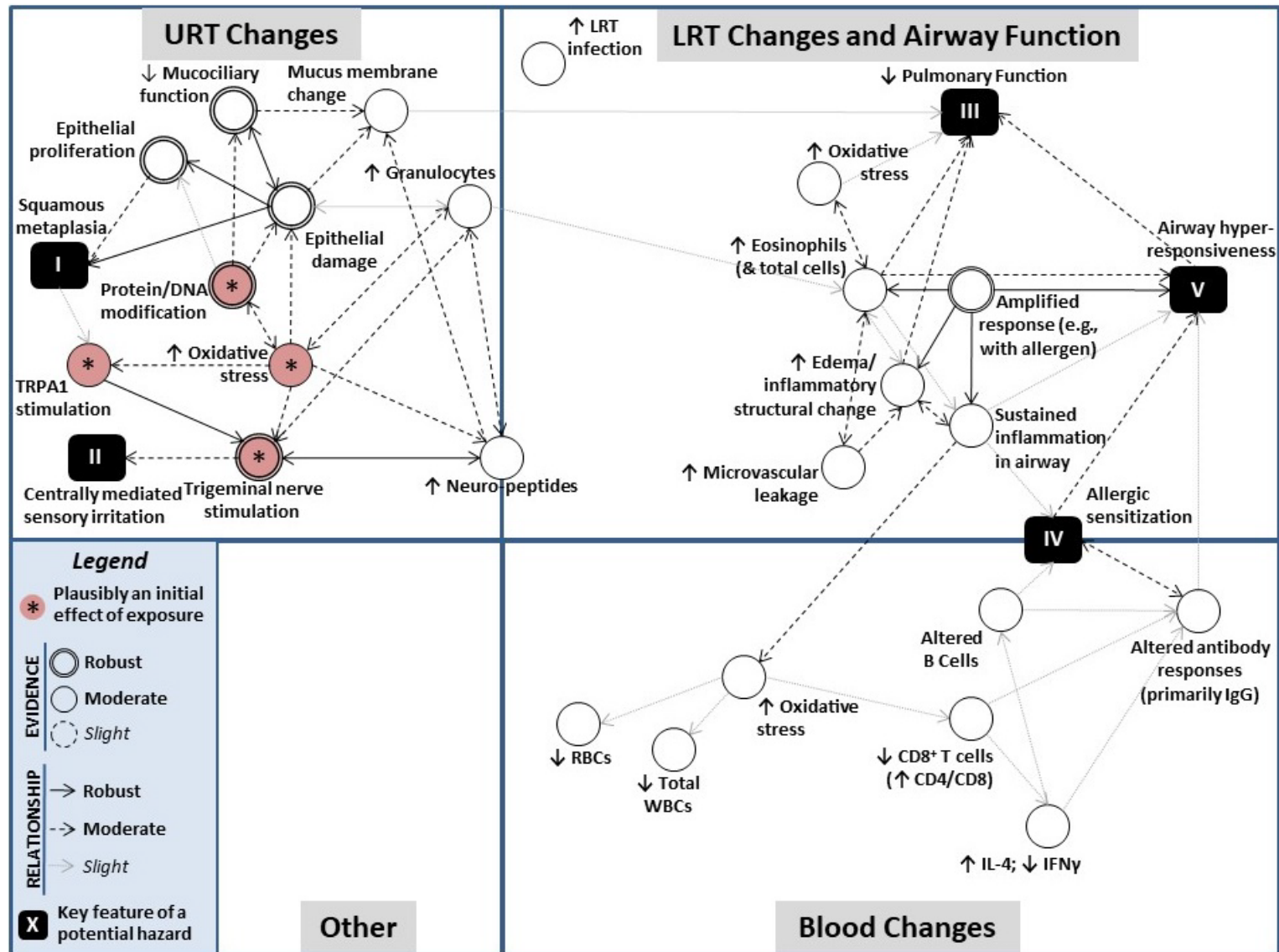


Figure C-23. Mechanistic events for respiratory effects of formaldehyde based on *robust* or *moderate* evidence.

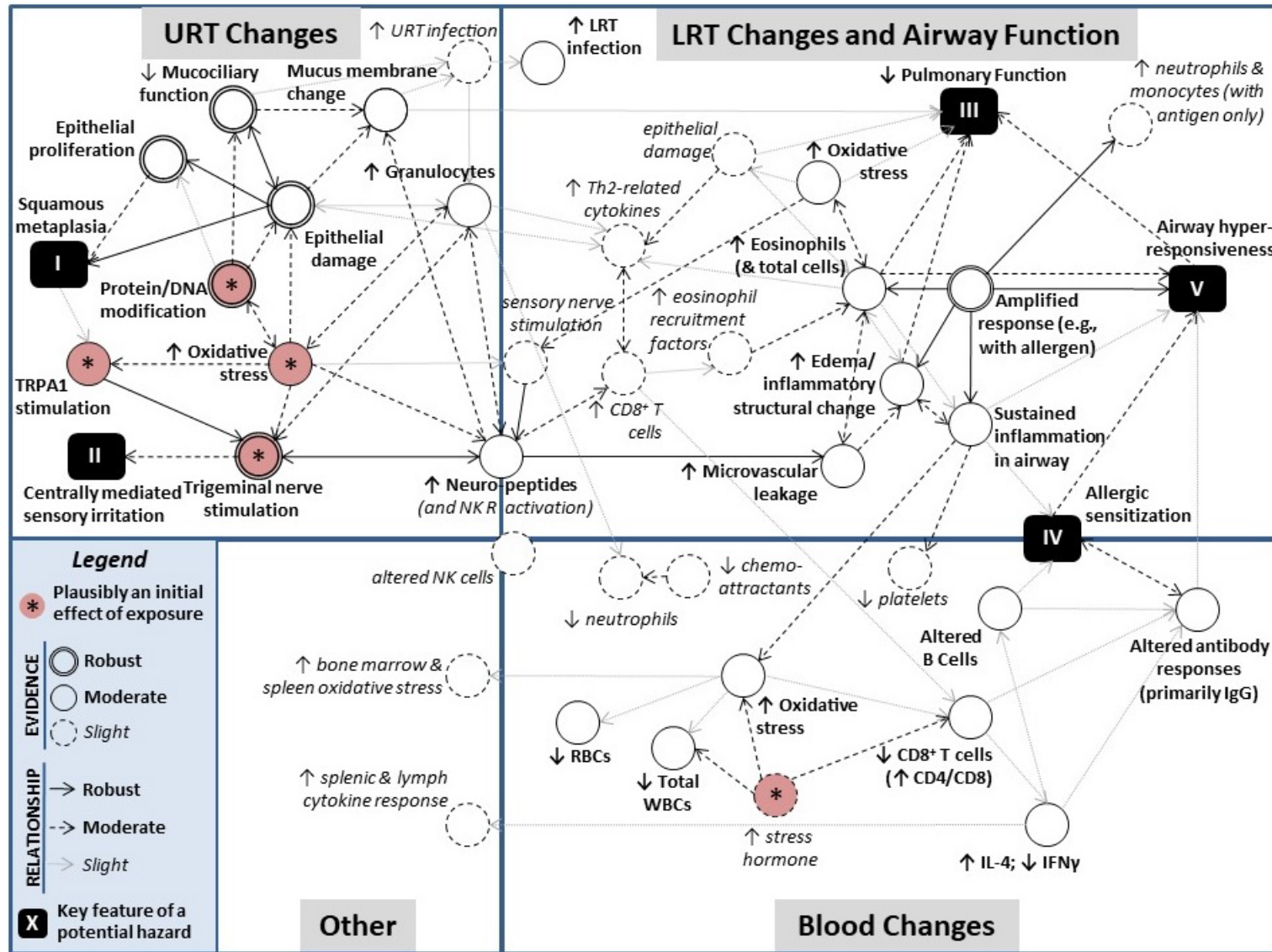


Figure C-24. Mechanistic events for respiratory effects of formaldehyde based on *robust*, *moderate*, or *slight* evidence.

Consideration of mechanistic changes across tissue compartments

Several interesting relationships across tissue compartments are suggested:

- Evidence of increased oxidative stress, in particular, appears to be conserved across each of the evaluated tissue compartments. As soluble inflammatory signals can be transmitted across tissue boundaries with relative ease, it is plausible that these indications of an increased body burden of free radicals may be an indirect consequence of inflammatory changes that could be relatively restricted to the airways.
- Observations of increased eosinophils, and to a somewhat lesser extent, neutrophils, in both the URT and LRT, suggest that the inflammation of the airways caused by formaldehyde exposure is not restricted to the URT sites directly contacted by the majority of inhaled formaldehyde.
- Although some more subtle changes appear to occur in the LRT (e.g., inflammation; altered airway permeability), the data suggest that overt damage to the airway epithelium by formaldehyde exposure is limited primarily to the URT.
- Key features of several potential health hazards appear to involve mechanistic changes occurring within multiple tissue compartments, including decreased pulmonary function and allergic sensitization.
- Although many uncertainties remain, the instances of opposing immune-related responses in the airways compared to those in the blood suggest immunological communication and possible recruitment of cells from one compartment to another. One exception to this pattern was the consistent observation of increased IL-4 in both the LRT and blood. IL-4 is associated with driving CD4+ T cells towards a Th2 response ([Kopf et al., 1993](#)). The evidence specific to changes in CD4+ T cell populations in either compartment were inadequate, limiting interpretations of the significance of this finding.
- While many immune-cell-related changes were observed, some only occurred in specific exposure contexts. For example, neutrophil and monocyte increases in the LRT were observed only with allergen sensitization, while eosinophil increases were not observed in studies of exposure less than several weeks; changes in NK cells and other lymphocytes subsets appeared to vary depending on concentration, and some antibody responses depended on the antigen (e.g., allergen) type and administration methods. In addition, immune system studies after developmental exposure represent a significant data gap.
- In general, the evidence becomes less convincing with increasing removal from the point-of-first-contact for inhaled formaldehyde, with the highest confidence for effects in the URT, slightly less confidence for effects in the LRT and blood, and a general inability to draw conclusions regarding the potential for effects in lymphoid organs.

Plausibility of potential associations between mechanistic changes and respiratory system health effects

Figure C-25 illustrates one or more potential sequences of events from formaldehyde inhalation to apical outcomes (i.e., key hazard features) described in each of the respiratory system

health effects sections in the Toxicological Review. Each of these sequences was developed based on the most reliable mechanistic evidence (i.e., *robust* or *moderate* evidence was preferred) that can plausibly link an initial effect of inhaled formaldehyde to each of these key hazard features, and which have been demonstrated in formaldehyde-specific studies. Thus, these sequences do not represent all possible scenarios for which data exist (see Figures C-23 and C-24 for more comprehensive illustrations), and data not considered in this analysis (e.g., studies of chemicals closely related to formaldehyde) could identify additional initial alterations and mechanistic events, as well as more interim changes or relationships between many of the depicted mechanistic events. As such, this figure may not illustrate the most biologically pertinent sequence of events, but it does illustrate biologically plausible pathways of effects based on data specific to formaldehyde exposure. Thus, this is a pragmatic attempt to link early mechanistic events with apical endpoints, similar to the AOP conceptual framework ([Villeneuve et al., 2014](#); [Ankley et al., 2010](#)). For each sequence, an interpretation regarding the likelihood of the presented sequence of events being a mechanism by which formaldehyde inhalation could cause respiratory system health effects is provided in the section below. As these interpretations are based on the robustness of the available evidence, they are primarily based on confidence in the individual studies and the consistency and coherence of observations across species and experimental paradigms. Other considerations outlined by Sir Bradford Hill ([1965](#)), including the magnitude and dose-dependency of the individual study findings, are discussed where the data are available, but these considerations generally had less of an impact on interpretations. This section references evidence conclusions from previous sections, as well as studies supporting biological understanding, but individual formaldehyde-specific studies are generally not referenced.

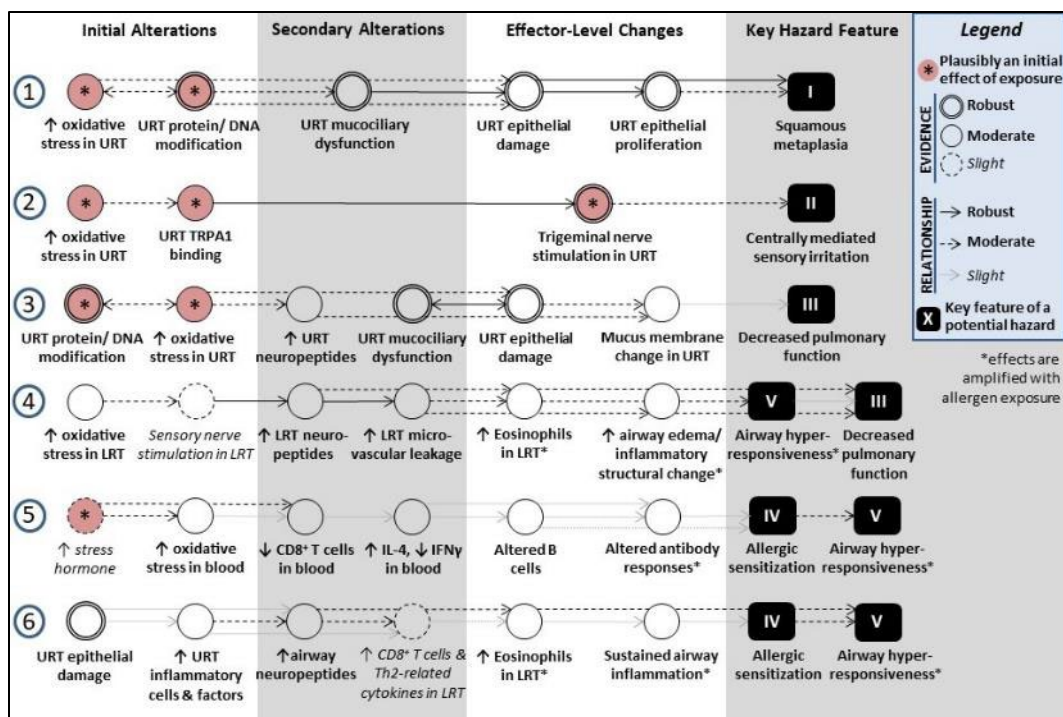


Figure C-25. Possible sequences of mechanistic events identified based on the most reliable evidence available.

This figure presents plausible mechanistic pathways illustrating the most reliable formaldehyde exposure-specific data (i.e., *robust* or *moderate* evidence was preferred) based on currently available information. The figure is organized by respiratory system health effect represented by key features of each hazard evaluated in the Toxicological Review. The pathways interpreted to most plausibly link possible initial effects of formaldehyde exposure to these apical events is presented, based on both the confidence in the relationships between events and confidence in the evidence for each of the linked mechanistic events. These pathways²⁰ are organized in a linear fashion from initial event(s) to key hazard feature(s), and each pathway is numbered, corresponding to the synthesis that follows. The mechanistic events are grouped into “initial events” and “secondary events” for endpoints that would be expected to occur earlier and later, respectively, along a sequential mechanistic progression. Generally, for the “initial” events, a preceding or precursor event other than a direct interaction with formaldehyde is unknown or has not been studied following formaldehyde exposure, or they have been described in previous pathways (e.g., see #6). “Effector-level changes” are those events that are most likely to be directly associated with the apical endpoint(s) of interest. The symbols, descriptors, and arrows are the same as those depicted in Figures C-23 and C-24.

²⁰ This approach draws some parallels to the AOP conceptual framework approach (Villeneuve et al., 2014; Ankley et al., 2010). As such, for those familiar with AOP terminology, it may be useful to think of the terms used herein according to related AOP terms (e.g., “plausible initial effects of exposure” and “initial alterations” relate to “molecular initiating events”; “mechanistic events” relate to “key events”; and “key hazard features” relate to “adverse outcomes”).

1) Respiratory tract pathology (squamous metaplasia) through epithelial cell damage

Interpretation: This is likely to be a major mechanism by which formaldehyde inhalation could cause squamous metaplasia.

Consistent with its known chemistry and reactivity, formaldehyde has been shown to react with DNA and other biological macromolecules at the point of first contact in the URT, where it also affects tissue redox capacity, presumably either through direct interactions with cellular macromolecules (e.g., lipids) or indirectly by impacting local tissue detoxification processes. These initial reactions have been shown to occur following acute and short-term exposure at concentrations $<0.5 \text{ mg/m}^3$, and generally, the magnitude of these effects is expected to be driven largely by formaldehyde concentration and distribution. Distribution of formaldehyde-induced nasal lesions progresses to more posterior locations with chronic exposure; presumably, this represents changes in formaldehyde deposition, although this has not been tested. Additionally, studies have not been performed to address whether long-term exposure may overcome the body's capacity to regulate or restrict the magnitude of these changes. Elevated oxidative stress could directly lead to cytotoxic or subcytotoxic epithelial cell damage and/or dysfunction through the modification of cellular proteins and DNA. Because similar endogenous defense mechanisms (e.g., glutathione) are responsible for the detoxification of some free radicals and formaldehyde, persistent oxidative stress may make these cells more prone to damage directly resulting from formaldehyde and other inhaled agents. DNA-protein crosslinks (DPXs), which have been observed at formaldehyde concentrations $\geq 0.3 \text{ mg/m}^3$ (rats) or $\geq 0.9 \text{ mg/m}^3$ (rhesus monkeys) and durations ≥ 3 hours (see Appendix C.3), can lead to cellular damage if they are not repaired. Formaldehyde can modify the structure and function of the mucociliary apparatus, potentially as a result of covalent modification of soluble factors in the mucus ([Morgan et al., 1984](#)) or ciliary proteins ([Hastie et al., 1990](#)). Studies of the mucociliary apparatus following acute exposure provide evidence for a concentration threshold for functional effects, again highlighting the importance of formaldehyde concentration and distribution. In rats, DPXs and regions of mucociliary dysfunction have both been demonstrated to correlate with locations of subsequent respiratory tract pathology and cell proliferation in the anterior portions of the nasal mucosa following formaldehyde exposure. The resultant, potentially adaptive, effects on cellular proliferation (i.e., hyperplasia) are typically dose- and duration-dependent and localized to regions of mucociliary dysfunction and epithelial damage. Cellular proliferation may be initiated, at least in part, in response to formaldehyde exposures not associated with histopathological evidence of epithelial cell damage, since some studies report effects on proliferation at $\approx 1 \text{ mg/m}^3$. Direct and overt epithelial cell damage or death associated with squamous metaplasia is not typically observed until formaldehyde concentrations are above 2 mg/m^3 . Squamous metaplasia is also localized initially to these high-flux, anterior regions, but these lesions increase in severity and advance to more posterior locations with longer exposure. Thus, although some early mechanistic events in this pathway are expected to be highly dependent on

formaldehyde concentration, the data supports a role for both exposure duration and concentration in the development of long-term lesions such as squamous metaplasia.

All of the events in this mechanism are based on *robust* or *moderate* evidence, with *robust* or *moderate* evidence for interactions between events, indicating that this mechanism is likely a major mechanism by which formaldehyde inhalation can cause squamous metaplasia. However, because modification of epithelial cell health and function in the URT can occur via multiple direct and indirect mechanisms following formaldehyde inhalation, which are expected to vary due to differences in both exposure duration and intensity, there are likely to be other important mechanisms by which formaldehyde exposure could cause respiratory tract pathology.

2) Sensory irritation through trigeminal nerve stimulation

Interpretation: This is likely to be the dominant mechanism by which formaldehyde inhalation could cause sensory irritation.

With distribution throughout the nasal mucosa, trigeminal nerve endings are well positioned for direct interactions with inhaled formaldehyde. Trigeminal nerve activation at unmyelinated C fibers occurs following acute formaldehyde exposure and the resultant physiological sensation of burning is known to be caused by afferent signaling to the CNS ([Mackenzie et al., 1975](#)). This afferent nerve activity has been demonstrated following formaldehyde inhalation. Based primarily on indirect evidence (e.g., ex vivo models), activation of the trigeminal nerve is probably at least partly dependent on direct activation of TRPA1 channels by formaldehyde (e.g., via binding). Further support for an “irritant receptor” response to formaldehyde exposure is provided by evidence of competitive inhibition of irritation caused by chlorine and acetaldehyde ([Chang and Barrow, 1984](#); [Babiuk et al., 1985](#)). However, other direct actions of formaldehyde at trigeminal nerve endings (e.g., binding to other receptors; modification of ion balance; protein modification) are possible and some other potential pathway scenarios are suggested. In addition, oxidative stress, such as that elicited in the URT by formaldehyde exposure, is known to activate TRP channels ([Bessac and Jordt, 2008](#)), providing another plausible indirect mechanism. Based on the proposed sequence of events, sensory irritation would be expected to be highly variable across individuals due to differences in TRPA1 channel sensitivity or access of formaldehyde to TRPA1 channels (e.g., due to differences in airway structure, mucus production, or TRPA1 channel density). Studies of related chemicals suggest that human sensitivity may also be dependent on demographic factors such as age, sex (women appear to be more sensitive), and allergy status ([Shusterman, 2007](#); [Hummel and Livermore, 2002](#)).

The threshold for activation of exposed rodent nerve endings has been reported at 0.31 mg/m³ formaldehyde. The levels necessary for in vivo activation following acute exposure may be somewhat higher. Although trigeminal nerve activation may worsen with constant, repeated exposure to low levels of formaldehyde, as has been demonstrated for other chemicals ([Brand and Jacquot, 2002](#)), constant exposure or high concentrations could conversely desensitize this

response by excessively stimulating the (presumed) irritant receptors. The potential for sensory irritation to attenuate over time due to processes such as desensitization (e.g., via internalization of TRPA1 receptors) is unclear, particularly with long-term exposure. Indirect evidence suggesting either the presence of extremely sensitive individuals in the population or a role for the duration of exposure in eliciting this effect is provided from residential studies identifying symptoms associated with sensory irritation at levels as low as 0.1 mg/m³ ([Zhai et al., 2013](#); [Liu et al., 1991](#); [Hanrahan et al., 1984](#)). Structural changes to the URT tissue (e.g., formaldehyde-induced modification of the epithelial cell layer altering accessibility of sensory nerve endings) and to the URT response of local immune cells (i.e., inflammatory cells may release mediators which can stimulate proliferation and/or sensitization of sensory nerve fibers ([Carr and Undem, 2001](#))) would be expected to be strong modifiers of this effect, introducing an exposure duration component to the concentration-dependence of receptor binding that is assumed for activation of TRPA1.

A strong biological understanding exists to identify the physiological sensation of sensory irritation as being related to stimulated sensory fibers of the trigeminal nerve. While the specific concentration and duration dependency of activation remain incomplete, based on the *robust* and *moderate* formaldehyde-specific evidence available to support activation of trigeminal nerve fibers and stimulation of TRPA1 receptors, respectively, along with a general lack of alternative explanations for chemical-induced sensory irritation, this mechanism is likely the dominant mechanism by which formaldehyde exposure can cause sensory irritation.

3) Decreased pulmonary function through URT epithelial damage

Interpretation: This is a possible mechanism by which formaldehyde inhalation could contribute to decreases in pulmonary function, but this is not a major pathway explaining this potential effect, and other changes are expected to be the primary drivers of any substantial functional changes.

Airway epithelial cells not only serve as a physical barrier to inhaled pathogens and antigens, they also participate in the regulation of airway inflammatory responses ([Holgate et al., 1999](#)). The demonstrated modification of the respiratory epithelium in the upper airways by formaldehyde exposure may affect pulmonary function through both physical, and humoral mechanisms, although definitive studies for the latter have not been conducted and such factors are generally tightly controlled and locally acting (e.g., [Mayer and Dalpke \(2007\)](#)). Modification to the URT epithelium by formaldehyde, particularly the observed effects on mucociliary function, is also likely to modify URT barrier and clearance processes, which could increase the impact of other inhaled antigens on pulmonary function; however, this possibility has not been well-studied. Physically, swelling of the mucus membrane has been observed in exposed humans at <1 mg/m³ formaldehyde, and this is expected to be highly influenced by the underlying respiratory status of the exposed individuals (e.g., allergy status; previous and/or current respiratory infections; etc.). This swelling can plausibly be linked to narrowing of the airways and impaired pulmonary function,

although this linkage has not been explicitly demonstrated by corresponding effects in the LRT following formaldehyde exposure and it is unclear to what extent URT swelling would need to progress before effects on lung function were experienced. Morphological changes in the mucous membrane can be related to changes in mucus secretion and, possibly, epithelial cell proliferation ([Reader et al., 2003](#)), both of which are observed following formaldehyde exposure. Dysfunction of airway epithelial cells can also modify their release of humoral factors, which help to regulate airway smooth muscle contraction and immune cell responses. For example, epithelial cells can release neutral endopeptidase, which is the major metabolizing enzyme for tachykinins such as substance P and neurokinin A ([Barnes, 1992](#)), and they are known to produce situation-specific signals that can either promote or inhibit the activity of local immune cells, including dendritic cells, which contribute to airway remodeling ([Lambrecht and Hammad, 2012](#)). In these ways, modification of the function of URT epithelial cells by formaldehyde exposure might result, in an indirect manner, in changes in humoral factors that could reach the lower airways and lungs in minimal amounts. However, direct formaldehyde-specific examinations of such potential associations, including the requisite exposure parameters (e.g., levels), were not identified.

This sequence of events can plausibly link structural damage and dysfunction of the epithelium in the URT to potential decrements in pulmonary function. However, a large amount of missing information, particularly regarding LRT changes, is assumed, and evidence linking these formaldehyde-induced mechanistic events in the URT to changes in pulmonary function has not been reliably demonstrated. While these events might contribute to some minimal level of decrease in pulmonary function, the data are insufficient to identify this sequence of events as a major mechanism.

- 4) Airway hyperresponsiveness and/or decreased pulmonary function through LRT inflammatory changes resulting from sensory nerve activation

Interpretation: This is likely to be an incomplete mechanism by which formaldehyde inhalation could cause airway hyperresponsiveness and decreased pulmonary function, although whether certain events occur at low exposure levels is unclear.

Activation of airway sensory nerve endings is known to cause the release of neuropeptides, including substance P. Short-term formaldehyde exposure appears to cause increases in substance P, and perhaps other neuropeptides, in the lower airways. In addition, several lines of evidence identify potential substance P-related changes in the LRT that are at least partially dependent on TRP channel activation. As discussed previously, while certain, very rare human exposure scenarios might result in weak activation of the vagus nerve in proximal regions of the LRT (e.g., the trachea) due to direct interactions with formaldehyde, it is expected that the predominant explanation (and that most relevant to interpretations) for activation remains unidentified and involves indirect pathway(s). One possible explanation involves indirect activation of LRT sensory nerve endings in association with the formaldehyde exposure-induced increases in LRT oxidative stress and/or

inflammation, as certain electrophilic oxidative byproducts and inflammatory factors can stimulate TRPA1 channels ([Taylor-Clark et al., 2008](#); [Andersson et al., 2008](#)). Alternatively, substance P could also be directly released from certain subsets of activated immune cells, including eosinophils ([Joos et al., 2000](#)), which are increased in the LRT, although this hypothesis has not been examined and may be somewhat less plausible, given the apparent discrepancy in the exposure duration required for substance P increases versus LRT eosinophil increases in the available studies. Regardless, any indirect pathway(s) would require prior modification of the LRT microenvironment after formaldehyde exposure through a separate, undefined mechanism.

Locally, substance P can cause vasodilation and leakage or constriction of airway smooth muscle, the latter of which appears to be enhanced in asthmatics (who also exhibit elevated substance P-immunoreactivity in airway nerves; ([Ollerenshaw et al., 1991](#)), all of which can contribute to airway narrowing or obstruction ([Joos et al., 1994](#); [Joos et al., 1995](#)). It should be noted that airway obstruction typically requires much higher doses of agonist than does leakage (e.g., ([Yiamouyiannis et al., 1995](#))). Formaldehyde-induced increases in substance P contribute to microvascular leakage in the LRT (i.e., trachea and main bronchi) following acute formaldehyde exposure, which has been observed at >1 mg/m³. Specifically, although the effects of prolonged exposure were not examined, at higher formaldehyde levels (i.e., >10 mg/m³) and with acute exposure, microvascular leakage was blocked by inhibition of the neurokinin 1 (NK₁) receptor, and perhaps also by inhibiting mast cell activation, but not by inhibition of histamine, cyclooxygenases, or bradykinin. Substance P is the preferred substrate for NK₁ receptors. Although activation of NK₁ receptors can contribute to structural changes in human airways, these receptors are more commonly associated with increases in airway inflammation ([Schuiling et al., 1999](#)). As introduced above, NK₁ receptors are also implicated in establishing the successful recruitment and adhesion of eosinophils and neutrophils to inflamed airways ([Baluk et al., 1995](#)), at which point these cells can release bronchoconstrictors. Thus, the increase in LRT eosinophils observed following formaldehyde exposure (and the *slight* evidence for increased neutrophils with allergen sensitization) could be related to elevated substance P. In addition, substance P itself can increase the responsiveness of the airways to bronchoconstrictors ([Cheung C et al., 1994](#)). Thus, either directly, or indirectly, the release of neuropeptides, presumably from stimulated sensory nerve endings, could result in airway hyperresponsiveness. Perhaps relatedly, possible consequences of increased microvascular leakage and inflammation include airway edema and related structural changes, which have been reported following short-term formaldehyde exposures ranging from >0.3 to >3 mg/m³ across studies, although these events have not been experimentally linked to sensory nerve stimulation or substance P signaling. Taken together, it is plausible that substance P-mediated inflammatory alterations to the lower airways, were they of sufficient severity, could also lead to decreases in pulmonary function.

Several notable uncertainties exist for this plausible mechanistic pathway. As discussed above, an understanding of the sequence of events preceding the observed changes in the LRT

remains largely incomplete. In addition, and perhaps most importantly, while most of the evidence is *moderate*, the data are based almost exclusively on acute or short-term experiments. Similarly, while evidence for some events at low formaldehyde levels (e.g., $<1 \text{ mg/m}^3$) exists, some of the more convincing associations, including the requirement of NK₁ receptor activation for microvascular leakage, have only been tested at very high formaldehyde concentrations (e.g., $>10 \text{ mg/m}^3$). Taken together, these limitations raise uncertainties for the relevance of this specific pathway to chronic, low-level exposure scenarios. Further, several important events related to this pathway have not been well studied. For example, the available studies have not examined the potential for sensory nerve activation to modify smooth muscle tone (e.g., regulation of contractile responses through the electrical activity; release of factors with direct action on smooth muscle cells, such as acetylcholine), and information does not exist to ascertain whether NK₂ receptor activation by neurokinin A, which can be a more potent bronchoconstrictor than substance P ([Kraneveld et al., 2002](#)), might be involved. Also, while substance P can stimulate mast cell degranulation and release of bronchoconstrictors such as histamine ([Suzuki et al., 1995](#); [Lilly et al., 1995](#)), in vivo evidence of changes in mast cells was not identified. However, given the recruitment of other immune cells to the airways after formaldehyde exposure, an event that can be mediated by mast cells ([Dawicki and Marshall, 2007](#)), data on mast cells may represent critical information that is missing from the present analysis. Overall, based on the consistent *moderate* evidence for changes in the LRT that are commonly associated with changes in pulmonary function and airway responsiveness, this incomplete sequence of events is likely one of the mechanisms by which formaldehyde exposure could cause airway hyperresponsiveness and decreased pulmonary function. However, the pertinence of some or all of the components in this pathway with long-term, low-level formaldehyde exposure is unknown, and it is considered likely that other important mechanistic events would be identified with additional studies, particularly those testing longer exposure durations. It remains unclear how directly translatable this pathway, based largely on animal data, might be to interpreting complex human diseases such as asthma, and notable events thought to be important to the development or progression of asthma have not been observed.

5) Allergic sensitization and airway hyperreactivity through altered antibody-related responses in the blood

Interpretation: It is unclear whether this is a possible mechanism by which formaldehyde inhalation could cause these effects, as an understanding of the potential mechanistic relationships is incomplete.

Many reactive oxygen and nitrogen species (ROS, RNS) can be essential immunomodulatory signaling molecules. However, prolonged, or excessive exposure to these factors can modify the structural and functional integrity of a wide range of cell and tissue types. Elevated indicators of oxidative stress have been identified in nearly all tissues examined following formaldehyde exposure, including the blood. In the blood of exposed humans, formaldehyde concentrations as

low as 0.1 mg/m³ have been shown to cause lipid peroxidation in peripheral immune cells, typically with prolonged exposure. The data are not available to demonstrate what might be causing this increase in free radicals, although factors released into the circulation as a result of pronounced or sustained airway inflammation would be expected to be capable of causing such an effect.

Specifically, regarding the elevated corticosterone levels, which have been reported in rats exposed for several weeks to much higher formaldehyde levels (3 mg/m³), an excess of glucocorticoids is typically associated with the inhibition of T cell cytokine secretion and function, although they may more specifically enhance the Th2 lineage and suppress the Th1 lineage ([Taves and Ashwell, 2020](#); [Elenkov, 2004](#)). However, the varied roles for stress hormones (and free radicals) in the regulation of immune responses are complex ([Glaser and Kiecolt-Glaser, 2005](#)). Formaldehyde-specific studies examining the dynamics of this potential interplay were not identified.

Immunomodulatory effects of circulating stress hormones (and free radicals) could plausibly be associated with changes in circulating immune cells. As previously mentioned, although formaldehyde-induced changes in circulating immune cells were consistently observed, they varied in magnitude and direction across studies, suggesting a complex regulatory mechanism(s) for these effects. For example, decreases in CD8⁺ T cells were primarily observed in the blood of individuals exposed to higher levels of formaldehyde (>0.5 mg/m³), but not in studies testing lower exposure levels for comparable durations. CD8⁺ T cells are composed of five subpopulations with numerous roles for both cell-mediated immunity and Th2-mediated allergies ([Mittrücker et al., 2014](#)). However, the majority of formaldehyde-specific studies evaluating T cell responses did not distinguish subpopulations of CD4⁺ or CD8⁺ T cells, since a number of these subpopulations have only recently been discovered, and some studies only assessed total T cells (see Table C-35). This complicates interpretations of these responses and raises the possibility that more consistency in changes across studies may exist for specific T cell subpopulations. Perhaps more importantly, the evidence for changes in CD4⁺ T cells, which would be highly informative to this analysis as they are viewed as critical to the development of hypersensitivity ([Cohn et al., 2004](#)), was mixed and uninterpretable. Stimulated CD8⁺ T cells produce IFN- γ , providing a plausible linkage between the decreases in CD8⁺ T cells and the decrease in IFN- γ at >0.75 mg/m³ formaldehyde in several studies. The observed increase in IL-4 at similar formaldehyde levels is more complicated, as its regulation is tightly controlled and likely to be mediated by multiple mechanisms. B cell proliferation and production of IgE and certain IgG subtypes is dependent on IL-4 and inhibited by IFN- γ ([Paul et al., 1987](#)), providing support for a relationship between these cytokine changes and altered IgG-related responses. The evidence of alterations in the number of B cells, as well as the potential relationship between B cell levels and Ig levels, would benefit from additional study.

Understanding the regulation and function of IgE and IgG responses continues to evolve. IgE has a clear role in the development of allergic diseases that affect the airways, including allergic asthma, although IgE may not always be essential (e.g., in other types of asthma; in other allergic

disorders). In contrast, IgG responses are poorly understood. While IgG may help to exacerbate IgE responses (e.g., patients with increases in both IgE and IgG are at greatest risk for developing allergic responses) and IgGs alone might induce allergic reactions to certain antigens ([Wu and Zarrin, 2014](#); [Williams et al., 2012](#); [Finkelman, 2007](#)), an excess of IgG antibodies can prevent IgE-mediated hypersensitivity and persons with increases in IgG alone are not typically at increased risk for allergic-related responses ([Williams et al., 2012](#); [Strait et al., 2006](#); [Pandey, 2013](#)). The evidence from formaldehyde-specific studies is insufficient to clarify whether IgE-mediated responses are involved (i.e., the evidence was considered *slight*, and was generally mixed and inconclusive), nor is it clear that changes in IgG are related to the development of sensitization or airway hyperresponsiveness. Further clarification of the observed IgG changes is also necessary, as some of the changes noted in response to formaldehyde exposure may depend on the duration of exposure or the specific IgG subtype examined. The antibody-related responses discussed herein have only been measured in the blood, as compared to samples that might be more directly informative to immune responses in the airways (e.g., nasal lavage or BAL). This is a notable data gap, given the somewhat disparate findings regarding immune cell counts in the airways and the blood. Overall, there are still critical uncertainties in the formaldehyde-specific antibody data.

In typical allergic disorders, changes in CD4⁺ Th2 cells are present and are thought to play a prominent role, whereas CD8⁺ T cell responses are generally lacking. Similarly, although IgG might contribute to allergic sensitization, the prototypical antibody response in allergy is thought to be largely driven by IgE. While it is possible that formaldehyde exposure may cause sensitization-related responses through a predominant IgG response rather than through IgE, the data demonstrating or proving such a linkage are not currently available. Overall, the available formaldehyde-specific studies do not provide information sufficient to disentangle the complex interplay between CD4⁺ and CD8⁺ T cells and B cells, regulatory cytokines such as IL-4, and the IgG and IgE responses that might underly the potential for formaldehyde to induce the interrelated immune effects of allergic sensitization and airway hyperresponsiveness.

Overall, the potential sequence(s) of events that may underly the observed changes in circulating antibodies remains poorly defined. Further, although a linkage between IgG responses and hypersensitivity is plausible, additional clarification is needed regarding the potential role for these types of changes in the pathogenesis of airway disease. Thus, based largely on an incomplete understanding of the necessity and ability of changes in IgG to induce these responses, and a lack of convincing formaldehyde-specific evidence demonstrating changes in IgE, it is unclear whether this is a possible mechanism by which formaldehyde exposure might cause these immune effects.

- 6) Airway hyperresponsiveness and allergic sensitization through airway eosinophilia and/or sustained airway inflammation

Interpretation: This is a likely a mechanism by which formaldehyde inhalation could cause airway hyperresponsiveness in those sensitized to allergens, although additional unidentified

events are expected to contribute. It is also a possible mechanism by which formaldehyde inhalation could cause airway hyperresponsiveness in nonsensitized individuals. Whether this mechanism is useful for explaining the development of allergic sensitization is unclear.

A number of studies demonstrate that short-term formaldehyde exposure, and possibly longer-term exposure (the data are sparse), can cause an increase in eosinophils in both the upper and lower airways, particularly in animals sensitized to allergens. As previously mentioned, an understanding of how this recruitment occurs remains unclear. Although specific events proving a linkage have not been demonstrated, other formaldehyde-specific observations may be associated with this change. For example, airway epithelial cells, which are modified as a result of formaldehyde exposure, can release immuno-stimulatory factors, including the Th2 cytokines, IL-4 and IL-13, when exposed to allergens ([Li et al., 1999](#)). While changes in IL-4 have been noted in the LRT and could plausibly be related to altered epithelial cells mediating recruitment of eosinophils, the more important, and thus more convincing, evidence of such a linkage would involve increases in IL-3, IL-5, IL-13, GM-CSF, and/or eotaxin ([Wang et al., 2007a](#); [Trivedi and Lloyd, 2007](#); [Jacobsen et al., 2014](#)); however, the formaldehyde-specific evidence related to these latter factors is limited and generally inconsistent. Alternatively, eosinophil recruitment could be related to increased neuropeptide release from stimulated sensory nerve endings, as previously discussed. Bidirectional communication exists between sensory nerve endings and immune cells of the airways, and neuropeptide release can be enhanced by various cytokines and neurotrophins, including nerve growth factor (NGF) ([Nockher and Renz, 2006](#)). NGF, which can also induce mast cell degranulation and shift T cells towards a Th2 response ([Mostafa, 2009](#); [de Vries et al., 2001](#)) and drive antigen-induced and tachykinin-mediated increases in inflammatory cells such as eosinophils ([Quarcoo et al., 2004](#)), may also be modified in the airways following formaldehyde exposure ([Fujimaki et al., 2004b](#)) (not shown in Figures C-23 and C-24). Specifically, regarding eosinophils, released neuropeptides such as substance P have been shown to prime eosinophils for chemotaxis by other factors such as leukotrienes or IL-5, and these neuropeptides can induce accumulated eosinophils to release factors associated with cellular activation, such as eosinophil cationic protein ([Kraneveld and Nijkamp, 2001](#)). Similar to the lack of evidence supporting a linkage with altered epithelial cell function, formaldehyde-specific data are not available to inform such potential linkages. Indirectly, neuropeptide release could also be associated with facilitating the recruitment of eosinophils to the airway by increasing the permeability of the microvasculature, although this evidence still fails to identify the immuno-attractant stimuli. Given the gaps in these linkages, it is likely that this sequence of events is incomplete. Of specific note, evidence of changes in CD4⁺ Th2 cells in the LRT would be expected for each of these potential scenarios leading to eosinophil recruitment, as these cells release factors such as IL-5 and are known to aid eosinophil recruitment in multiple experimental scenarios ([Trivedi and Lloyd, 2007](#); [Hogan et al., 1998](#)).

Regardless of the mechanism of recruitment, the evidence indicates that airway eosinophils are increased by formaldehyde exposure, and activated eosinophils are known to affect airway

contractile responses. Thus, even a short-lived increase in eosinophils could increase bronchoconstriction (e.g., through the release of mediators such as leukotrienes, major basic protein and M2 receptor antagonists, and through the activation of other immune cells such as mast cells and basophils, all of which can act on smooth muscle). However, the relationship of increased eosinophils to airway hyperresponsiveness or allergic sensitization to nonspecific stimuli is more complicated and depends on a combination of factors, many of which the formaldehyde-specific data do not address. For example, the longevity of this eosinophilic response following formaldehyde exposure, particularly in healthy individuals, remains unclear. Short-term eosinophil effects on pulmonary function with subsequent clearance of these cells from the airways would be unlikely to lead to prolonged hypersensitivity of the airways, which would be expected to involve persistent activation of these cells and continued production of pro-inflammatory mediators. A single animal study suggests that eosinophils persist with subchronic formaldehyde exposure at 2.3 mg/m³ (but not at ≤ 0.5 mg/m³) in animals sensitized to allergen ([Fujimaki et al., 2004b](#)), and other indirect evidence indicates that inflammation of the airways persists with long term formaldehyde exposure, particularly in those sensitized to allergens (see Appendix C.7.2). However, it remains unknown whether these latter findings reflect the involvement of the populations of immune cells and secreted factors believed to be critical to the development of airway hyperresponsiveness. As previously described, the evidence examining the involvement of other important immunomodulatory events expected to affect airway responsiveness and allergic sensitization, including activation of basophils and mast cells, recruitment and/or development of a Th2 phenotype in CD4⁺ T cells, evidence of remodeling²¹ in the bronchi and/or alveoli, and changes in secreted factors known to affect smooth muscle reactivity, is generally *slight* or *inadequate*. These represent important data gaps.

Some experimental animal studies also report data suggesting increases in CD8⁺ T cells in the LRT at very high levels of formaldehyde (>5 mg/m³) with short term exposure. Similar to the observed LRT increases in eosinophils, the mechanism(s) mediating this recruitment to the airways is unknown, but likely to be downstream of formaldehyde-induced changes to epithelial cells and/or sensory nerve fibers. The observation of this change alongside the *moderate* evidence of decreases in CD8⁺ T cells in the blood, generally suggesting a threshold for this effect around 0.5 mg/m³, is of interest (note: similar trends in changes in other cells populations, including NK cells, were also observed). Recruitment of lymphocytes to inflamed airways from the blood in response to acute insults is assumed for multiple respiratory disorders ([Medoff et al., 2005](#)) and has been demonstrated with different pathogenic stimuli, including exacerbation of asthma or COPD by rhinovirus infection ([Message et al., 2008](#); [Mallia et al., 2014](#)). In these models, rhinovirus challenge

²¹ “Airway remodeling” has a specific meaning in human airway disease (see ([Bergeron and Boulet, 2006](#))). Several formaldehyde-specific animal studies defined the observed airway structural changes as remodeling (e.g., ([Wu et al., 2013](#); [Qiao et al., 2009](#); [Liu et al., 2011](#))). Although the studies’ data may relate to some aspects of airway remodeling, they are more generally described herein as inflammatory histologic changes to avoid misinterpretation.

generally causes an increase in BAL cells, including eosinophils and CD8⁺ lymphocytes (and possibly neutrophils), while cell counts in the blood, including CD4⁺ and CD8⁺ T cells (and possibly NK cells) are decreased. In these types of studies, the specific relationship and magnitude of these changes appears to depend on the “dose” (e.g., viral load), as well as the sequence of pathology (e.g., viral challenge in symptomatic individuals). While the exact mechanisms underlying these complementary changes are unclear, hypotheses include modifications to epithelial cell function that leads to exaggerated immune responses in the absence of cytotoxicity ([Proud and Leigh, 2011](#); [Gavala et al., 2013](#)). Thus, some of the observed airway inflammatory responses could be mediated through a sequence of events resulting from recruitment of certain immune cell populations from the blood to the airways, which may be directly relevant to changes observed in acutely challenged humans with airway disorders.

Overall, the evidence for persistent increases in airway immune cells and other immunomodulatory factors following formaldehyde exposure in individuals with prior allergen sensitization is interpreted as likely to represent an incomplete mechanism that could lead to airway hyperresponsiveness, as relevant observations have been reported after long-term exposure. However, the currently available data are insufficient to indicate this sequence of events as a likely mechanism for airway hyperresponsiveness in nonsensitized individuals. Owing to the lack of reliable formaldehyde-specific evidence demonstrating changes in IgE and other immunomodulatory factors assumed to be essential to the development of allergic responses, it is unclear whether this is a possible mechanism by which formaldehyde might cause allergic sensitization. Similarly, it remains unclear how useful this pathway might be to interpreting complex human diseases such as asthma. Additional studies are needed, particularly those employing long-term, low-level formaldehyde exposure.

Consideration of mechanistic pathways that may be associated with each potential respiratory system health effect

Several conclusions are suggested by the analyses of potential mechanistic pathways that might be associated with individual respiratory health effects, based on the most reliable formaldehyde-specific data:

- The confidence in the suggested mechanistic associations varies across the respiratory system health effects. While some uncertainties remain, important mechanistic events associated with sensory irritation, squamous metaplasia, and to a lesser extent, decreased pulmonary function, are supported by robust or moderate formaldehyde-specific data, and the relationships described are largely well-understood biological phenomena or have been demonstrated following formaldehyde exposure. Comparatively, the understanding of mechanisms for potential immune effects is less complete. While moderate evidence exists for several mechanistic events that are likely to be involved in the development of airway hyperresponsiveness, the effect(s) at the point of contact that leads to these events is unclear. The mechanistic evidence describing the potential development of allergic sensitization is the most limited, as it includes slight evidence for several events, and the

majority of the potential mechanistic relationships have not been experimentally validated and a clear scientific consensus regarding the relationships does not exist.

- The primary mechanism for sensory irritation is considered well understood, although it is based largely on acute or short-term exposures, and sensitivity is expected to vary between individuals. While studies clarifying the effects of tissue modification with longer term exposure in humans would be useful, it is likely that rodents exposed to $\approx 0.2 \text{ mg/m}^3$ formaldehyde under normal conditions would exhibit this effect. However, as exposure to formaldehyde appears to cause airway inflammation, which can increase the sensitivity and response magnitude of sensory nerve fibers, inflammation is viewed as a likely modifier of sensory irritation.
- At least one of the mechanisms by which formaldehyde exposure could cause squamous metaplasia is considered well understood, and it appears to depend on both exposure level and duration. Based on the pathway presented, these events are likely to occur at similar or slightly higher formaldehyde levels than those causing sensory irritation, and while cumulative tissue modifications with longer exposure or differences in human anatomy may increase sensitivity, the available experimental animal evidence suggests that pronounced effects leading to metaplasia are unlikely below 0.5 mg/m^3 .
- Several contributing mechanistic pathways appear to impact pulmonary function, and the complex interactions within and across these pathways are expected to involve additional, unidentified factors. While some important mechanistic changes occur at low formaldehyde exposure levels (e.g., $\leq 0.2 \text{ mg/m}^3$ in rodents), data are not available to quantitatively relate these changes to decrements in pulmonary function. In addition, sensitivity is expected to be influenced by the respiratory health of exposed individuals. As with the mechanistic evidence supporting other health effects, much of the data is based on short term exposure. As exposure duration increases, and in the absence of potential compensatory mechanisms (which remains largely unexamined), amplification of these mechanistic events is expected.
- Given the lack of clear explanatory mechanisms for allergic sensitization, in particular, and uncertainties in data that may help to explain airway hyperresponsiveness, as well as an expectation of a large amount of important information that has not yet been identified in formaldehyde-specific studies, it is difficult to speculate on the exposure level- and duration-dependence of these potential pathways. However, some of the important events that may be involved (e.g., eosinophil increases) suggest a duration-dependence for the development of persistent changes in the sensitivity of the airways (note: transient hyperresponsiveness may be possible with short-term exposure), while other important data suggest that a concentration threshold likely exists in regard to critical changes in the cellular immune responses. Individual variability, including underlying respiratory health, is expected to be a significant modifier of these effects.

C.8. CANCER (SUPPORTING INFORMATION FOR HAZARD)

C.8.1. Cancers other than Upper Respiratory Tract and LHP Cancers

Cancer sites for which data were reported that were not formally reviewed in this assessment included lung, non-Hodgkin lymphoma, brain, bladder, colon, pancreas, prostate, and skin cancers (see Section 2.2.9 of the Toxicological Review). A summary of the studies available on

lung, non-Hodgkin lymphoma, and brain are provided below for information. The data on bladder, colon, pancreas, prostate, and skin cancers were sparse and, as such, these studies are not summarized.

Lung Cancer

Evidence describing an association between formaldehyde exposure and the risk of dying from lung cancer is available from 31 epidemiologic studies ([Walrath and Fraumeni, 1983](#); [Walrath and Jr, 1983](#); [Walrath and Fraumeni, 1984](#); [Stroup et al., 1986](#); [Stern, 2003](#); [Stellman et al., 1998](#); [Solet et al., 1989](#); [Robinson et al., 1987](#); [Partanen et al., 1990](#); [Meyers et al., 2013](#); [Marsh et al., 2001](#); [Logue et al., 1986](#); [Liebling et al., 1984](#); [Levine et al., 1984b](#); [Jakobsson et al., 1997](#); [Hayes et al., 1990](#); [Hansen and Olsen, 1995](#); [Gérin et al., 1989](#); [Edling et al., 1987b](#); [Divine and Barron, 1987](#); [Dell and Teta, 1995](#); [De Stefani et al., 2005](#); [Coggon et al., 2014](#); [Chiazze et al., 1997](#); [Checkoway et al., 2011](#); [Bond et al., 1986](#); [Blair et al., 1990](#); [Bertazzi et al., 1986](#); [Bertazzi et al., 1989](#); [Beane Freeman et al., 2013](#); [Band et al., 1997](#); [Andjelkovich et al., 1995](#)). Currently, these are the only primary studies that provide informative evidence of the effect of formaldehyde exposure on the risk of dying from lung cancer. A few studies are interpreted as unlikely to be informative (i.e., [Wesseling et al., 1996](#); [Harrington and Oakes, 1984](#); [Hansen et al., 1994](#); [Hall et al., 1991](#); [Fryzek et al., 2005](#))), based on considerations used to evaluate observational studies in the toxicological review.

Non-Hodgkin Lymphoma

The most specific level of non-Hodgkin lymphoma diagnosis that is commonly reported across the epidemiologic literature has been based on the first three digits of the Eighth or Ninth Revision of the ICD code [i.e., non-Hodgkin lymphoma ICD-8 and ICD-9: Codes 200 and 202 ([WHO, 1967, 1977](#)); however, early studies reported results for lymphosarcoma/reticulosarcoma alone (ICD-8/9: Code 200)]. Evidence describing the association between formaldehyde exposure and the specific risk of non-Hodgkin lymphoma was available from 19 epidemiologic studies—four case-control studies ([Wang et al., 2009b](#); [Tranah et al., 2009](#); [Gérin et al., 1989](#); [Blair et al., 1993](#)) and 15 cohort studies ([Walrath and Fraumeni, 1983](#); [Walrath and Jr, 1983](#); [Walrath and Fraumeni, 1984](#); [Stroup et al., 1986](#); [Stellman et al., 1998](#); [Robinson et al., 1987](#); [Meyers et al., 2013](#); [Matanoski, 1989](#); [Hayes et al., 1990](#); [Hansen and Olsen, 1995](#); [Edling et al., 1987b](#); [Dell and Teta, 1995](#); [Coggon et al., 2014](#); [Beane Freeman et al., 2009](#); [Band et al., 1997](#); [Andjelkovich et al., 1995](#)). One study was interpreted as unlikely to be informative (i.e., [Matanoski, 1989](#))).

Brain Cancer

Evidence describing an association between formaldehyde exposure and the risk of dying from brain cancer is available from 17 epidemiologic studies ([Walrath and Fraumeni, 1983](#); [Walrath and Jr, 1983](#); [Walrath and Fraumeni, 1984](#); [Stroup et al., 1986](#); [Stellman et al., 1998](#); [Robinson et al., 1987](#); [Meyers et al., 2013](#); [Matanoski, 1989](#); [Levine et al., 1984b](#); [Hayes et al., 1990](#); [Hauptmann et al., 2009](#); [Hansen and Olsen, 1995](#); [Hagmar et al., 1986](#); [Dell and Teta, 1995](#); [Coggon](#)

Supplemental Information—Formaldehyde (Inhalation)

[et al., 2003](#); [Beane Freeman et al., 2013](#); [Band et al., 1997](#); [Andjelkovich et al., 1995](#)). Currently, these are the only primary studies that provide evidence of the effect of formaldehyde exposure on the risk of dying from brain cancer. A few studies were interpreted as unlikely to be informative (i.e., ([Wesseling et al., 1996](#); [Harrington and Oakes, 1984](#); [Hansen et al., 1994](#); [Hall et al., 1991](#))).

APPENDIX D. DOSE-RESPONSE ASSESSMENT SUPPLEMENT

The technical detail on dose-response evaluation and determination of points of departure (POD) for relevant toxicological endpoints are provided in this Appendix for Noncancer (Appendix D.1) and Cancer (Appendix D.2) endpoints. Some of the endpoints were modeled using the U.S. EPA's Benchmark Dose Software (BMDS, version 3.3). The common practices used in evaluating the model fit and selecting the appropriate model for determining the POD, as outlined in the *Benchmark Dose Technical Guidance Document* ([U.S. EPA, 2012](https://www.epa.gov/bmds/benchmark-dose-software)) were used²². For some data, alternative methods were used, and these are noted as necessary in the summary of the modeling results.

D.1. DOSE-RESPONSE ANALYSIS FOR NONCANCER HEALTH EFFECTS

A thorough understanding of the exposure-response functions for any association between exposure and health outcomes supports both the derivation of the traditional toxicity values (e.g., RfC) as well as potentially allowing for the estimation of risk above and below those values, and thus provides a more comprehensive understanding of the effects of formaldehyde exposure on various health outcomes. The following details on the estimation of points of departure for the derivation of candidate reference concentrations (cRfCs) are provided to support the derivation of toxicity values as well as to directly inform the potential computation of benefits analyses which require detailed information describing the shape of the exposure-response function across a range of exposures. Such benefits analyses may be used to support a variety of rulemakings.

²² For each dichotomous endpoint, BMDS dichotomous models were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p -value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR. For each continuous endpoint, BMDS continuous models were fitted to the data using the maximum likelihood method. Model fit was assessed by a series of tests as follows. For each model, first the homogeneity of the variances was tested using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected (χ^2 p -value $\geq$ 0.10), the model was fitted to the data assuming constant variance. If Test 2 was rejected (χ^2 p -value < 0.10), the variance was modeled as a power function of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance, models for the mean response were tested for adequacy of fit using a likelihood ratio test (BMDS Test 4, with χ^2 p -value < 0.10 indicating inadequate fit). Other factors were also used to assess the model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

D.1.1. Sensory Irritation

Estimates from Observational Epidemiology Studies

Hanrahan et al. (1984)

[Hanrahan et al. \(1984\)](#) conducted a cross-sectional study and reported a concentration-response relationship for the prevalence of ocular discomfort (i.e., burning eyes/eye irritation) in a study of 61 teenage and adult residents of mobile homes in Wisconsin. In-home formaldehyde measurements were obtained for all participants, and measured formaldehyde levels (average of two approximately 1-hour air samples²³—one from the kitchen or living room and one from a bedroom) were used to characterize average in-home exposures.

[Hanrahan et al. \(1984\)](#) reported that prevalent symptoms²⁴ of burning eyes and eye irritation were significantly associated with in-home formaldehyde exposures, and the authors provided a graphical representation of the best-fitting logistic regression model results of predicted prevalence of “burning eyes” for exposures at 100 ppb increments from 100 to 800 ppb. From inspection of this graph, EPA determined the prevalence of burning eyes predicted at 100 ppb is approximately 4%. While the published exposure-response results were shown truncated at 100 ppb, [\(Hanrahan et al., 1984\)](#) reported that exposures ranged from <100 ppb to 800 ppb, and the indoor median formaldehyde concentration was 160 ppb. The reconstruction of that underlying functional form can show the results of the [\(Hanrahan et al., 1984\)](#) model extended below 100 ppb.

²³ Exposure assessment methodology was consistent with NIOSH Method 3500 ([NIOSH, 1994](#)).

²⁴ [\(Hanrahan et al., 1984\)](#) reported on the “prevalence” of symptoms that had occurred since moving into the home.

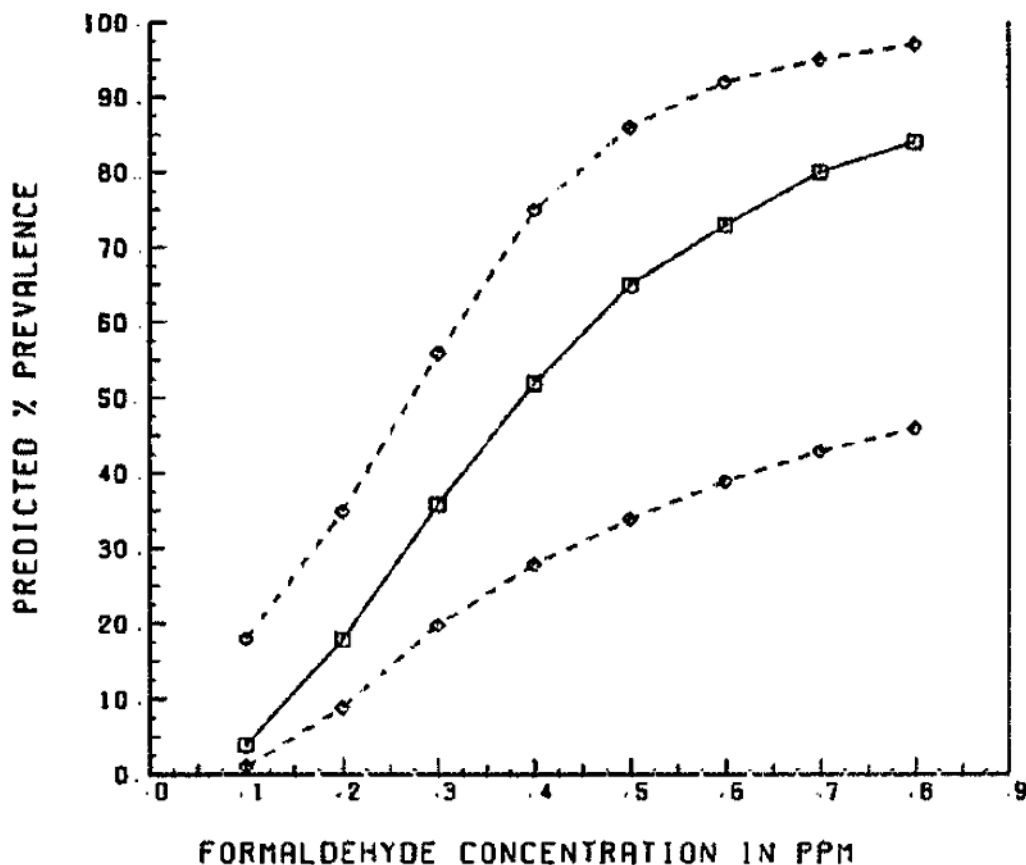


Figure D-1. Regression of prevalence of “burning eyes” versus indoor formaldehyde concentration (ppm) in mobile homes (approximately 1-hour air samples). Dashed lines show upper and lower 95th percentile confidence intervals on model results.

In Figure D-1, the dependent variable is displayed as a predicted percentage prevalence of burning eyes. However, the general epidemiologic method used to model prevalence data is logistic regression, which predicts the log odds of prevalence, which the authors then transformed to prevalence for graphing. In order to describe the underlying functional form of the results displayed, EPA converted the prevalence data back to prevalence odds. Table D-1 shows the prevalence values which EPA visually estimated from the plot, as well as the associated prevalence odds, which EPA calculated as estimated prevalence divided by the complement of estimated prevalence, that is $p/(1-p)$. Figure D-2 plots the estimated prevalence odds against the residential concentration of formaldehyde.

Table D-1. Concentration-response information for the central estimate of the effect extracted from (Hanrahan et al., 1984).

Residential formaldehyde concentration (ppm)	Prevalence (p)	Prevalence odds ($p/[1-p]$)
0.1	0.038	0.039
0.2	0.182	0.223
0.3	0.362	0.567
0.4	0.525	1.104
0.5	0.655	1.897
0.6	0.735	2.777
0.7	0.806	4.159
0.8	0.846	5.508

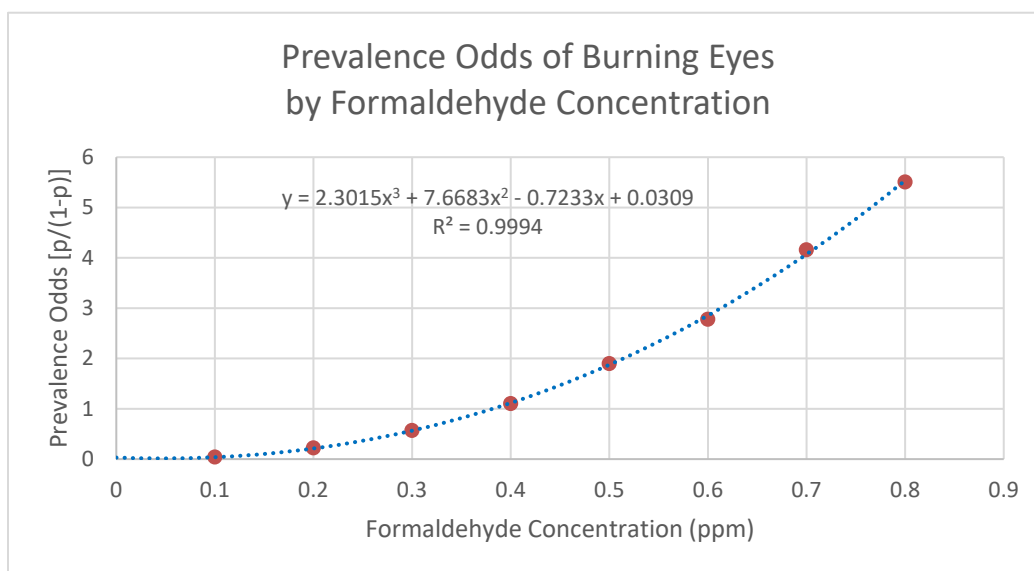


Figure D-2. Plot of the central estimate of the prevalence odds by residential concentration-response information from Table D-1.

In order to describe the underlying functional form of the model-predicted results from (Hanrahan et al., 1984), EPA fit polynomial trend lines from linear up to cubic functions with the intercept fixed at a background prevalence of burning eyes of 3% (prevalence odds of 0.0309)²⁵ (using Microsoft Excel) to the discrete prevalence odds data in Figure D-2 and found that a third-degree polynomial function fit with an R^2 value of 0.9994. This indicates nearly a perfect fit to the

²⁵Setting the intercept to other value such as 0.01, 0.02, 0.03 made little difference (e.g., at 0.03, the R^2 had the same value of 0.9991, and the model was $y = 6.1949x^3 + 3.7689x^2 + 0.0309x + 0.03$).

published model results. Such a high value of R^2 would not have been achieved from analysis of the raw data (unavailable), but the objective here was to recreate the functional form of the modeled data presented by ([Hanrahan et al., 1984](#)). The following describes the functional form for the prevalence odds:

$$\frac{p}{1-p} = 2.3015 * (exposure)^3 + 7.6683 * (exposure)^2 - 0.7233 * (exposure) + 0.0309$$

(D-1)

Table D-2 shows the prevalence values for the upper bound on the central estimate of the published concentration-response function, which EPA visually estimated from the plot, as well as the associated prevalence odds, which EPA calculated as estimated prevalence divided by the complement of estimated prevalence, that is $p/(1-p)$. Figure D-3 plots the estimated prevalence odds against the residential concentration of formaldehyde.

Table D-2. Concentration-response information for the upper bound on the central estimate of the effect extracted from ([Hanrahan et al., 1984](#))

Residential formaldehyde concentration (ppm)	Prevalence (p)	Prevalence odds ($p/[1-p]$)
0.1	0.18	0.22
0.2	0.35	0.54
0.3	0.55	1.22
0.4	0.74	2.85
0.5	0.84	5.25
0.6	0.91	10.11
0.7	0.94	15.67
0.8	0.96	24.00

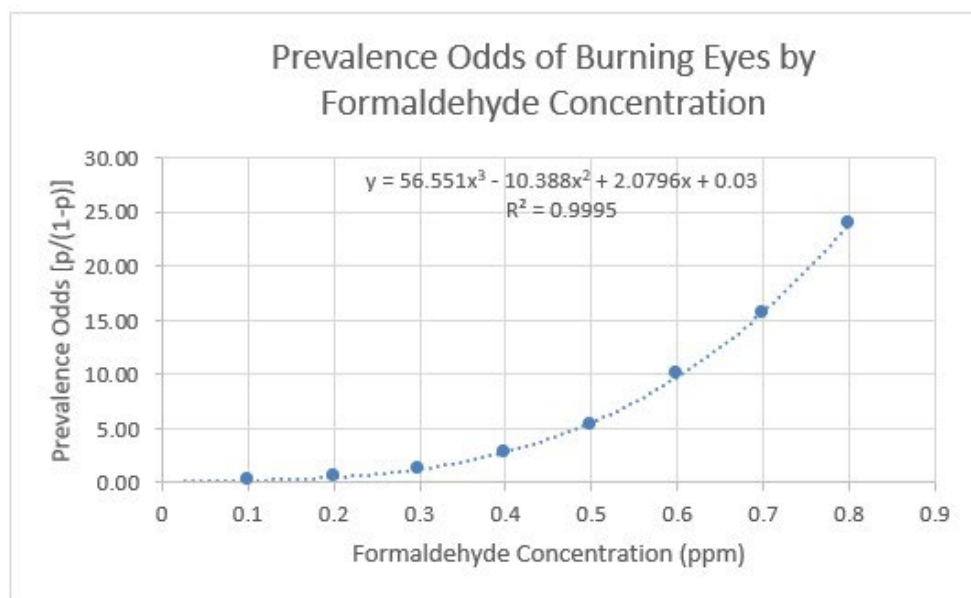


Figure D-3. Plot of the upper bound on the central estimate of the prevalence odds by residential concentration-response information from Table 2.

In order to describe the underlying functional form of the model-predicted results from Hanrahan et al. (1984), EPA fit polynomial trend lines from linear up to cubic functions with the intercept fixed at zero (using Microsoft Excel) to the discrete prevalence odds data in Figure D-3 and found that a third-degree polynomial function fit with an R^2 value of 0.9995. This indicates nearly a perfect fit to the published model results. The following describes the functional form for the prevalence odds:

$$\frac{p}{1-p} = 56.551 * (exposure)^3 - 10.388 * (exposure)^2 + 2.0796 * (exposure) + 0.03 \quad (D-2)$$

Selecting a benchmark response (BMR) for the derivation of a reference concentration (RfC) involves making judgments about the statistical and biological characteristics of the data set. A BMR representing an extra risk of 10% is generally recommended as a standard reporting level for quantal data. Biological considerations may warrant the use of a BMR of 5% or lower for some types of effects (e.g., frank effects), or a BMR greater than 10% (e.g., for early precursor effects) as the basis of the point of departure (POD) for a reference value (U.S. EPA, 2012).

EPA calculated the concentration at which a 10% extra risk of “burning eyes” would have been observed in these data using the polynomial functions for the main effect to estimate the BMC and for the upper-bound to estimate the BMCL. In this derivation, 10% extra risk is the benchmark response (BMR) and the BMC and BMCL for a 10% BMR are noted as the BMC₁₀ and BMCL₁₀. Note

that in Hanrahan et al. (1984), the prevalence of “burning eyes” was similar to that of “eye irritation.” As there is little information available in the literature to estimate the background prevalence of “burning eyes,” the background prevalence of “burning eyes” was estimated at 3% (in the absence of formaldehyde exposure) based on the prevalence of “eye irritation.” A background prevalence of 3% was considered to be a reasonable estimate. Sensitivity analyses using a background prevalence of 1% and 2% were also evaluated and yielded BMC and BMCL estimates.²⁶

Because the extra risk is a function of the prevalence in the exposed (P_{Exposed}) and the prevalence in the unexposed ($P_{\text{Unexposed}}$) was estimated at 3%, EPA derived P_{Exposed} for 10% extra risk above background.

$$\text{Extra Risk} = 0.10 = [P_{\text{Exposed}} - P_{\text{Unexposed}}]/[1 - P_{\text{Unexposed}}] \text{ and } P_{\text{Unexposed}} = 0.03, \text{ then } P_{\text{Exposed}} = 0.127.$$

(D-3)

Because the exposure-response function from Hanrahan et al. (1984) is in terms of the prevalence odds, that value is derived based on $P_{\text{Exposed}} = 0.127$. Thus, the prevalence odds = $[P_{\text{Exposed}}]/[1 - P_{\text{Exposed}}] = 0.145$. To derive the BMC, solve for the exposure value, which yields prevalence odds of 0.145:

$$0.145 = 6.1949 * (\text{exposure})^3 + 3.7689 * (\text{exposure})^2 + 0.0309 * (\text{exposure}) + 0.03$$

(D-4)

Of the three roots, only one is within the exposure range of the data.

Exposure = 0.153 ppm formaldehyde = 0.188 mg/m³ formaldehyde (see footnote²⁷)

To derive the interim BMCL, solve for:

$$0.145 = 56.551 * (\text{exposure})^3 - 10.388 * (\text{exposure})^2 + 2.0796 * (\text{exposure}) + 0.03$$

(D-5)

Of the three roots, only one is within the exposure range of the data.

Exposure = 0.0706 ppm formaldehyde = 0.0868 mg/m³ formaldehyde

The BMC₁₀ is 0.188 mg/m³. The BMCL₁₀ is 0.0868 mg/m³.

²⁶Using a 1% background prevalence to estimate the exposure-response function and the BMC, yields an estimate of 0.154 ppm = 0.190 mg/m³ formaldehyde, and a BMCL estimate of 0.0768 = 0.0945 mg/m³; using a 2% background prevalence to estimate the exposure-response function and the BMC, yields an estimate of 0.154 ppm = 0.189 mg/m³ formaldehyde, and a BMCL estimate of 0.0739 = 0.0909 mg/m³.

²⁷Concentration (mg/m³) = Concentration (ppm) * (Molecular mass/Molar volume) = 0.155 ppm * [(30.03 g/mol)/(24.45 L)] = 0.191 mg/m³ at 25°C = Concentration (ppm) * 1.23.

([Liu et al., 1991](#)) combined with ([Hanrahan et al., 1984](#))

[Hanrahan et al. \(1984\)](#) provided graphical dose-response information of formaldehyde concentrations of 0.1 ppm to 0.8 ppm and the prevalence of “burning eyes” (sensory irritation) which EPA extracted for modeling. Liu et al. (1991) also provided graphical dose-response information at lower exposure levels using a cumulative exposure metric of ppm-hours during the weeklong sampling period for the prevalence of “burning eyes”. The 2014 National Research Council (NRC) report recommended that dose-response estimation be based on data from compatible studies of similar exposure and outcome when feasible and the specific idea to estimate the dose-response function from the combination of these two studies was recommended by NASEM ([NASEM, 2023](#)). The data from ([Liu et al., 1991](#)) and ([Hanrahan et al., 1984](#)) complemented each other, although several assumptions were necessary to fit the two related datasets together.

In order to convert these cumulative exposure metrics to concentration values, the duration time needed to be divided out. ([Liu et al., 1991](#)) does not specifically say what the duration time was, however, ([Liu et al., 1991](#)) noted that “[f]or a person who spends 60% of the time inside his or her own home, a weekly HCHO exposure of 7 ppm-hours can be translated into a weekly average HCHO concentration of 0.07 ppm. From this statement, EPA assumed that ([Liu et al., 1991](#)) expected that their study subjects spent 60% of their week inside their homes which is 100.8 hours per week. ([Liu et al., 1991](#)) graphical results showed the prevalence of “burning eyes” as a function of cumulative formaldehyde concentration for three categories of exposure: 0–7.0 ppm-hours; 7.0–12.0 ppm-hours; and >12.0 ppm-hours. Dividing the three categories of exposure by 100.8 hours, the three exposure categories are: 0.01-0.06944 ppm; 0.06944-0.1195 ppm; and >0.1195 ppm.

More granular exposure data from the same study was published in ([Sexton et al., 1986](#)) which provided graphical data for the two seasons when homes were sampled: summer (Jul/Aug) and winter (Feb/Mar) although the increments of the exposure categories did not match up evenly with the increments of exposure from ([Liu et al., 1991](#)). For example, the increment of exposure from ([Sexton et al., 1986](#)) were shown in a histogram per 0.025 ppm from zero to > 0.45 ppm in Summer and to 0.35 ppm in winter. While the graphic of exposure data in Figure 3 of ([Sexton et al., 1986](#)) shows the lowest exposure at zero, Liu et al. (1991) reported that the limit of detection was 0.01 ppm and ([Sexton et al., 1986](#)) reported that the maximum exposure was 0.464 ppm which provides an upper bound on the summer exposure category of “>0.45 ppm.” Similarly, for Winter, the minimum exposure was 0.017 ppm and the maximum was 0.314 ppm. EPA extracted data from Figure 3 in ([Sexton et al., 1986](#)) to estimate the percentage of mobile homes corresponding to the three exposure categories in ([Liu et al., 1991](#)). This information is included in Table D-3 to describe the mid-point of each exposure category and then the estimated exposure for each group from ([Liu et al., 1991](#)) is the average of the exposure in each group from ([Sexton et al., 1986](#)) weighted by the estimated number of homes for each exposure group in ([Sexton et al., 1986](#)).

Table D-3. Extracted data from (Liu et al., 1991) and (Sexton et al., 1986) used to estimate additional data point for dose-response of sensory irritation

Season	Exposure group (ppm) from Liu et al. (1991)	Exposure group (ppm) from (Sexton et al., 1986)	Exposure group mid-point (ppm)	Estimated number of homes in (Sexton et al., 1986) intervals ^a	Number of homes per Liu et al. (1991) intervals	Estimated group exposure ^b in Liu et al. (1991) intervals (ppm)	Prevalence of “Burning eyes” by exposure category in Liu et al. (1991)	Prevalence odds ($p/[1-p]$)
Summer	0.01-0.0694	0.01-0.025	0.0175	40	315	0.0431	13.3%	0.153
		0.025-0.05	0.0375	160				
		0.05-0.0694	0.05972	115				
	0.0694-0.119	0.0694-0.075	0.0722	33	192	0.0906	17.1%	0.206
		0.075-0.1	0.0875	109				
		0.1-0.119	0.1095	50				
	0.1195-0.464	0.119-0.125	0.12205	16	156	0.1943	21.4%	0.272
		0.125-0.15	0.1375	40				
		0.15-0.175	0.1625	33				
		0.175-0.20	0.1875	17				
		0.20-0.225	0.2125	15				
		0.225-0.25	0.2375	7				
		0.25-0.30	0.275	9				
		0.30-0.35	0.325	9				
		0.35-0.40	0.375	5				
		0.40-0.45	0.425	3				
		0.45-0.464	0.457	2				
Winter	0.01-0.06944	0.017-0.025	0.021	8	205	0.04748	10.8%	0.121
		0.025-0.05	0.0375	99				
		0.05-0.06944	0.05972	98				
	0.06944-0.1195	0.06944-0.075	0.0722	28	208	0.09115	14.7%	0.172
		0.075-0.1	0.0875	126				
		0.1-0.119	0.1095	54				
	0.1195-0.314	0.119-0.125	0.122	17	110	0.1698	20.6%	0.259
		0.125-0.15	0.1375	31				
		0.15-0.175	0.1625	26				
		0.175-0.2	0.1875	10				
		0.2-0.225	0.2125	10				
		0.225-0.25	0.2375	10				
		0.25-0.30	0.275	3				

Supplemental Information—Formaldehyde (Inhalation)

Season	Exposure group (ppm) from Liu et al. (1991)	Exposure group (ppm) from (Sexton et al., 1986)	Exposure group mid-point (ppm)	Estimated number of homes in (Sexton et al., 1986) intervals ^a	Number of homes per Liu et al. (1991) intervals	Estimated group exposure ^b in Liu et al. (1991) intervals (ppm)	Prevalence of “Burning eyes” by exposure category in Liu et al. (1991)	Prevalence odds (p/[1-p])
		0.30-0.314	0.307	3				

^a Estimated from the extracted % of mobile homes in Figure 3 of (Sexton et al., 1986) and rounded to whole numbers.

^b Estimated exposure for each group from Liu et al. (1991) is the average of the exposure in each group from Sexton et al. (1986) weighted by the estimated number of homes for each exposure group in Sexton et al. (1986).

^c A conversion of 1 ppm = 1.23 mg/m³ formaldehyde is used.

In Figure D-4 below, EPA combined the eight points from the dose-response data from (Hanrahan et al., 1984) with the six additional points from (Liu et al., 1991), which shows that the two sets of reconstructed dose-response data overlapped between 0.1 and 0.2 ppm formaldehyde concentration.

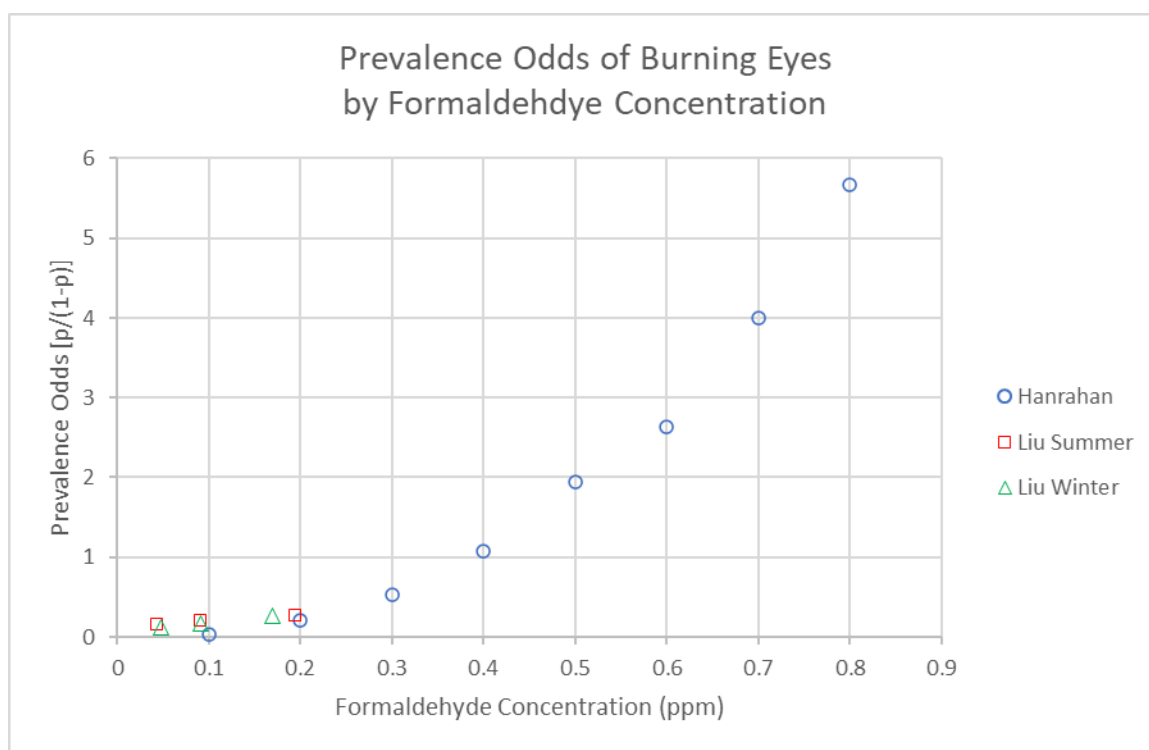


Figure D-4. Plot of the prevalence odds by residential concentration-response information from the central estimate of from Hanrahan et al. (1984) combined with complementary information from the central estimate of from Liu et al. (1991). Blue circles (Hanrahan et al., 1984); Red squares ‘Summer’ Liu et al. (1991); Green triangles ‘Winter’ Liu et al. (1991).

Supplemental Information—Formaldehyde (Inhalation)

Assuming a background prevalence of “burning eyes” of 3%, EPA fit a cubic polynomial function with the intercept fixed at the log-odds of 0.03 (0.0309), to the discrete prevalence odds data in Figure D-4 and found that a third-degree polynomial function fit with an R^2 value of 0.998 (see Figure D-5). The following formula describes the functional form for the prevalence odds:

$$\frac{p}{1-p} = 7.8077 * (exposure)^3 + 1.6517 * (exposure)^2 + 0.7104 * (exposure) + 0.0309 \quad (D-6)$$

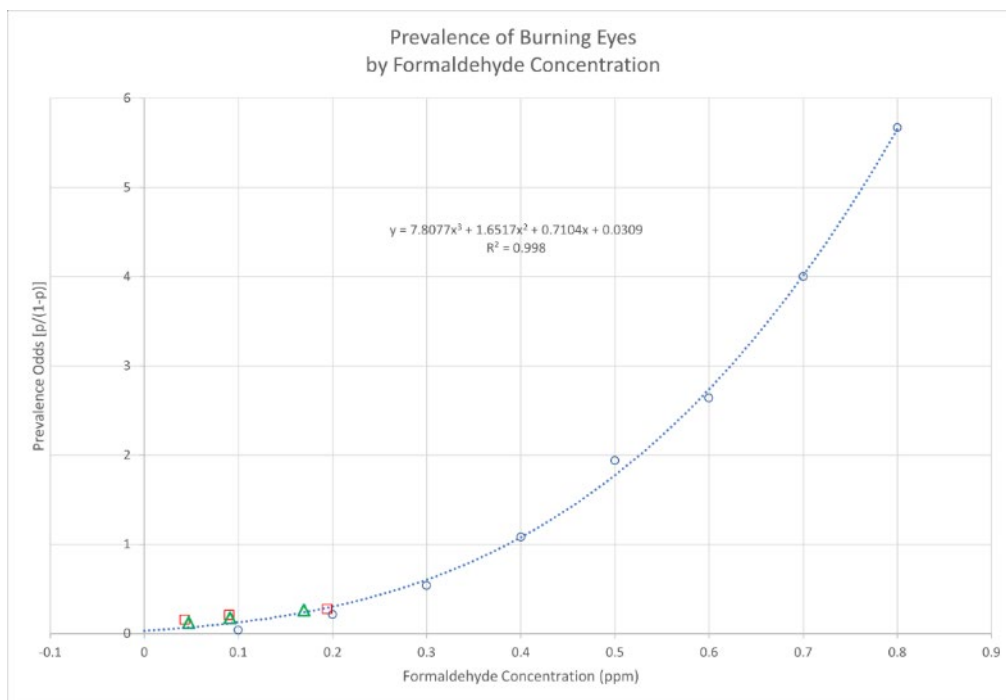


Figure D-5. Plot of the prevalence odds by residential concentration-response information from the central estimate of from ([Hanrahan et al., 1984](#)) combined with information from the central estimate of from ([Liu et al., 1991](#)) with third degree polynomial function fit to these data.

EPA used a BMR of 10% extra risk of “burning eyes” and calculated the concentration at which a 10% extra risk of “burning eyes” would have been observed in these data using the polynomial functions for the main effect to estimate the BMC_{10} . EPA calculated the concentration at which a 10% extra risk of “burning eyes” would have been observed in these data using the polynomial functions for the main effect to estimate the BMC. Since extra risk is a function of the prevalence in the exposed ($P_{Exposed}$) and the prevalence in the unexposed ($P_{Unexposed}$), which was estimated at 3%, EPA derived $P_{Exposed}$ for 10% extra risk above background.

Extra Risk = 0.10 = $[P_{Exposed} - P_{Unexposed}] / [1 - P_{Unexposed}]$ and $P_{Unexposed} = 0.03$, then $P_{Exposed} = 0.127$.

(D-7)

Because the exposure-response function from Hanrahan et al. (1984) is in terms of the prevalence odds and the prevalence odds were also estimable from Liu et al. (1991), the BMC₁₀ is derived based on $P_{\text{Exposed}} = 0.127$. Thus, the prevalence odds = $[P_{\text{Exposed}}]/[1-P_{\text{Exposed}}] = 0.145$. To derive the BMC, solve for the exposure value, which yields prevalence odds of 0.145:

$$0.145 = 7.8077 * (\text{exposure})^3 + 1.6517 * (\text{exposure})^2 + 0.7104 * (\text{exposure}) + 0.0309$$

(D-8)

Of the three roots, only one is within the exposure range of the data.

Exposure = 0.1141 ppm formaldehyde = 0.1403 mg/m³ formaldehyde ²⁸

The BMCL₁₀ for the combined data from (Hanrahan et al., 1984) and (Liu et al., 1991) is 0.1403 mg/m³ formaldehyde. EPA used the data extracted from the Hanrahan study to estimate a BMC₁₀ and BMCL₁₀ for sensory irritation since Hanrahan et al. (1984) provided the upper bound of the risk estimates which could be used to estimate the BMCL₁₀. Liu et al. (1991) did not report the confidence intervals for the estimated dose-response so no BMCL₁₀ for the combined data is possible.

Estimates from Controlled Exposure Studies in Humans

Kulle et al. (1993, 1987)

Modeling results are presented that support the derivation of PODs for sensory irritation based on two controlled human exposure studies. Kulle et al. (1993) reanalyzed results of a study of eye, nose, and throat irritation among participants exposed to 0, 0.5, 1.0, 2.0, and 3.0 ppm for 3 hours once a week with exposure order randomly assigned (Kulle et al., 1987). The results of the different modeling approaches are summarized in Table D-4 and explained below.

Table D-4. Modeling summary for irritation symptoms in (Kulle et al., 1987; Kulle, 1993)

Approach	Endpoint ^a	Selected model ^b	BMC ^c (ppm)	BMCL (95%) ppm	BMCL (99%) ppm	Notes
CatReg	Combined ^d Eye Irritation or Nose/Throat Irritation	Probit	0.56	0.42	0.36	Selected POD
CatReg	Eye Irritation	CLogLog	0.80	0.57	0.47	
CatReg	Nose/Throat Irritation	-	-	-	-	Model fit not acceptable ^d
BMDS	Eye Irritation	Probit	0.69	0.50	0.44	

²⁸Value converted based on 1 ppm = 1.23 mg/m³, assuming 25°C and 760 mm Hg.

Supplemental Information—Formaldehyde (Inhalation)

Approach	Endpoint ^a	Selected model ^b	BMC ^c (ppm)	BMCL (95%) ppm	BMCL (99%) ppm	Notes
BMDS	Nose/Throat Irritation	Multistage 3 ^o	1.27	0.45	0.27	Large BMC: BMCL ratio

^aEye irritation results throughout this table reflect modeling of males and females combined. Nose/throat irritation results throughout this table reflect modeling of females only given the negative slope observed for males.

^bThe selected model for each approach and endpoint was the viable model with the lowest AIC (see details below).

^cBMC and BMCL are termed ERC and LERC in CatReg. In both, a BMR of 10% extra risk was used for the modeling.

^dThe preferred model used eye irritation data to predict the slope and combined eye and nose/throat irritation data for the intercept. A second modeling combination using nose/throat irritation for the slope and combined irritation data for the intercept yielded a slightly higher POD of 0.48 ppm at the 99th percentile (see details below). The selected CatReg model results were preferred primarily due to the lower POD, although the BMC/BMCL ratio for these results was also lower, indicating a narrower confidence interval and a lower level of uncertainty.

The datasets modeled from this study ([Kulle et al., 1987](#); [Kulle, 1993](#)) are presented in Table D-5 below.

Table D-5. Incidence and severity of sensory irritation symptoms in humans after controlled formaldehyde exposure

Sensory irritation endpoint	Formaldehyde (ppm)	Symptom prevalence and severity ^a							
		Males				Females			
		None	Mild	Moderate	Severe	None	Mild	Moderate	Severe
Eye Irritation	0	9/10	1/10	–	–	9/9	–	–	–
	0.5	4/4	–	–	–	6/6	–	–	–
	1	8/10	2/10	–	–	6/9	2/9	1/9	–
	2	5/10	2/10	3/10	–	4/9	4/9	1/9	–
	3	–	3/6	3/6	–	--	2/3	1/3	–
Nose/Throat Irritation	0	9/10	1/10	–	–	7/9	2/9	–	–
	0.5	4/4		--	--	5/6	1/6	--	--
	1	9/10	1/10	--	--	9/9		--	--
	2	8/10	2/10	--	--	4/9	5/9	--	--
	3	6/6		--	--	1/3	2/3	--	--

^aThe authors also report results for a “symptom score difference” (pre- and post-exposure), pooling results across severity levels; however, these pooled data were not preferred for use quantitatively without information on the underlying within-subject severity information at both timepoints or the score calculations.

CatReg Model Forms

CatReg offers two choices of models, Model 1 (cumulative odds model) and Model 2 (unrestricted cumulative model). For additional details see the CatReg User guide (U.S. EPA, 2017).

The Cumulative Odds Model is described by the following equation:

$$\Pr(Y \geq s|C, T) = H[\alpha_s + \beta_1 * f_1(C) + \beta_2 * f_2(T)] \quad \text{Model 1} \quad (D-9)$$

The Unrestricted Cumulative Model is described by the following equation:

$$\Pr(Y \geq s|C, T) = H[\alpha_s + \beta_{1s} * f_1(C) + \beta_{2s} * f_2(T)] \quad \text{Model 2} \quad (D-10)$$

CatReg refers to any model of the form of Model 1 as a cumulative odds model because the model is expressed in terms of the cumulative probabilities, or odds, for $Y \geq s$.

Note that Model 1 is a special case of Model 2 wherein parameters β_{1s} and β_{2s} do not depend on s (which denotes severity level). That is, Model 1 is a simplification of Model 2 in which only the intercept term can vary across severity levels, not the coefficients of dose or time (a restriction called parallelism, meaning the fitted slopes for severity levels will be parallel to one another).

In other words, the cumulative odds model (Model 1) states that:

- the probability that a severity level s or higher will occur at a given dose or concentration (C) and time (T) is given by the exposure-response curve (logistic, normal, or Gumbel, determined by the choice of link function), and
- the intercept parameters may differ by severity level (i.e., a different intercept for each severity level), but the coefficients for C and T do not differ by severity.

A primary use of fitting Model 2 is to test whether the simpler Model 1 is adequate. Model 2 has the undesirable feature that the regression lines for different severity levels are not parallel and may cross. Often the crossing is well outside the range of values of interest, so the model can be used to make empirical risk estimates. The user has the option to add an additional parameter, γ , which represents a hypothetical background concentration or dose, in some circumstances.

Understanding the Model Equations

The left-hand side of the Model 1 and Model 2 equations above is read as follows: the probability that a response of severity level s or greater occurs, given that concentration or dose is C and time is T (time refers to exposure duration). No expression for $s = 0$ is included because this is the minimal category, and Y is always greater than or equal to 0 (i.e., $\Pr(Y \geq 0|C, T) = 1$).

The right-hand side is described as follows:

- H is a probability function taking values between 0 and 1, for which the user has three choices: logistic, normal, and Gumbel.

Supplemental Information—Formaldehyde (Inhalation)

- The parameter α_s is the intercept for severity level s , $s = 2, \dots, S$ (to be called the intercept or severity parameters). The severity parameters are ordered as $\alpha_1 \leq \alpha_2 \leq \dots \leq \alpha_S$. This constraint is a consequence of the requirement that the probability of exceeding a lower score is larger than the probability of exceeding a higher score for any fixed levels of C and T .
- In Model 1, the parameter β_1 determines the dependence of the response on concentration or dose (I), whereas β_2 determines the dependence on time (T). In Model 2, the parameters are also indexed by s because they may change values with severity level s .
- Current choices for f_1 and f_2 are "untransformed" and "base-10 logarithm." Other transformations of C and T may be obtained by transforming the input data.

Parameters are α_s , β_{1s} (to be called the coefficient of concentration or dose), β_{2s} (to be called the coefficient of time or duration), for severity levels s running from 1 to S . All parameters may be stratified on variables, as discussed in the CatReg User Guide ([U.S. EPA, 2017](#))

CatReg Results

As described in Section 8.0 and 8.1 of the CatReg User Guide, hypothesis tests built into CatReg are designed to support the user with respect to model choice, parameter stratification and stratification/dataset configuration (e.g., whether intercept, dose and time parameter coefficients should be estimated separately by endpoint and/or sex). With respect to model choice, CatReg Model 2 results for the data shown in Table D-5 indicate that the simpler cumulative odds model (Model 1) is adequate for evaluating the ([Kulle, 1993](#)) eye and nasal/throat irritation datasets. Table D-6 shows the results of the CatReg hypothesis testing for a categorical regression analysis of the two irritation datasets, as well as a dataset combining the two endpoints, when intercept and slope parameters are stratified (estimated separately) by sex. The male nose/throat irritation data could not be analyzed separately because the estimated dose-response slope was negative. For both the eye irritation and combination datasets, the hypothesis test results suggest that the males and female data can be combined for the purpose of deriving the intercept and slope parameters ($p > 0.05$).

Table D-6. CatReg hypothesis test results for irritation datasets when intercept and slope parameters are stratified by sex

Dataset	Parameter	<i>p</i> -value	Conclusion
Eye Irritation	Intercept	0.91	Combine Intercept Data for Male and Female
	Slope	0.99	Combine Slope Data for Male and Female
Nose/Throat Irritation	Intercept*	--	--
	Slope*	--	--
	Intercept	0.88	Combine Intercept Data for Male and Female

Supplemental Information—Formaldehyde (Inhalation)

Dataset	Parameter	p-value	Conclusion
Combined (Eye & Nose/Throat Irritation)	Slope	0.61	Combine Slope Data for Male and Female

*Could not model male nose/throat irritation separately due to negative slope.

The CatReg hypothesis test results for intercept and slope parameters stratified by eye and nose/throat irritation shown in Table D-7 suggest that, if male and female data are combined, the intercept parameter estimates for eye irritation and nose/throat irritation are similar but the slope parameters are not similar ($p < 0.5$), suggesting that the irritation data can be combined for the estimation of the intercept parameter but that separate slope parameters should be derived.

Table D-7. CatReg hypothesis test results for irritation datasets when intercept and slope parameters are stratified by endpoint, eye and nose/throat irritation

Dataset	Parameter	p-value	Conclusion
Male & Female	Intercept	0.188	Combine Intercept Data for Eye and Nose/Throat
	Slope	0.0022	Stratify Slope Data by Eye and Nose/Throat
Male	Intercept*	--	--
	Slope*	--	--
Female	Intercept	0.30	Combine Intercept Data for Eye and Nose/Throat
	Slope	0.11	Combine Slope Data for Eye and Nose/Throat

*Could not model male nose/throat irritation separately due to negative slope

Table D-8 shows the CatReg modeling results for the combined male and female data using the combined eye and nose/throat irritation data (stratifying slope by endpoint) and separate endpoints results (female only is shown for nose/throat irritation given the negative slope in males). This table shows both the 95th and 99th percentile lower bound, as discussed above. The selected modeling results, based on the lowest AIC, are highlighted, with the selected POD of 0.36 ppm bolded. Also italicized are PODs representing the best-fitting 99th percentile results from the other CatReg modeling outputs.

Table D-8. Summary of CatReg modeling results for sensory irritation

Endpoint and Dataset	Model ^a	Severity ^b	ERC10 ^c , ppm	95th Percentile LERC10, ppm	99th Percentile LERC10, ppm	AIC ^d	P-value
Eye irritation (male & female)	Logit	mild	0.74	0.50	0.41	99.52	<0.001
		moderate	1.71	1.33	1.17		
	Probit	mild	0.67	0.46	0.38	99.28	<0.001

Supplemental Information—Formaldehyde (Inhalation)

Endpoint and Dataset	Model ^a	Severity ^b	ERC10 ^c , ppm	95th Percentile LERC10, ppm	99th Percentile LERC10, ppm	AIC ^d	P-value
	CLogLog	moderate	1.64	1.27	1.12	97.69	<0.001
		mild	0.80	0.57	0.47		
		moderate	1.71	1.32	1.16		
Eye irritation data (male & female) to estimate slope and combined eye (male & female) and nose/throat irritation (female) data to estimate intercept	Logit	mild	0.62	0.46	0.39	177.73	0.0013
		moderate	1.81	1.42	1.26		
	Probit	mild	0.56	0.42	0.36	176.19	0.0010
		moderate	1.70	1.33	1.18		
	CLogLog	mild	0.69	0.53	0.46	177.48	0.0012
		moderate	1.85	1.45	1.29		
Nose/throat irritation data (female) to estimate slope and combined eye (male & female) and nose/throat irritation (female) data to estimate intercept	Logit	mild	1.44	0.84	0.59	177.73	0.0013
		moderate	4.21	2.07	1.19		
	Probit	mild	1.41	0.75	0.48	176.19	0.0010
		moderate	4.30	1.96	0.99		
	CLogLog	mild	1.48	0.90	0.66	177.48	0.0012
		moderate	3.98	2.09	1.30		
Nose/Throat Irritation (female)	Logit ^e	mild	0.76	0.42	0.28	41.42	0.0602
		moderate	--	--	--		
	Probit ^e	mild	0.72	0.38	0.24	41.61	0.0656
		moderate	--	--	--		
	CLogLog ^e	mild	0.81	0.49	0.36	41.26	0.0561
		moderate	--	--	--		

^aAll of the Link function models use Linear scale for dose and time.

^bCatReg was used to estimate the probability of a 10% increase (the BMR) in any symptom incidence of this severity or greater.

^cERC and LERC are CatReg equivalents of the BMDS BMC and BMCL.

^dThe AIC values for the two different methods of combining the irritation datasets are identical because the same model was used (i.e., the model stratified dose on endpoint and gave results for both).

^eThe p-value of the model fit is > 0.05. This is generally considered not significant, indicating that the current model fit is not acceptable. These cells are shown in gray as they were not considered for use in derivation or comparison.

BMDS Results

BMDS²⁹ was used to provide a comparison to the CatReg results. For the purposes of doing so, the data were pooled to provide a dichotomized comparison. Specifically, any incidence of symptoms with severity greater than mild were pooled into a single group for modeling purposes

²⁹ United States Environmental Protection Agency. (2023). BMDS-Online (Build d5f505c8; Model Library Version 2023.03.1) [Web App]. Accessed on or around November 08, 2023.

Supplemental Information—Formaldehyde (Inhalation)

against the control (“none”) group. Table D-9 below shows the endpoints and data that were modeled. As with other modeling of these endpoints, a BMR of 10% extra risk was used, and results are presented for combined sexes for eye irritation and females alone for nose/throat irritation (both sexes were modeled individually for both endpoints with similar results, data not shown). Results were modeled and are shown with both the 95th and 99th percentile lower bound (Table D-10).

Table D-9. Incidence and severity of sensory irritation symptoms in humans after controlled formaldehyde exposure

Sensory irritation endpoint	Formaldehyde (ppm)	Symptom (any ^a) prevalence					
		Males		Females		Males & Females	
		None	Symptoms	None	Symptoms	None	Symptoms
Eye Irritation	0	9/10	1/10	9/9	0/9	18/19	1/19
	0.5	4/4	0/4	6/6	0/6	10/10	0/10
	1	8/10	2/10	6/9	3/9	14/19	5/19
	2	5/10	5/10	4/9	5/9	9/19	10/19
	3	0/6	6/6	0/3	3/3	0/9	9/9
Nose/Throat Irritation	0	9/10	1/10	7/9	2/9	16/19	3/19
	0.5	4/4	0/4	5/6	1/6	9/10	1/10
	1	9/10	1/10	9/9	0/9	18/19	1/19
	2	8/10	2/10	4/9	5/9	12/19	7/19
	3	6/6	0/6	1/3	2/3	7/9	2/9

^aAny person with a symptom of severity rated ‘mild’ or greater was combined into a single ‘symptoms’ count.

Table D-10. BMD modeling summary for eye irritation in males and females

Model	BMC (ppm)	BMCL (95%)	BMCL (99%)	P-value	AIC	Notes
Hill	0.852	0.510	0.362	0.147	67.596	Viable
Gamma	0.853	0.497	0.333	0.182	66.839	Viable
LogLogistic	0.852	0.510	0.362	0.147	67.596	Viable
Multistage 1°	0.270	0.191	0.167	0.063	71.876	Questionable P-value ^a
Multistage 2°	0.676	0.395	0.273	0.373	65.09	Viable
Multistage 3°	0.863	0.369	0.268	0.226	66.134	Viable
Multistage 4°	0.825	0.330	0.254	0.104	67.783	Viable
Weibull	0.886	0.501	0.348	0.211	66.225	Viable
Logistic	0.760	0.546	0.476	0.364	64.737	Viable

Supplemental Information—Formaldehyde (Inhalation)

Model	BMC (ppm)	BMCL (95%)	BMCL (99%)	P-value	AIC	Notes
LogProbit	0.850	0.541	0.399	0.159	67.254	Viable
Probit	0.694	0.502	0.441	0.369	64.645	Viable, Lowest AIC ^b
Quantal Linear	0.270	0.191	0.167	0.063	71.876	Questionable P-value ^a

^aGoodness of fit *p* value was less than 0.1, so these results were non-preferred (gray). Other models were viable.

^bBMDS recommended and EPA selected model (green highlighting), with the selected POD bolded.

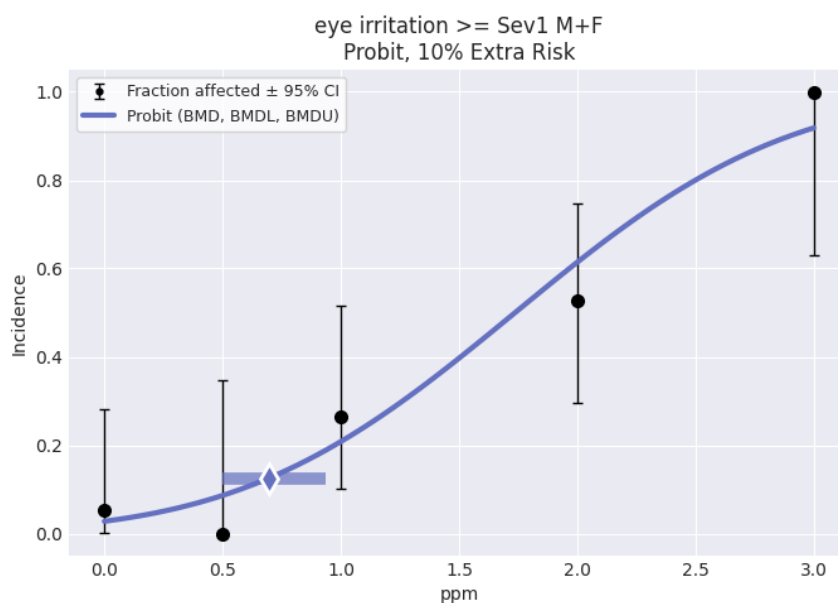


Figure D-6. Selected BMDS model results (Probit model) for eye irritation (incidence) versus ppm formaldehyde exposure (ppm) in male and female adults (Kulle et al., 1993; 1987). BMD = BMC.

Table D-11 BMD modeling summary of nose or throat irritation in females

Model	BMC (ppm)	BMCL (95%)	BMCL (99%)	P-value	AIC	Notes
Hill	1.693	0.85	0.368	0.34	40.27	Viable
Gamma	1.369	0.601	0.292	0.208	41.493	Viable
LogLogistic	1.354	0.624	0.264	0.21	41.471	Viable
Multistage 1°	0.564	0.271	0.214	0.146	42.829	Viable, Lowest BMCL ^a
Multistage 2°	0.977	0.388	0.256	0.295	40.683	Viable
Multistage 3°	1.267	0.453	0.274	0.332	40.021	Viable, Lowest AIC ^b
Multistage 4°	1.267	0.448	0.274	0.332	40.021	Viable, Lowest AIC
Weibull	1.296	0.495	0.274	0.178	42.015	Viable
Logistic	0.758	0.493	0.414	0.213	41.417	Viable
LogProbit	1.37	0.697	0.236	0.231	41.222	Viable

Supplemental Information—Formaldehyde (Inhalation)

Model	BMC (ppm)	BMCL (95%)	BMCL (99%)	P-value	AIC	Notes
Probit ^a	0.715	0.467	0.396	0.205	41.608	Viable
Quantal Linear	0.564	0.271	0.214	0.146	42.829	Viable

All models were viable.

^aBMD5 recommended model based on range of 95% BMCL values being > 3-fold (note: range of 99% BMCL values within 3-fold)

^bSelected model (green highlighting) for the 99% BMCL values, with the selected POD bolded. An identical POD would be estimated using a more complex model with the same AIC.

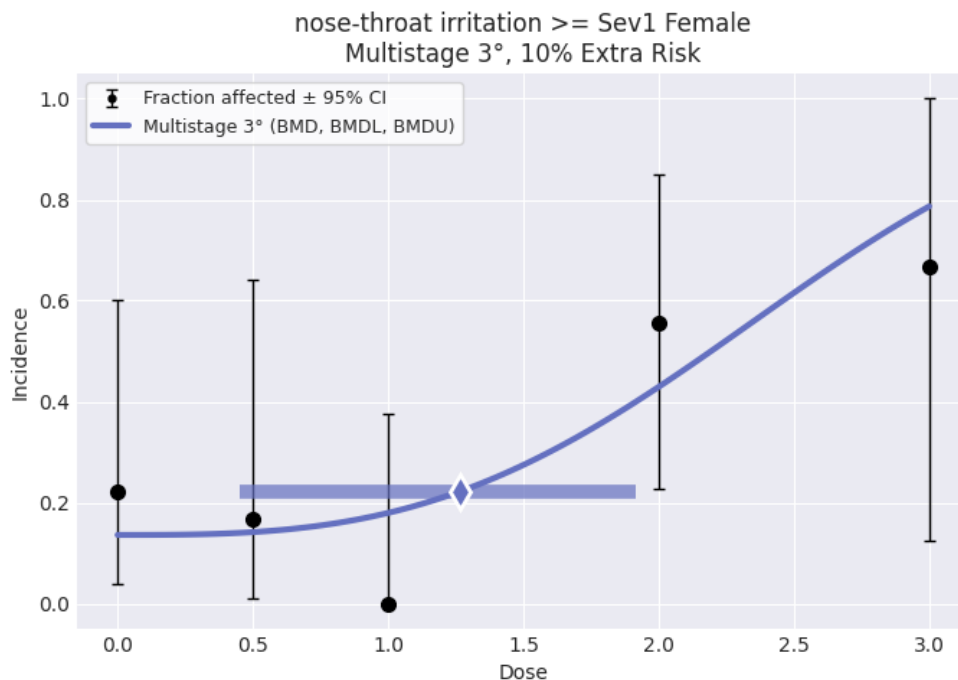


Figure D-7. Selected BMD5 model results (Multistage 3° model) for nose or throat irritation (incidence) versus ppm formaldehyde exposure (dose) in female adults (Kulle et al., 1993; 1987). BMD =BMC.

Andersen and Molhave (1983, 1979)

Another experimental study exposed a group of 16 subjects to 0.3, 0.5, 1.0, and 2.0 mg/m³ formaldehyde for 5-hour periods with a 2-hour clean air exposure prior to each trial ([Andersen, 1979](#); [Andersen and Molhave, 1983](#)). The order of exposure concentrations was randomized. The occurrence of irritation symptoms during the clean air exposure was not reported. The results of the different modeling approaches are summarized in Table D-12 and explained below.

Table D-12. Modeling summary for irritation symptoms in ([Andersen, 1979](#); [Andersen and Molhave, 1983](#))

Approach	Endpoint	Selected Model ^a	BMC (mg/m ³)	BMCL (95%)	BMCL (99%)	Notes
0% Background Prevalence	Prevalence of conjunctival redness and nose or throat dryness	LogLogistic	0.26	0.16	0.11	
3% Background Prevalence	Prevalence of conjunctival redness and nose or throat dryness	LogLogistic	0.28	0.17	0.12	Selected POD (bolded)
6% Background Prevalence	Prevalence of conjunctival redness and nose or throat dryness	LogLogistic	0.30	0.18	0.13	
9% Background Prevalence	Prevalence of conjunctival redness and nose or throat dryness	Hill	0.45	0.25	0.18	

^aThe selected model for each approach was the viable model with the lowest AIC (see details below).

The datasets modeled from this study ([Andersen, 1979](#); [Andersen and Molhave, 1983](#)) are presented in Table D-13 below.

Table D-13. Sensory irritation symptom data from Andersen and Molhave (1983, 1979)

	Formaldehyde Level (mg/m ³)				
	0	0.3	0.5	1.0	2.0
Prevalence of Discomfort^a (%)	Not reported ^b	3/16 (19%)	5/16 (31%)	15/16 (94%)	15/16 (94%)

^aSymptoms characterized as conjunctival irritation and dryness in the nose and throat.

^bClean-air exposure symptoms were not measured; Modeling assumed either 0% (incidence of 0/16 in the control group), 3% (incidence of 0.46/16 in the control group), 6%, or 9% background prevalence of symptoms.

BMD modeling was performed assuming a background prevalence of symptoms in controls of either 0% (see Table D-14 and Figure D-8 for results), 3% (see Table D-15 and Figure D-9 for results), 6% (see Table D-16 and Figure D-10 for results), or 9% (see Table D-17 and Figure D-11 for results).

Table D-14. BMD modeling summary of conjunctival irritation and dryness in the nose and throat dryness in males and females; 0 % background prevalence

Model	BMC (mg/m ³)	BMCL (95%) (mg/m ³)	BMDL (99%) (mg/m ³)	P-value	AIC	Notes
Hill	0.281	0.167	0.119	0.295	58.823	Viable

Supplemental Information—Formaldehyde (Inhalation)

Model	BMC (mg/m ³)	BMCL (95%) (mg/m ³)	BMDL (99%) (mg/m ³)	P-value	AIC	Notes
Gamma	0.208	0.091	0.062	0.195	58.846	Viable, Large ratio ^a
LogLogistic	0.257	0.157	0.112	0.401	57.33	Viable, Lowest AIC^b
Multistage 1°	0.08	0.06	0.053	0.152	60.262	Viable, Large ratio ^a
Multistage 2°	0.138	0.068	0.057	0.114	60.321	Viable, Large ratio ^a
Multistage 3°	0.138	0.068	0.057	0.114	60.321	Viable, Large ratio ^a
Multistage 4°	0.138	0.068	0.057	0.114	60.321	Viable, Large ratio ^a
Weibull	0.169	0.077	0.06	0.17	59.527	Viable, Large ratio ^a
Logistic	0.256	0.182	0.158	<0.001	62.408	Questionable P-value ^c
LogProbit	0.249	0.153	0.11	0.32	57.965	Viable
Probit ^a	0.239	0.175	0.155	<0.001	65.167	Questionable P-value ^c
Quantal Linear	0.08	0.06	0.053	0.152	60.262	Viable, Large ratio ^a

^aLowest dose/BMCL and/or lowest dose/BMC ratio greater than 3, but results recommended as viable (no shading)

^bBMDS recommended model and Selected model (green highlighting), with the selected POD bolded.

^cGoodness of fit *p* value was less than 0.1, so these results were non-preferred (gray).

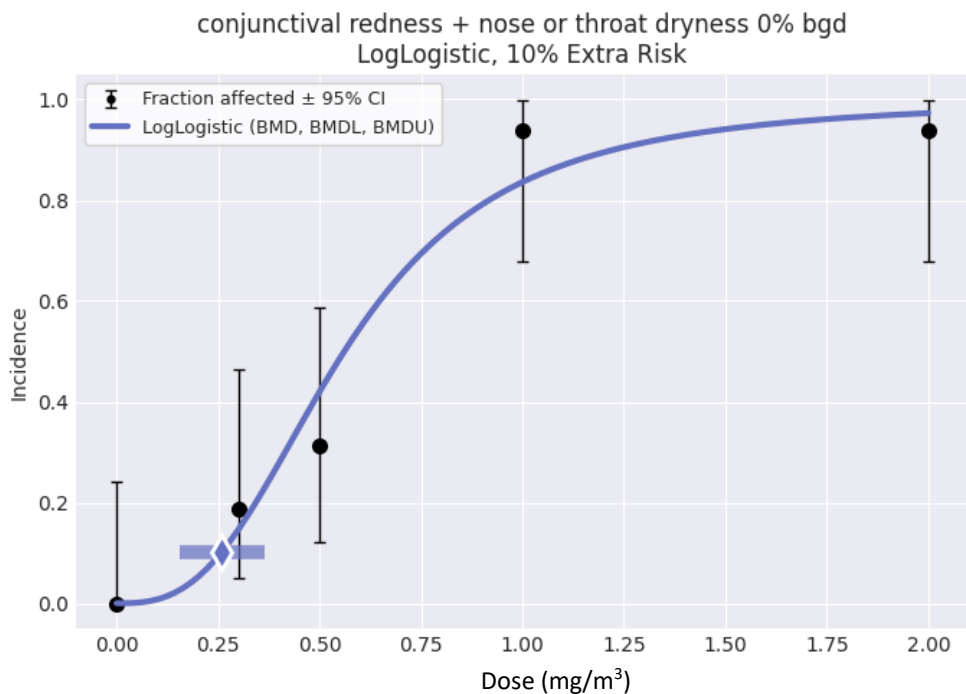


Figure D-8. Selected BMDS model results (LogLogistic model) for conjunctival irritation and dryness in the nose and throat dryness (incidence) versus mg/m^3 formaldehyde exposure (dose) in male and female adults assuming a 0% background prevalence (Andersen and Molhave, 1983, 1979). BMD = BMC.

Table D-15. BMD modeling summary of conjunctival irritation and dryness in the nose and throat in males and females; 3% background prevalence

Model	BMC (mg/m^3)	BMCL (95%) (mg/m^3)	BMDL (99%) (mg/m^3)	P-value	AIC	Notes
Hill	0.372	0.188	0.134	0.155	64.613	Viable
Gamma	0.224	0.099	0.064	0.088	65.24	Questionable P-value and ratio ^a
LogLogistic	0.279	0.167	0.12	0.23	63.536	Viable, Lowest AIC ^b
Multistage 1°	0.082	0.06	0.054	0.069	66.961	Questionable P-value and ratio ^a
Multistage 2°	0.151	0.071	0.059	0.045	66.808	Questionable P-value and ratio ^a
Multistage 3°	0.151	0.071	0.059	0.045	66.808	Questionable P-value and ratio ^a
Multistage 4°	0.151	0.071	0.059	0.045	66.808	Questionable P-value and ratio ^a
Weibull	0.179	0.082	0.061	0.074	66.01	Questionable P-value and ratio ^a

Supplemental Information—Formaldehyde (Inhalation)

Model	BMC (mg/m ³)	BMCL (95%) (mg/m ³)	BMDL (99%) (mg/m ³)	P-value	AIC	Notes
Logistic	0.244	0.174	0.152	0.002	65.032	Questionable P-value ^a
LogProbit	0.266	0.161	0.116	0.169	64.262	Viable
Probit ^a	0.229	0.169	0.15	0.003	67.549	Questionable P-value ^c
Quantal Linear	0.082	0.06	0.054	0.069	66.961	Questionable P-value and ratio ^a

^aGoodness of fit *p* value was less than 0.1 and lowest dose/BMCL ratio and/or lowest dose/BMC ratio greater than 3, so these results were non-preferred (gray).

^bBMDs recommended model and Selected model (green highlighting), with the selected POD bolded.

^cGoodness of fit *p* value was less than 0.1, so these results were non-preferred (gray).

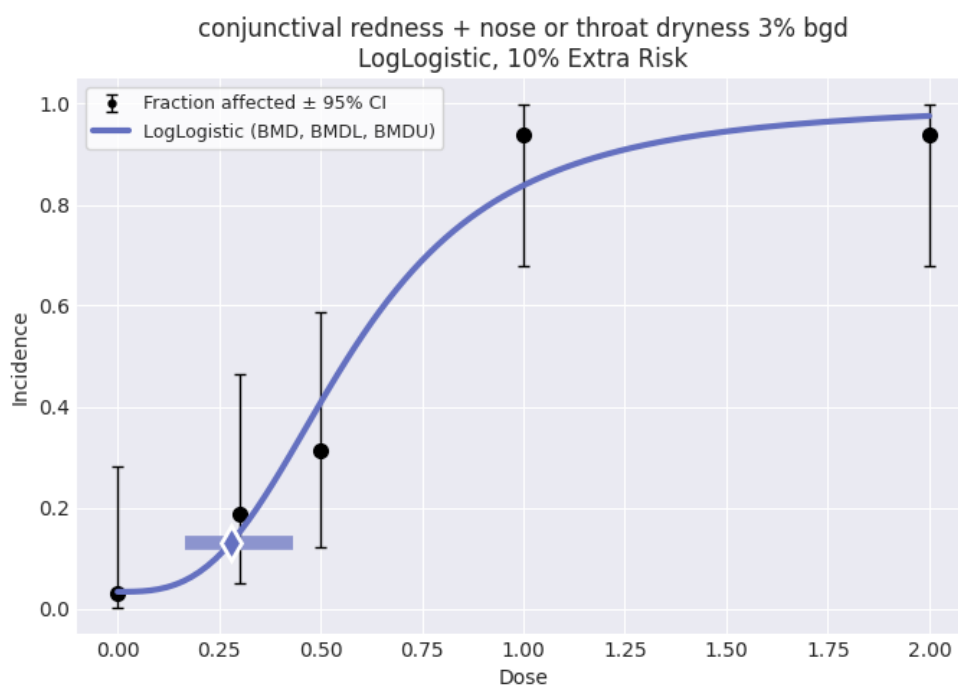


Figure D-9. Selected BMDS model results (LogLogistic model) for conjunctival irritation and dryness in the nose and throat dryness (incidence) versus mg/m³ formaldehyde exposure (dose) in male and female adults assuming a 3% background prevalence (Andersen and Molhave, 1983, 1979). BMD = BMC.

Table D-16. BMD modeling summary of conjunctival irritation and dryness in the nose and throat in males and females; 6 % background prevalence

Model	BMC (mg/m ³)	BMCL (95%) (mg/m ³)	BMDL (99%) (mg/m ³)	P-value	AIC	Notes
Hill	0.43	0.223	0.157	0.284	66.712	Viable
Gamma	0.24	0.107	0.067	0.081	68.294	Questionable P-value ^a

Supplemental Information—Formaldehyde (Inhalation)

Model	BMC (mg/m ³)	BMCL (95%) (mg/m ³)	BMDL (99%) (mg/m ³)	P-value	AIC	Notes
LogLogistic	0.3	0.178	0.128	0.223	66.407	Viable, Lowest AIC ^b
Multistage 1°	0.083	0.061	0.054	0.058	70.321	Questionable P-value and ratio ^a
Multistage 2°	0.166	0.074	0.06	0.038	69.945	Questionable P-value and ratio ^a
Multistage 3°	0.166	0.074	0.06	0.038	69.945	Questionable P-value and ratio ^a
Multistage 4°	0.166	0.074	0.06	0.038	69.945	Questionable P-value and ratio ^a
Weibull	0.189	0.087	0.063	0.068	69.155	Questionable P-value and ratio ^a
Logistic	0.233	0.168	0.147	0.01	67.49	Questionable P-value ^a
LogProbit	0.284	0.17	0.124	0.162	67.217	Viable
Probit ^a	0.22	0.164	0.146	0.01	69.778	Questionable P-value ^c
Quantal Linear	0.083	0.061	0.054	0.058	70.321	Questionable P-value and ratio ^a

^aGoodness of fit *p* value was less than 0.1 and lowest dose/BMCL ratio and/or lowest dose/BMC ratio greater than 3, so these results were non-preferred (gray).

^bBMDS recommended model and Selected model (green highlighting), with the selected POD bolded.

^cGoodness of fit *p* value was less than 0.1, so these results were non-preferred (gray).

Supplemental Information—Formaldehyde (Inhalation)

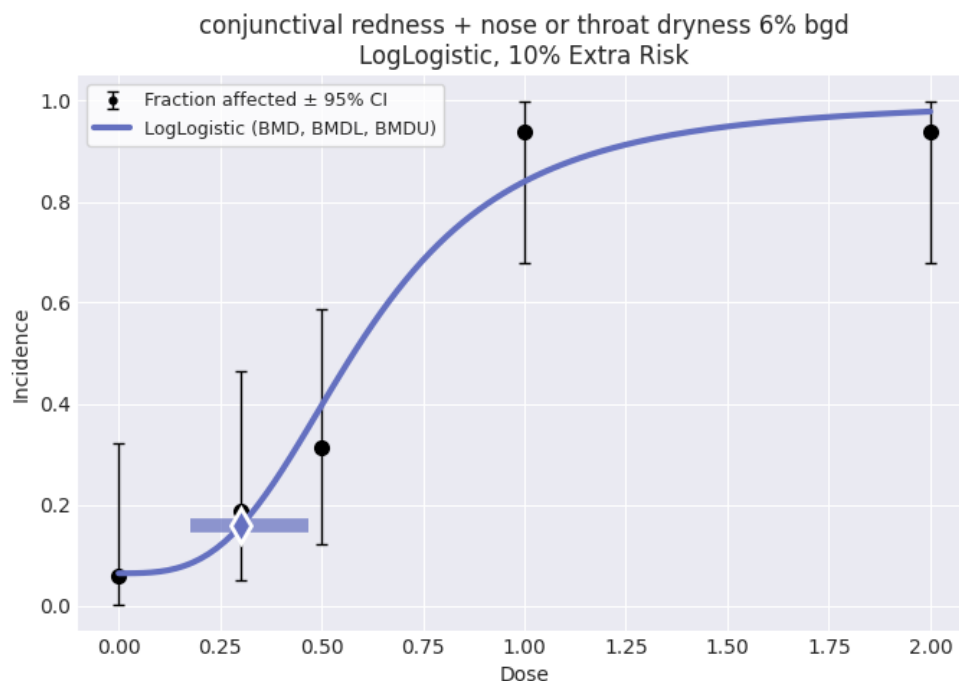


Figure D-10. Selected BMDS model results (LogLogistic model) for conjunctival irritation and dryness in the nose and throat dryness (incidence) versus mg/m³ formaldehyde exposure (dose) in male and female adults assuming a 6% background prevalence (Andersen and Molhave, 1983, 1979). BMD = BMC.

Table D-17. BMD modeling summary of conjunctival irritation and dryness in the nose and throat in males and females; 9% background prevalence

Model	BMC (mg/m ³)	BMCL (95%) (mg/m ³)	BMDL (99%) (mg/m ³)	P-value	AIC	Notes
Hill	0.446	0.252	0.177	0.435	68.576	Viable, Lowest AIC ^b
Gamma	0.256	0.115	0.069	0.073	70.838	Questionable P-value ^a
LogLogistic	0.32	0.188	0.137	0.209	68.787	Viable
Multistage 1°	0.085	0.062	0.055	0.049	73.168	Questionable P-value and ratio ^a
Multistage 2°	0.183	0.077	0.062	0.032	72.56	Questionable P-value and ratio ^a
Multistage 3°	0.183	0.077	0.062	0.032	72.56	Questionable P-value and ratio ^a
Multistage 4°	0.183	0.077	0.062	0.032	72.56	Questionable P-value and ratio ^a
Weibull	0.2	0.091	0.065	0.061	71.792	Questionable P-value and ratio ^a
Logistic	0.224	0.162	0.142	0.025	69.796	Questionable P-value ^a

Supplemental Information—Formaldehyde (Inhalation)

Model	BMC (mg/m ³)	BMCL (95%) (mg/m ³)	BMDL (99%) (mg/m ³)	P-value	AIC	Notes
LogProbit	0.301	0.179	0.131	0.153	69.666	Viable
Probit ^a	0.213	0.16	0.142	0.02	71.87	Questionable P-value ^c
Quantal Linear	0.085	0.062	0.055	0.049	73.168	Questionable P-value and ratio ^a

^aGoodness of fit *p* value was less than 0.1 and lowest dose/BMCL ratio and/or lowest dose/BMC ratio greater than 3, so these results were non-preferred (gray).

^bBMDS recommended model and Selected model (green highlighting), with the selected POD bolded.

^cGoodness of fit *p* value was less than 0.1, so these results were non-preferred (gray).

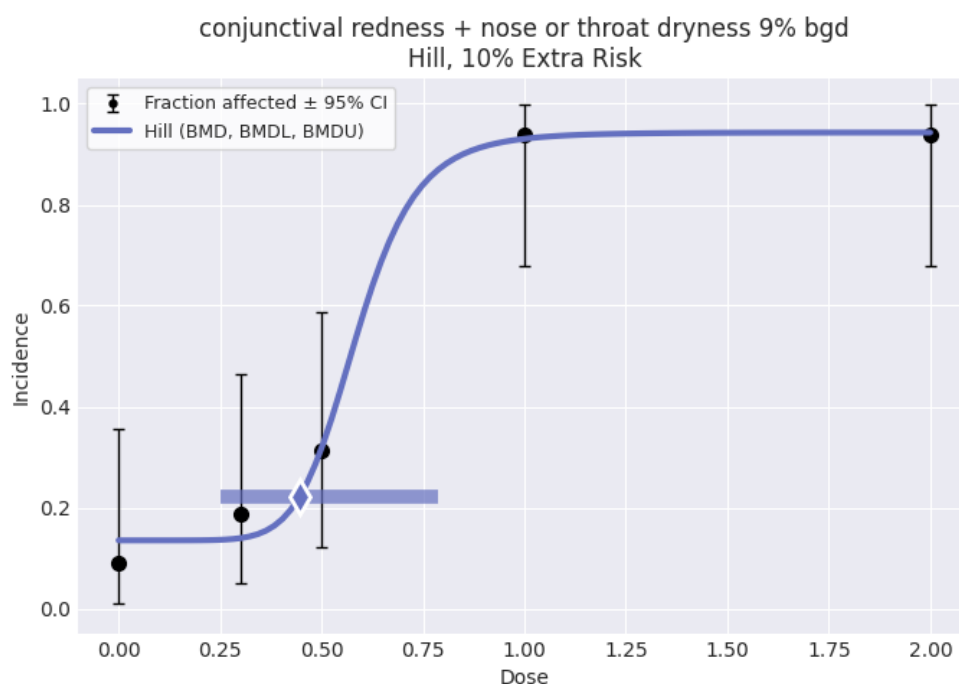


Figure D-11. Selected BMDS model results (Hill model) for conjunctival irritation and dryness in the nose and throat dryness (incidence) versus mg/m³ formaldehyde exposure (dose) in male and female adults assuming a 9% background prevalence (Andersen and Molhave, 1983, 1979). BMD = BMC.

D.1.2. Pulmonary Function

Estimates from Observational Epidemiology Studies

A cross-sectional study of residential formaldehyde exposure in a large population-based sample observed a linear relationship between increased formaldehyde exposure and decreased peak expiratory flow rate (PEFR) among children exposed to average concentrations of 0.032 mg/m³ (26 ppb) ([Krzyzanowski et al., 1990](#)). This study of effects in a residential population used a thorough exposure assessment protocol and repeated measurements of PEFR, thus, enhancing the

ability to detect an association at the lower concentrations found in the homes. Declines in peak expiratory flow rate (PEFR) were associated with increases in 2-week average indoor residential formaldehyde concentrations, with greater declines observed in children (5–15 years of age, $n = 208$ in analytical data set) compared to adults ([Krzyzanowski et al., 1990](#)). Mean formaldehyde levels were 26 ppb (0.032 mg/m³), and more than 84% of the homes had concentrations 40 ppb (0.049 mg/m³) and lower.

EPA calculated the concentration at which a 10% decrement in pulmonary function would be expected. In this derivation, 10% decrement in a continuous response is considered to be the benchmark response (BMR). A BMC_{10%} and BMCL_{10%} were determined from the regression coefficient from a random effects model of PEFR among children reported by the study authors. Statistical models which adjusted for important covariates (including smoking status, SES, NO₂ levels, episodes of acute respiratory illness, and the time of day) did not identify any potential confounders and those covariates were not included in the final model.

$$\begin{aligned}
 y = & 349.6 - 1.28 * (\text{household formaldehyde}) - 6.1 * (\text{morning}) + 0.09 \\
 & * (\text{bedroom formaldehyde}) * (\text{morning}) + 0.0031 * (\text{bedroom formaldehyde})^2 \\
 & * (\text{morning}) + 4.59 * (\text{morning}) * (\text{asthma}) - 1.45 * (\text{bedroom formaldehyde}) \\
 & * (\text{morning}) * (\text{asthma}) + 0.031 (\text{bedroom formaldehyde})^2 * (\text{morning}) \\
 & * (\text{asthma})
 \end{aligned}
 \tag{D-11}$$

where y = PEFR (L/min); household formaldehyde = 2-week household mean concentration; morning = time of PEFR measurement (0,1); 2-week bedroom mean concentration; current asthma = doctor's diagnosis and current status (0,1).

For the purpose of deriving a point of departure for indoor formaldehyde, the primary estimate of the point of departure was computed for household formaldehyde with *morning* = 0 and *asthma* = 0. The regression coefficient (β) for household formaldehyde was -1.28 ± 0.46 L/minute-ppb and the 95% one-sided upper bound on the regression coefficient was -2.04 L/minute-ppb;

$$\begin{aligned}
 \beta - (\text{critical value for one-tailed } \alpha \text{ of } 0.05 * \text{s.e. of } \beta) = & -1.28 - (1.645 * \\
 & 0.46) = -2.04
 \end{aligned}
 \tag{D-12}$$

Based on the background PEFR of 349.6 L/minute, a 10% decrement is 35 L. Dividing 35 L by the regression coefficient for household formaldehyde of -1.28 L/minute-ppb (i.e., -1.28 L/(minute*ppb)), the change in formaldehyde concentration resulting in a 10% decrement in PEFR is 27 ppb which is equivalent to 0.033 mg/m³. The BMCL resulting in a 10% decrease from a background of 349.6 L/minute is 17 ppb (35 L/minute divided by -2.04 L/minute-ppb), which is equivalent to 0.021 mg/m³.

Supplemental Information—Formaldehyde (Inhalation)

In order to estimate how much more sensitive asthmatic children were to formaldehyde, household and bedroom formaldehyde concentrations were assumed to be the same and *morning* = 1 and *asthma* = 1. Solving the final regression model for these realizations of *household formaldehyde*, *bedroom formaldehyde*, *morning*, and *asthma* yield the following:

$$\begin{aligned} -35 \text{ L/min} = & -1.28 * (\text{household formaldehyde}) - 6.1 * (1) + 0.09 \\ & * (\text{household formaldehyde}) * (1) + 0.0031 * (\text{household formaldehyde})^2 * (1) \\ & + 4.59 * (1) * (1) - 1.45 * (\text{household formaldehyde}) * (1) * (1) \\ & + 0.031 (\text{household formaldehyde})^2 * (1) * (1) \end{aligned} \quad (\text{D-13})$$

which simplifies to:

$$-35 \frac{\text{L}}{\text{min}} = 0.0341 * (\text{household formaldehyde})^2 - 2.64 * (\text{household formaldehyde}) - 1.51 \quad (\text{D-14})$$

Solving for *household formaldehyde* yields a BMC_{10%} (asthmatics) resulting in a 10% decrease from a background PEFR of 349.6 L/minute of 16 ppb given that asthmatic children were more sensitive to the respiratory effects of formaldehyde exposure than were children in general who had BMC_{10%} of 27 ppb.

D.1.3. Asthma Exacerbation

Estimates from Observational Epidemiology Studies

[Venn et al. \(2003\)](#) studied how indoor formaldehyde exposures affected the proportion of childhood asthma cases who reported symptoms of asthma attacks (asthma exacerbation). During an asthma attack, the muscles of the airways constrict thereby limiting air flow and the cells in the airway produce mucus which further restricts the passage of air. Symptoms included any of the following: wheezing, chest tightness, breathlessness, or cough ([Venn et al., 2003](#)). According to the Centers for Disease Control and Prevention ([Moorman et al., 2012](#)), more than 50% of children with asthma experienced at least one asthma attack in the previous 12 months yielding an annual rate of asthma attacks in the general population of children of more than 5%. Approximately 10% of children with asthma suffer an asthma attack resulting in a visit to the emergency room each year. The annual mortality rate from asthma among children is 2–3 per million ([Moorman et al., 2012](#)).

[Venn et al. \(2003\)](#) (see Table D-18) divided the children's bedroom formaldehyde exposures into quartiles and reported a statistically significant exposure-response trend of increasing risk of symptoms of an asthma attack with increasing quartiles of formaldehyde concentrations ($p = 0.03$) and then fit a regression model to estimate the “per quartile” increase in risk. Venn et al. ([2003](#)) identified similar exposure-response functions for night-time and daytime

symptoms of an asthma attack (asthma exacerbation) in children with asthma³⁰: for night-time symptoms, the odds ratio (OR) per exposure quartile increase in formaldehyde concentration was 1.45 (95% CI: 1.06–1.98); for daytime symptoms, the OR per exposure quartile was 1.40 (95% CI: 1.00–1.94)³¹. Results were adjusted for age, sex, and socioeconomic status. Dampness was also reported to be a risk factor for symptoms of an asthma attack; however, further adjustment of the formaldehyde results for dampness made little difference (Venn et al., 2003). No effect of other volatile organic compounds or nitrogen dioxide on the risk of asthma attacks was found.

As the formaldehyde measures were taken in the children’s bedrooms, the RfC derivation is based on the exposure-response function for night-time symptoms of an asthma attack. The following table summarizes the results from Venn et al. (2003) specific to the exposure-response relationship for night-time symptoms of asthma attacks in children with asthma. Note that, by definition, the OR reported for each exposure level is relative to the odds of being a case in the reference category, which is the lowest quartile of exposure. In Venn et al. (2003), the reference category is defined as exposures within the range 0–16 µg/m³. The median concentration within this range was 12.24 µg/m³ (Venn, 2012). In order to estimate the OR per unit increase in formaldehyde concentration from the reported effect per unit increase in quartile of formaldehyde exposure, the difference in each quartile’s median formaldehyde concentration was computed by subtracting 12.24 µg/m³ from each quartile median.

Table D-18. Modeled effect estimates for night-time symptoms of an asthma attack; data from (Venn et al., 2003)

Exposure quartile ^a (µg/m ³)	Quartile median ^b (µg/m ³)	Quartile median > reference quartile (µg/m ³)	OR by quartile ^a	Lower bound OR by quartile	Upper bound OR by quartile	Modeled OR ^c	Lower bound modeled OR ^c	Upper bound modeled OR ^c
0–16	12.24	0	1			1		
16.1–22	19.23	6.99	1.4	0.54	3.62	1.45	1.06	1.98
22.1–32	26.55	14.31	1.61	0.62	4.19	2.10	1.12	3.92
32+	41.02	28.78	3.33	1.23	9.01	3.05	1.19	7.73

^a(Venn et al., 2003);

^b(Venn, 2012);

^c(Venn et al., 2003) OR per increasing quartile = 1.45 (95% CI: 1.06–1.98).

³⁰Cases were defined as those whose doctors had prescribed asthma drug treatment at the time of the study (including the preceding year) (Venn et al., 2003).

³¹Exposure measurements, pulmonary function measurements, and symptoms of asthma attacks were measured over a 4-week period.

EPA considered multiple methodologies for identifying a point of departure for this health endpoint. If the information provided by Venn et al. (2003) had been limited to just the quartile-specific results, then the one method might have used the results from Table D-18 of Venn et al. (2003) which show the first statistically significant effect occurring in the highest exposure group with a quartile mean of 41.02 $\mu\text{g}/\text{m}^3$ which could represent the LOAEL and thus the corresponding NOAEL could be the quartile mean of the third exposure group at 26.55 $\mu\text{g}/\text{m}^3$. However, because Venn et al. (2003) also reported a statistically significant exposure-response function (p -trend = 0.02) with OR=1.45 per exposure quartile (95% CI: 1.06–1.98), it is not reasonable to assume there is no effect at the median of the third quartile because the reported OR for this quartile was 1.61 (95% CI: 0.62–4.19) and the reported exposure-response function corresponds to a modeled OR=2.10 (95% CI: 1.12–3.92). Likewise, for the second quartile with a quartile-specific result of OR=1.4 (95% CI: 0.54–3.62), rather than evidence of “no effect,” the reported exposure-response function indicates a modeled OR = 1.45 (95% CI: 1.06–1.98), which is consistent with the second quartile-specific results of OR = 1.4 but has narrower confidence intervals due to the use of data from all the quartiles rather than just a comparison of the second quartile to the first.

The reported exposure-response function from Venn et al. (2003) appears to be a more precise estimate of the exposure-response relationship for night-time symptoms of poor asthma control in children with asthma. In order to estimate a point of departure, the units of ‘per quartile’ need to be defined in terms of “per $\mu\text{g}/\text{m}^3$.” As the magnitude of the increase in exposure from the median of the first quartile to the median of the second quartile is 6.99 $\mu\text{g}/\text{m}^3$, an estimate of the effect of exposure per $\mu\text{g}/\text{m}^3$ can be obtained by scaling the $\ln(\text{OR})$ and its standard error by the difference in quartile medians. The OR = 1.45 per quartile (95% CI: 1.06–1.98) is first converted to the natural log scale as $\ln(\text{OR}) = 0.37156$ per quartile (95% CI: 0.05827–0.68310), and then each term is multiplied by unity as expressed by $[(1 \text{ quartile})/(6.99 \mu\text{g}/\text{m}^3)]$ to yield an effect of $\ln(\text{OR}) = 0.053156$ (95% CI: 0.008336–0.09773), which when exponentiated back to the OR scale is equivalent to an OR = 1.05 per $\mu\text{g}/\text{m}^3$ (95% CI: 1.01–1.10). This equivalent exposure-response function in terms of “per $\mu\text{g}/\text{m}^3$ ” retains the same p -trend value of 0.02 because the scaling cancels out.

According to Table B-8 in Venn et al. (2003), the prevalence of night-time asthma symptoms among the cases in the reference group is 0.41. Because the symptoms of an asthma attack among children with asthma is considered to be a frank effect (an overt or clinically apparent effect), a BMR of 5% was used to derive the POD for the derivation of the RfC (U.S. EPA, 2012). Using a BMR=5% extra risk for symptoms of an asthma attack, the prevalence of symptoms among the exposed at 5% extra risk compared to the prevalence of symptoms at zero exposure is:

$$\text{Extra Risk} = 0.05 = [P_{\text{Exposed}} - P_{\text{Unexposed}}] \div [1 - P_{\text{Unexposed}}] \text{ and } P_{\text{Unexposed}} = 0.41, \text{ then } P_{\text{Exposed}} = 0.4395.$$

(D-15)

$$\begin{aligned}\text{Find OR} &= [P_{\text{Exposed}}/(1 - P_{\text{Exposed}})]/[P_{\text{Unexposed}}/(1 - P_{\text{Unexposed}})] \\ &= [0.4395/(1 - 0.4395)]/[0.41/(1 - 0.41)] = 1.13\end{aligned}$$

(D-16)

For the derivation of the point of departure, here the benchmark concentration or BMC, note that the exposure-response function is defined relative to the reference group (those exposed to the first quartile of formaldehyde exposures) which experienced a median formaldehyde concentration of 12.24 µg/m³ ([Venn, 2012](#)), personal communication personal communication. So, in deriving the BMC, the first step is to estimate the magnitude of the concentration above the reference concentration of 12.24 µg/m³, which corresponds to a 5% extra risk. For clarity, that value will be called the “interim BMC₀₅.” The second step is to add that interim BMC₅ to the median formaldehyde concentration in the reference group. While it is possible that there are adverse effects of formaldehyde below the median formaldehyde concentration in the reference group, it should be understood that the methodology used in this derivation restricts the BMC to be greater than the median formaldehyde concentration in the reference group. The alternative would be to extrapolate the exposure-response function down from 12.24 µg/m³ to either the background ambient formaldehyde concentration, or down to a concentration of zero.

To derive the interim BMC using the linear concentration-response function, solve for:

$$\text{OR corresponding to a 5\% extra risk} = 1.13 = (1.05 \text{ per } \mu\text{g}/\text{m}^3) * (\text{Interim BMC}_5)$$

$$\text{Interim BMC}_5 = 1.08 \mu\text{g}/\text{m}^3$$

To derive the interim BMCL using the linear concentration-response function, the one-sided 95% upper bound is needed (rather than the upper bound of the two-sided 95% CI around the OR). Using the one-sided 95% upper bound, which is 1.09 (calculation below)³², solve for:

$$\text{OR corresponding to a 5\% extra risk} = 1.13 = (1.09 \text{ per } \mu\text{g}/\text{m}^3) * (\text{Interim BMCL}_5)$$

$$\text{Interim BMCL}_5 = 1.04 \mu\text{g}/\text{m}^3$$

Adding back the median formaldehyde concentration in the reference category (12.24 µg/m³), the BMCL₅ value is 13.28 µg/m³ and this value is selected as the point of departure for the cRfC.

³²To calculate the standard error of the ln(OR): $[(\ln(1.10) - \ln(1.01))/3.92] = 0.02178$. Therefore, the 95% one-sided upper bound of the ln(OR) is $[\ln(\text{OR}) + 1.645(0.02178)] = 0.08461$ and the 95% one-sided upper bound of the OR is 1.09.

D.1.4. Respiratory Tract Pathology

Estimates from Experimental Animal Studies

This appendix provides support to the decisions and details of modeling the respiratory pathology data in rats and mice in Section 5.1.2 for deriving candidate RfCs based on these endpoints. These involve the following endpoints and studies: squamous metaplasia in F344 rats ([Kerns et al., 1983](#)), basal hyperplasia in Wistar rats ([Woutersen et al., 1989](#)), and squamous metaplasia in Wistar rats ([Woutersen et al., 1989](#)).

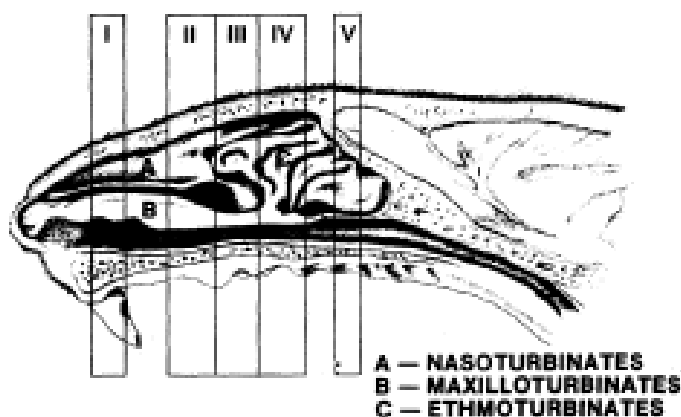


Figure D-12. Midsagittal section of rat nose showing section levels from ([Kerns et al., 1983](#)) (nostril is to the left).

Formaldehyde flux to the nasal lining was used in analyzing the dose-response data from Kerns et al. ([1983](#)) at the Level 1 cross section (Figure D-12) of the F344 rat nose, which is located in the front portion of the rat nose behind the nasal vestibule ([Young, 1981](#)). The 18-month data was chosen for modeling; while it would have been preferable to use the 24-month dose-response, these data could not be modeled because of a very steeply rising dose-response curve resulting in unbounded confidence limits (see Table D-19).

Table D-19. Incidence of squamous metaplasia in nasal cavity of F344 rats

Level I				
Duration	0 mg/m ³	2.5 mg/m ³	6.9 mg/m ³	17.6 mg/m ³
6 months	NA	4/20	10/20	NA
12 months	NA	7/20	11/20	NA
18 months	0/40	24/40	35/40	38/39
24 months	1/101	91/94	81/82	27/27
27 months	3/19	4/20	8/19	5/5
30 months	1/10	2/5	1/8	NR

[Kimbell et al. \(2001b\)](#) modeled formaldehyde flux to the nasal lining in the F344 rat and human; their flux estimates are shown in Figure D-13 as a contour plot of flux per ppm of exposure (note: only the lateral view of the three-dimensional surface is presented). The plot in the top panel of the figure indicates that formaldehyde flux per ppm of exposure to the surface of the Level 1 section would correspond to the upper range (greater than approximately 1,750 pmol/mm²-h-ppm) of flux estimates per ppm exposure. [\(Kimbell et al., 2001b\)](#) divided their total flux (per ppm of exposure) range in the rat into 20 flux bins with the mean flux in bin 14 equal to 1,764 pmol/mm²-hour-ppm of exposure (see Table 1, [\(Kimbell et al., 2001b\)](#)). Therefore, we use flux estimates from flux bins 14-20 of their paper; the surface-area-weighted average flux per ppm of exposure in these flux intervals is 1,879.66 pmol/mm²-hour per ppm (i.e., 1,528.18 pmol/mm²-hour per mg/m³) of exposure. Therefore, average flux in the Level 1 region corresponding to the BMCL₁₀ of 0.448 mg/m³ is estimated to be $1,528.18 \times 0.448 = 685$ pmol/mm²-hour.

In order to extrapolate the above BMCL to the human, one is interested in knowing the human exposure concentration at which some region in the human nose (see Figure D-13) is exposed to a formaldehyde flux of 685 pmol/mm²-hour. This is estimated from Table 3 in [\(Kimbell et al., 2001b\)](#), which tabulates formaldehyde flux to the human nasal lining at various inspiratory rates. At any given exposure, the anterior regions of the nose are subject to the highest concentrations of formaldehyde; therefore, we averaged the data from flux bins 17-20 in their tabulation, which receive the highest levels of flux. The average flux per ppm of exposure concentration in bins 17-20 in the human is 1,741 pmol/mm²-hour per ppm of exposure. Thus, the exposure concentration at which these regions would receive a flux of 685 pmol/mm²-hr is 0.484 mg/m³. This is the human BMCL corresponding to 0.10 extra risk, which was selected because the observed squamous metaplasia was determined to be of minimal-to-mild adversity. As an aside, it is instructive to note that the values for the rat and human BMCLs are roughly the same, a result that one would expect if the principle of ppm-equivalence across species is applied.

As discussed in Section 3.2.4 of the Toxicological Review, squamous metaplasia occurred in several sagittal cross sections (Level 1-5, depicted in Figure D-13) of the F344 rat nose in the Kerns et al. [\(1983\)](#) study. However, accurate estimates of formaldehyde flux over the nasal lining other than Level 1 were not available to EPA, and flux estimates provided in [Kimbell et al. \(2001b\)](#) cannot be reliably used for the other cross-sections because of a lack of correspondence with the nasal regions in their paper. Therefore, only the squamous metaplasia data reported for Level 1 was carried forward in calculating a candidate RfC. Details of benchmark dose modeling for data on squamous metaplasia in F344 rat and squamous metaplasia and basal hyperplasia in Wistar rat are shown in Table D-20 and Figures D-14 to D-16.

Table D-20. Benchmark dose modeling of rat respiratory histopathological effects

Model ^a	BMR	AIC	BMC	BMCL	P-value	Best model ^b	Notes
Squamous metaplasia in F344 rat (Level 1)							
Mstage k=2	0.10	97.779	0.351	0.281	0.253		
Mstage k=1	0.10	97.779	0.351	0.281	0.253		
Log-logistic	0.10	97.322	0.492	0.119	0.981		BMC/BMCL > 3
Log-Probit ^c	0.10	95.619	0.576	0.448	0.947	✓	Lowest AIC
Basal hyperplasia in Wistar rat (anterior, Levels 1 & 2)^d							
Dichotomous Hill	0.10	65.975	1.634	0.487	0.309		Lowest BMCL, BMC/BMCL > 3
Mstage k=1 ^e	0.10	63.846	1.676	1.108	0.620	✓	Lowest AIC
Squamous metaplasia in Wistar rat (anterior, Levels 1 & 2)^{d,f}							
Probit	0.10	70.725	1.291	0.939	0.406		Lowest AIC
Log-probit ^c	0.10	71.779	0.821	0.459	0.298	✓ ^f	Lowest residual ^f
Multistage k=1	0.10	71.722	0.395	0.267	0.226		Lowest BMCL

^aFigures D-14 to D-16 show fits for all the models evaluated; only models that provided a reasonable fit are tabulated.

^bShown in bottom panels of Figures D-14 to D-16 along with the BMD/BMDL.

^cModel was restricted to prevent the slope of the dose-response from becoming unbounded at zero dose.

^dExposure concentrations were 0, 0.123, 1.23 & 12.05 mg/m³; observed incidences were 0/26, 1/26, 2/28 & 14/26 for basal hyperplasia, and 3/26, 1/26, 6/28 & 25/26 for squamous metaplasia.

^eThis model gives identical AIC, BMD and BMDL values as the Gamma and Weibull models.

^fThis data set is noisy in the lower dose groups. The probit model has the lowest AIC but gives a scaled residual of 0.72 which is high compared to the value of 0.002 from the log-probit for the dose group near the BMD. The AIC for the log-probit model, while higher, is within a value of 2.0 from that of the probit, and better fits the trend. The multistage model results in a scaled residual of -0.90, an even higher absolute value.

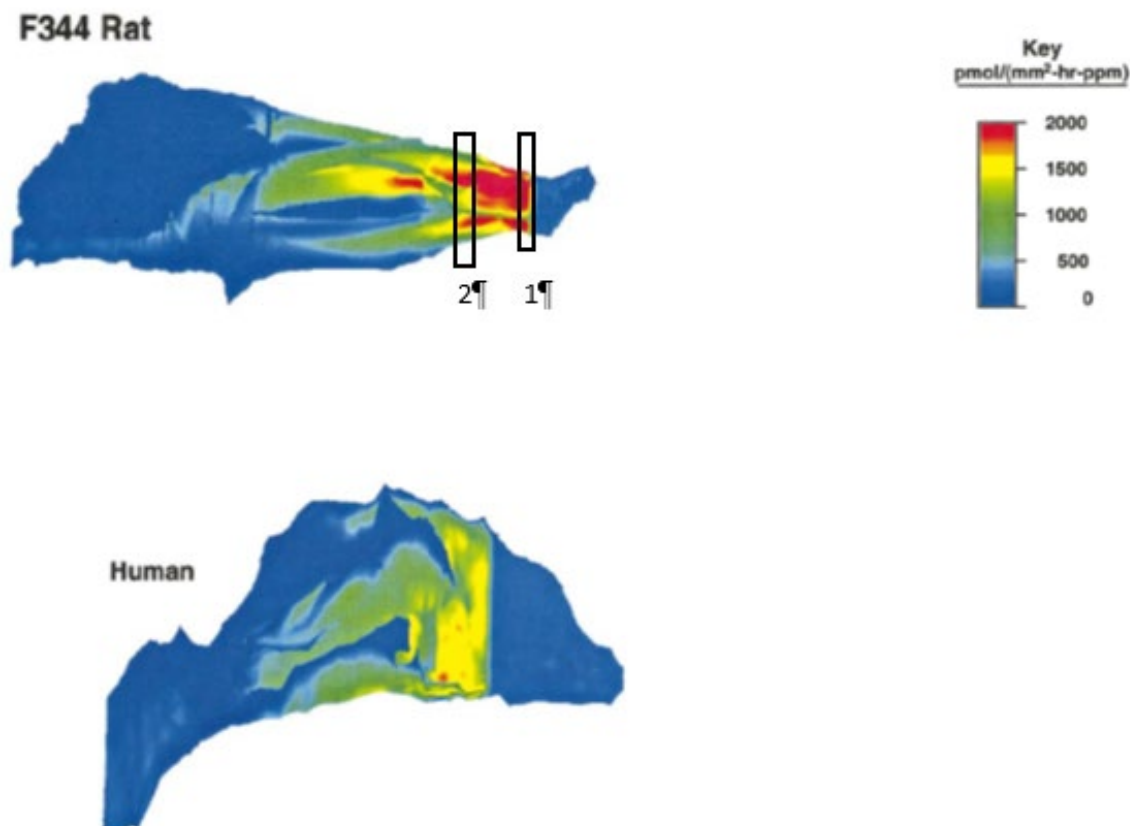


Figure D-13. Lateral view of contour plot of formaldehyde flux to the rat (on the top) and human nasal lining (on the bottom) using CFD modeling ([Kimbell et al., 2001b](#)) (nostril is to the right). The actual surface is three-dimensional. Flux at a site is linear with exposure concentration and is shown here in terms of per ppm; therefore, values shown here need to be multiplied by exposure concentration. Rectangular boxes on the rat mesh roughly estimate location of section Levels 1 & 2 in ([Kerns et al., 1983](#)) (corresponding to Figure D-12).

Supplemental Information—Formaldehyde (Inhalation)

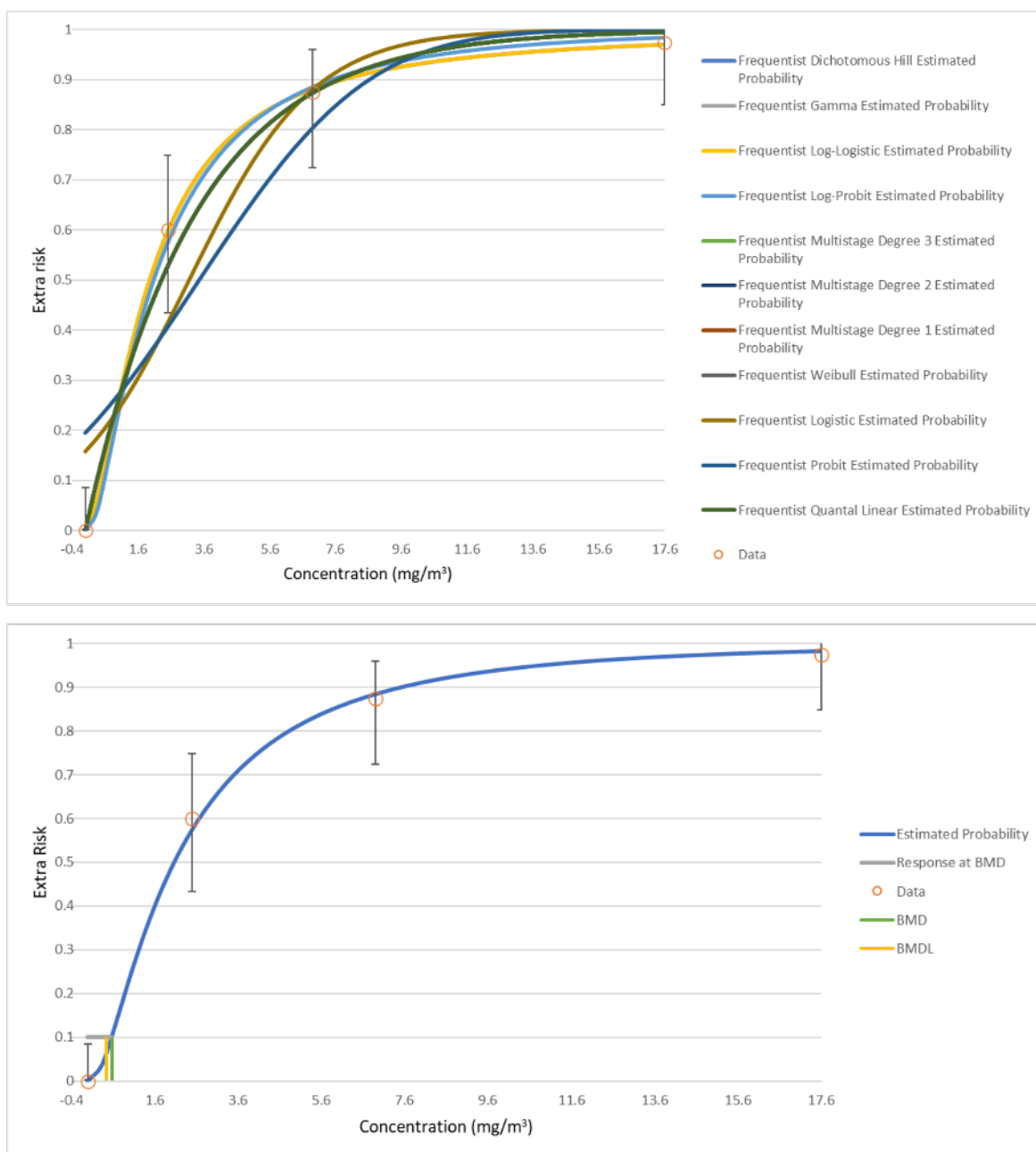


Figure D-14. All model fits (top panel) and best fitting log-probit model (bottom panel) for level 1 squamous metaplasia (F344 rat). BMR is at 0.1 extra risk, BMDL is at 95% lower confidence bound.

Supplemental Information—Formaldehyde (Inhalation)

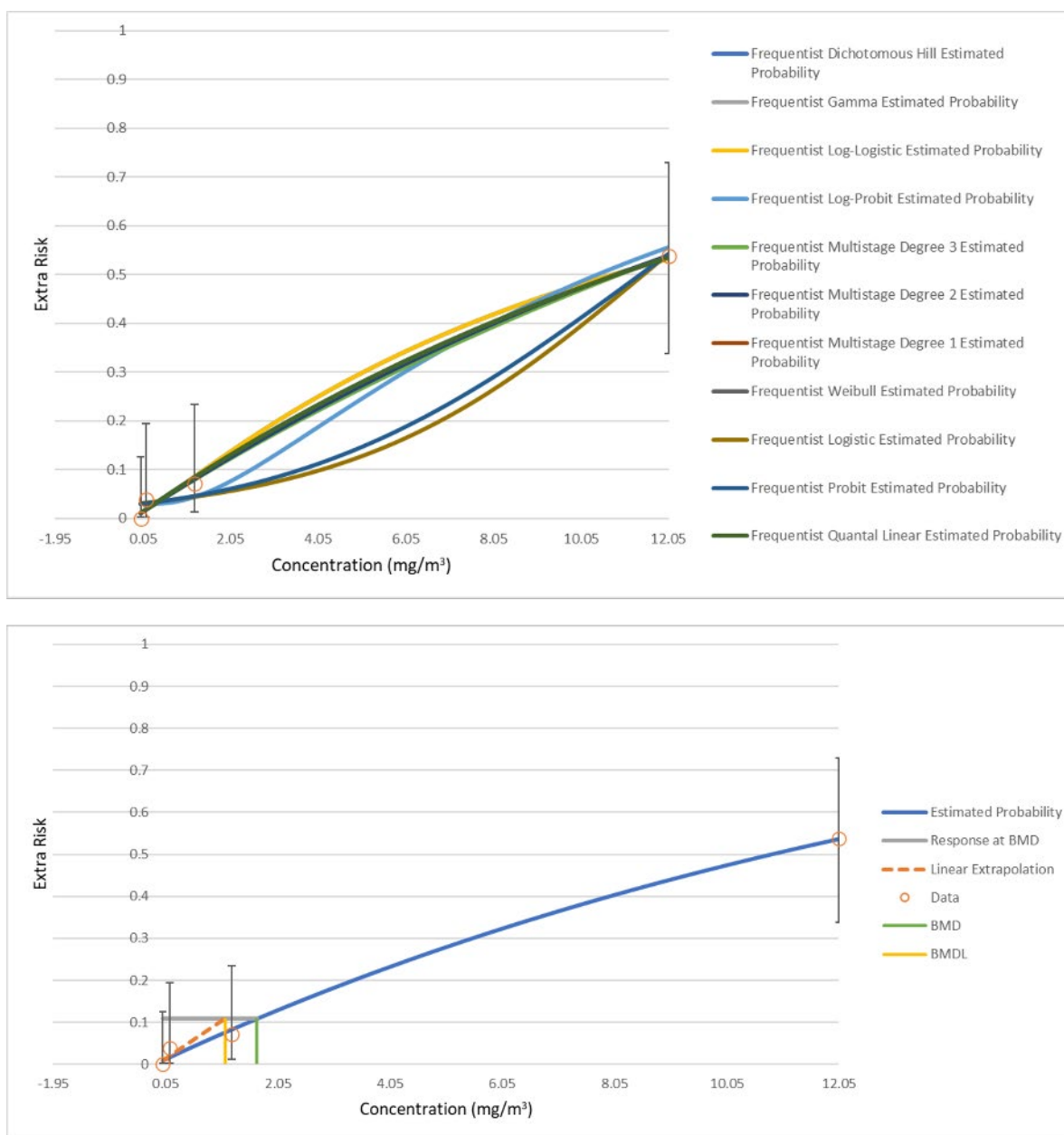


Figure D-15. All model fits (top panel) and best fitting Multistage (k=1) model (bottom panel) for basal hyperplasia (Wistar rat). BMR is at 0.1 extra risk, BMDL is at 95% lower confidence bound.

Supplemental Information—Formaldehyde (Inhalation)

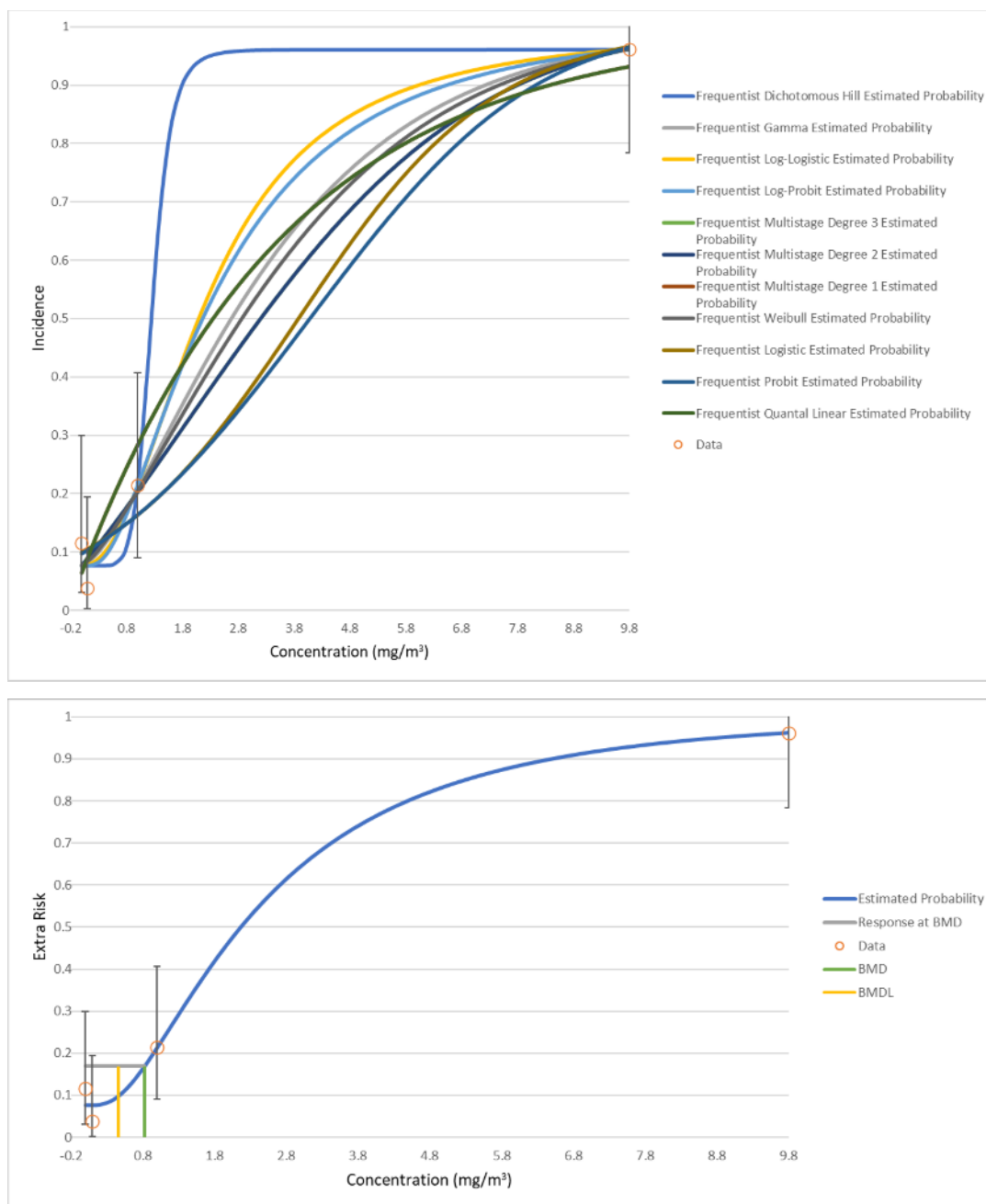


Figure D-16. All model fits (top panel) and best fitting log-probit model (bottom panel) for squamous metaplasia (Wistar rat). BMR is at 0.1 extra risk, BMDL is at 95% lower confidence bound.

D.1.5. Reproductive Toxicity in Males

Estimates from Experimental Animal Studies

Two *medium* confidence studies reporting effects on the male reproductive system in rats were considered for candidate reference value derivation ([Ozen et al., 2002](#); [Ozen et al., 2005](#)) (see modeled study details in Table D-21). For each endpoint, the BMDL estimate (95% lower confidence limit on the BMD, as estimated by the profile-likelihood method) and AIC value were used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL estimates were “sufficiently close,” that is, differed by at most 3-fold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD. One study ([Ozen et al., 2005](#)) included findings after both four and 13 weeks of formaldehyde exposure; however, only the 13-week data were advanced for POD derivation given the goal of developing a lifetime toxicity value.

Table D-21. Endpoints selected for dose-response modeling for reproductive and developmental toxicity in animals

Species (strain)/Sex Endpoint	Adjusted Formaldehyde Concentrations and Effect Data			
Ozen et al. (2005) , Table 1				
Rat (Wistar)/adult males, 13-wk exposure	Concentration (mg/m³) ^a	0	6.15	12.3
Serum testosterone (ng/L)	No. of animals Mean ± SD	6 406.5 ± 41.20	6 244.0 ± 58.44	6 141.3 ± 20.97
Ozen et al. (2002) , Table 2				
Rat (Wistar)/adult males, 13-wk exposure	Concentration (mg/m³)	0	12.2	24.4
Testis weight as percent of body weight	No. of animals Mean ± SD	7 0.91 ± 0.01	7 0.84 ± 0.03	7 0.82± 0.03

^aReported as 0, 5, and 10 ppm. Conversion: ppm*1.23.

Serum Testosterone in Male Rats

For the BMD modeling of serum testosterone in male Wistar rats exposed to formaldehyde by inhalation for 13 weeks ([Ozen et al., 2005](#)), model fit to the mean responses was good. Fit of the models for variance was marginal because the reported sample estimates of standard deviations (SD) did not change monotonically with concentrations (see Table D-22).

Table D-22. Summary of BMDS modeling for serum testosterone in male Wistar rats exposed to formaldehyde by inhalation for 13 weeks ([Ozen et al. 2005](#)); BMR = 1 SD change from the control mean

Model	BMC (mg/m ³)	BMCL (95%)	BMCL _{ADJ} ^a	P-value	AIC	Notes
Exponential (M3)	1.322	0.878	0.209	-	191.221	Questionable ^b
Exponential (M5)	1.34	0.797	0.190	-	193.221	Questionable ^b
Hill	3.53	0.713	0.170	-	193.221	Questionable ^b
Polynomial 2°	1.931	1.464	0.349	0.139	191.405	Viable
Power	1.935	1.465	0.349	0.140	191.402	Viable, Lowest AIC ^c
Linear	1.935	1.465	0.349	0.140	191.402	Viable

^aConversion: (mg/m³)*(8 hrs/24 hrs)*(5 d/7 d).

^bZero degrees of freedom, saturated model, therefore goodness of fit cannot be calculated.

^cBMDS recommended and EPA selected model (green highlighting), with the selected POD and PODADJ bolded.

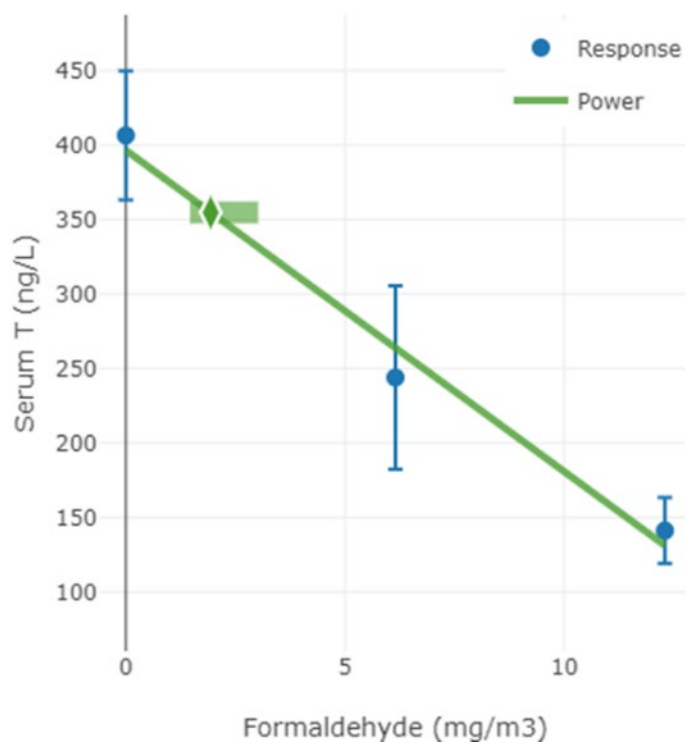


Figure D-17. Plot of mean response (serum testosterone, serum testosterone, ([Ozen et al. 2005](#))) by formaldehyde concentration (mg/m³), with the fitted curve for the Power model. BMR = 1 SD change from the control mean. Concentrations are in mg/m³.

Supplemental Information—Formaldehyde (Inhalation)

Relative Testis Weight in Male Rats

As shown in Tables D-23 and D-24, BMDS models could not be fitted successfully to data for testis weight as a percentage of body weight ([Ozen et al., 2002](#)) at the 13-week exposure duration because they reduce to linear models that had large-scaled residuals (poor fit).

Table D-23. Summary of BMDS modeling for relative testis weight in male Wistar rats exposed to formaldehyde by inhalation for 13 weeks ([Ozen et al., 2002](#)); BMR = 10% RD

Model	BMC (mg/m ³)	BMCL (95%)	BMCL _{ADJ} ^a	P-value	AIC	Notes
Exponential (M3)	24.232	19.223	4.577	0.0363	-87.915	Questionable ^{c,d}
Exponential (M5)	25.7	12.131	2.888	-	-90.297	Questionable ^{b,c}
Hill	25.376	13.192	3.141	-	-90.297	Questionable ^{b,c}
Polynomial 2°	24.397	19.582	4.523	0.0287	-87.512	Questionable ^{c,d}
Power	24.445	19.584	4.662	0.0287	-87.513	Questionable ^{c,d}
Linear	24.445	19.583	4.663	0.0287	-87.513	Questionable ^{c,d}

^aConversion: (mg/m³)*(8 hrs/24 hrs)*(5 d/7 d).

^bZero degrees of freedom, saturated model.

^cConstant variance test failed (Test 2 *p*-value < 0.05); control stdev fit > 1.5.

^dGoodness of fit *p*-value less than 0.1.

Table D-24. Summary of BMDS modeling for relative testis weight in male Wistar rats exposed to formaldehyde by inhalation for 13 weeks ([Ozen et al., 2002](#)); BMR = 1 SD change from the control mean

Model	BMC (mg/m ³)	BMCL (95%)	BMCL _{ADJ} ^a	P-value	AIC	Notes
Exponential (M3)	6.687	4.857	1.156	0.0363	-87.915	Questionable ^{c,d}
Exponential (M5)	2.644	0.177	0.042	-	-90.297	Questionable ^{b,c}
Hill	2.214	0	-	-	-90.297	Unusable, BMCL does not exist ^{b,c}
Polynomial 2°	7.064	5.185	1.235	0.0287	-87.512	Questionable ^{c,d}
Power	7.079	5.185	1.235	0.0287	-87.513	Questionable ^{c,d}
Linear	7.079	5.184	1.234	0.0287	-87.513	Questionable ^{c,d}

^aConversion: (mg/m³)*(8 hrs/24 hrs)*(5 d/7 d).

^bZero degrees of freedom, saturated model.

^cConstant variance test failed (Test 2 *p*-value < 0.05); control stdev fit > 1.5.

^dGoodness of fit *p*-value less than 0.1.

An alternative approach to POD derivation is the NOAEL/LOAEL approach. EPA calculations indicate that if the data are normally distributed (unverified, but plausible for relative weights), the response at the first concentration represents a decrease of 7.7% below control (95% confidence

interval 4.6% to 11%), and the response at the second concentration represents a decrease of 11% (95% confidence interval 7.9% to 14%). The response at the second concentration is closest to the target BMR for organ weights (10% decrease), so the second concentration (24.4 mg/m³, adjusted concentration 5.81 mg/m³) would be used as the biologically relevant LOAEL and is likely to be only marginally higher than the target BMC (i.e., at 10% RD). It is not clear that the lower concentration can be reasonably interpreted as a NOAEL, so no NOAEL is selected.

D.2. DOSE-RESPONSE ANALYSIS FOR CANCER

D.2.1. Supporting Information for Upper Respiratory Tract (Nasal) Cancer Estimates based on Epidemiology Data

Life-table Analysis for Nasopharyngeal Cancer (NPC) Risk

A spreadsheet illustrating the calculation for the derivation of the lower 95% bound on the effective concentration associated with a 0.05% extra risk (LEC₀₀₀₅) for nasopharyngeal carcinoma (NPC) incidence based on data in [Beane Freeman et al. \(2013\)](#) is presented in Table D-25.

Table D-25. Extra risk calculation^a for environmental exposure to 0.0550 ppm formaldehyde (the LEC₀₀₀₅ for NPC incidence)^b using a log-linear exposure-response model based on the cumulative exposure trend results of Beane Freeman (2013), as described in Section 5.2.1

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Interval number (i)	Age interval	All-cause mortality (×10 ⁵ /yr)	NPC incidence (×10 ⁵ /yr)	All cause hazard rate (h*)	Prob of surviving interval (q)	Prob of surviving up to interval (S)	NPC cancer hazard rate (h)	Cond prob of NPC incidence in interval (Ro)	Exp duration mid interval (xtime)	Cum exp mid interval (xdose)	Exposed NPC hazard rate (hx)	Exposed all cause hazard rate (h*x)	Exposed prob of surviving interval (qx)	Exposed prob of surviving up to interval (Sx)	Exposed cond prob of NPC in interval (Rx)
1	<1	623.4	0.02	0.0062	0.9938	1.0000	0.00000	0.000000	0	0.0000	0.0000	0.0062	0.9938	1.0000	0.00000
2	1-4	26.5	0.05	0.0011	0.9989	0.9938	0.00000	0.000002	0	0.0000	0.0000	0.0011	0.9989	0.9938	0.00000
3	5-9	11.5	0.06	0.0006	0.9994	0.9927	0.00000	0.000003	0	0.0000	0.0000	0.0006	0.9994	0.9927	0.00000
4	10-14	14.3	0.11	0.0007	0.9993	0.9922	0.00001	0.000005	0	0.0000	0.0000	0.0007	0.9993	0.9922	0.00001
5	15-19	49.4	0.15	0.0025	0.9975	0.9915	0.00001	0.000007	2.5	0.4182	0.0000	0.0025	0.9975	0.9915	0.00001
6	20-24	86.5	0.17	0.0043	0.9957	0.9890	0.00001	0.000008	7.5	1.2547	0.0000	0.0043	0.9957	0.9890	0.00001
7	25-29	96.0	0.18	0.0048	0.9952	0.9847	0.00001	0.000009	12.5	2.0911	0.0000	0.0048	0.9952	0.9847	0.00001
8	30-34	110.2	0.30	0.0055	0.9945	0.9800	0.00002	0.000015	17.5	2.9276	0.0000	0.0055	0.9945	0.9800	0.00002
9	35-39	138.8	0.54	0.0069	0.9931	0.9746	0.00003	0.000026	22.5	3.7641	0.0000	0.0069	0.9931	0.9746	0.00003
10	40-44	201.1	0.80	0.0101	0.9900	0.9679	0.00004	0.000039	27.5	4.6005	0.0001	0.0101	0.9900	0.9679	0.00005
11	45-49	324.0	1.07	0.0162	0.9839	0.9582	0.00005	0.000051	32.5	5.4370	0.0001	0.0162	0.9839	0.9582	0.00008
12	50-54	491.7	1.48	0.0246	0.9757	0.9428	0.00007	0.000069	37.5	6.2734	0.0001	0.0246	0.9757	0.9428	0.00011
13	55-59	711.7	1.70	0.0356	0.9650	0.9199	0.00009	0.000077	42.5	7.1099	0.0001	0.0356	0.9650	0.9198	0.00013
14	60-64	1,015.8	1.85	0.0508	0.9505	0.8878	0.00009	0.000080	47.5	7.9464	0.0002	0.0509	0.9504	0.8876	0.00014
15	65-69	1,527.6	2.19	0.0764	0.9265	0.8438	0.00011	0.000089	52.5	8.7828	0.0002	0.0765	0.9264	0.8436	0.00017
16	70-74	2,340.9	2.08	0.1170	0.8895	0.7817	0.00010	0.000077	57.5	9.6193	0.0002	0.1172	0.8894	0.7815	0.00016
17	75-59	3,735.4	1.85	0.1868	0.8296	0.6954	0.00009	0.000059	62.5	10.4557	0.0002	0.1869	0.8295	0.6951	0.00013
18	80-84	6,134.1	1.86	0.3067	0.7359	0.5769	0.00009	0.000046	67.5	11.2922	0.0002	0.3068	0.7358	0.5766	0.00011
							Ro =	0.000662						Rx =	0.001163
Extra Risk = (Rx-Ro)/(1-Ro) = 0.0005															

Column A:	Interval index number (i).
Column B:	5-year age interval (except <1 and 1–4) up to age 85.
Column C:	All-cause mortality rate for interval i ($\times 105/\text{year}$) (2010 data from NCHS).
Column D:	NPC incidence rate for interval i ($\times 105/\text{year}$) (2000-2010 SEER data).
Column E:	All-cause hazard rate for interval i (h^*i) (= all-cause mortality rate $\times$ number of years in age interval). ^c
Column F:	Probability of surviving interval i without being diagnosed with NPC (q_i) (= $\exp(-h^*i)$).
Column G:	Probability of surviving up to interval i without having been diagnosed with NPC (S_i) ($S_1 = 1$; $S_i = S_{i-1} \times q_{i-1}$, for $i > 1$).
Column H:	NPC incidence hazard rate for interval i (h_i) (= NPC incidence rate $\times$ number of years in interval).
Column I:	Conditional probability of being diagnosed with NPC in interval i (= $(h_i/h^*i) \times S_i \times (1-q_i)$), i.e., conditional upon surviving up to interval i without having been diagnosed with NPC [Ro, the background lifetime probability of being diagnosed with NPC, is the sum of the conditional probabilities across the intervals].
Column J:	Exposure duration (in years) at mid-interval (xtime).
Column K:	Cumulative exposure mid-interval (xdose) (= exposure level (i.e., 0.0550 ppm) $\times 365/240 \times 20/10 \times \text{xtime}$) [365/240 $\times 20/10$ converts continuous environmental exposures to corresponding occupational exposures].
Column L:	NPC incidence hazard rate in exposed people for interval i (h_{xi}) (= $h_i \times (1 + \beta \times \text{xdose})$, where $\beta = 0.04311 + (1.645 \times 0.01865) = 0.07379$ per ppm $\times$ year) [0.04311 per ppm $\times$ year is the regression coefficient obtained, along with its SE of 0.01865, from Dr. Beane Freeman (see Section 5.2.1). To estimate the LEC_{0005} (i.e., the 95% lower bound on the continuous exposure giving an extra risk of 0.05%), the 95% upper bound on the regression coefficient is used (i.e., $\text{MLE} + 1.645 \times \text{SE}$)].
Column M:	All-cause hazard rate in exposed people for interval i (h^*xi) (= $h^*i + (h_{xi} - h_i)$).
Column N:	Probability of surviving interval i without being diagnosed with NPC for exposed people (q_{xi}) (= $\exp(-h^*xi)$).
Column O:	Probability of surviving up to interval i without having been diagnosed with NPC for exposed people (S_{xi}) ($S_{x1} = 1$; $S_{xi} = S_{xi-1} \times q_{xi-1}$, for $i > 1$).
Column P:	Conditional probability of being diagnosed with NPC in interval i for exposed people (= $(h_{xi}/h^*xi) \times S_{xi} \times (1-q_{xi})$) [Rx, the lifetime probability of being diagnosed with NPC for exposed people = the sum of the conditional probabilities across the intervals].

^aUsing the methodology of BEIR IV ([NRC, 1988](#)).

^bThe estimated 95% lower bound on the continuous exposure level of formaldehyde that gives a 0.05% extra lifetime risk of NPC.

^cFor the cancer incidence calculation, the all-cause hazard rate for interval i should technically be the rate of either dying of any cause or being diagnosed with the specific cancer during the interval [i.e., (the all-cause mortality rate for the interval + the cancer-specific incidence rate for the interval – the cancer-specific mortality rate for the interval [so that a cancer case isn't counted twice, i.e., upon diagnosis and upon death]) $\times$ number of years in interval]. This adjustment was ignored here because the NPC incidence rates are small compared to the all-cause mortality rates.

MLE = maximum likelihood estimate; SE = standard error.

D.2.2. Supporting Information for Upper Respiratory Tract (Nasal) Cancer Estimates based on Animal Data

Combining Studies for Dose-response Modeling of Rat Nasal Tumors

The dose-response data from the two long term cancer bioassays using F344 rats ([Monticello et al., 1996](#); [Kerns et al., 1983](#)) were statistically evaluated for heterogeneity using EPA's Categorical Regression software ([U.S. EPA, 2000](#)). Data from the two studies were pooled (see Table D-26) and the dose-response analysis was stratified by study. The response was categorized into three severity levels corresponding to the tumor outcome being right censored (0), incidental (1) or fatal (2).

The probability that a severity level, s , or higher will occur is given as a function of exposure concentration and time in terms of a link function H ,

$$\Pr(Y \geq s|C, t) = H[a + b_1 \times f_1(C) + b_2 \times f_2(t)] \quad (\text{D-17})$$

where a , b_1 and b_2 were all allowed to vary across severity levels.

The regression was used to test the null hypothesis that the parameters for intercept (a), exposure concentration (b_1) and time parameters (b_2) are statistically equivalent between the severity levels Sev1 and Sev2 at a select p value (> 0.05). As indicated in Table D-27, the analyses were carried out in multiple ways: with and without including time as a parameter (i.e., considering the individual time to tumor data or the summary data, respectively), and also collapsing the incidental (I) and fatal (F) severity levels. Regardless of whether the data are grouped by dose-time or just dose, whether I and F responses are analyzed separately ("I, F") or lumped ("I-F") or whether time to tumor is considered, the hypothesis tests indicate that the model parameters derived from the individual studies are equivalent ($p > 0.05$). This equivalence across parameters indicates that the data from the studies can be combined for dose-response purposes as a mean of achieving greater power in the analysis.

Table D-26. Data used in dose-response modeling of SCC in F344 rat nose^a

Kerns et al study					Monticello et al study				
Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex	Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex
0	182	3	10	F	0	1	3	6	M
0	182	3	10	M	0	4	3	6	M
0	269	0	1	F	0	11	3	6	M
0	365	3	10	F	0	39	3	6	M
0	365	3	10	M	0	82	3	6	M

Supplemental Information—Formaldehyde (Inhalation)

Kerns et al study					Monticello et al study				
Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex	Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex
0	511	0	1	F	0	122	0	1	M
0	558	3	20	F	0	182	3	6	M
0	558	3	20	M	0	247	0	1	M
0	573	0	1	F	0	365	0	1	M
0	608	0	2	F	0	365	3	12	M
0	621	0	1	F	0	416	0	1	M
0	623	0	1	M	0	472	0	1	M
0	629	0	1	M	0	530	0	1	M
0	646	0	1	M	0	540	0	1	M
0	659	0	1	F	0	558	3	10	M
0	679	0	1	M	0	568	0	2	M
0	683	0	1	M	0	577	0	1	M
0	703	0	1	F	0	592	0	1	M
0	715	0	1	F	0	611	0	1	M
0	719	0	1	F	0	620	0	2	M
0	721	0	1	F	0	624	0	1	M
0	728	0	1	M	0	628	0	1	M
0	730	3	47	F	0	633	0	1	M
0	730	3	54	M	0	648	0	1	M
0	770	0	1	F	0	677	0	1	M
0	794	0	1	F	0	687	0	2	M
0	804	0	1	F	0	700	0	1	M
0	819	0	1	F	0	702	0	1	M
0	821	3	9	F	0	716	0	2	M
0	821	3	10	M	0	730	3	21	M
0	826	0	1	M	0.7	1	3	6	M
0	841	0	1	F	0.7	4	3	6	M
0	878	0	1	F	0.7	11	3	6	M
0	878	0	1	M	0.7	39	3	6	M
0	895	0	1	M	0.7	65	0	1	M
0	899	0	1	F	0.7	82	3	6	M
0	911	3	4	F	0.7	182	3	6	M
0	911	3	6	M	0.7	365	3	12	M

Supplemental Information—Formaldehyde (Inhalation)

Kerns et al study					Monticello et al study				
Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex	Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex
2	182	3	10	F	0.7	449	0	1	M
2	182	3	10	M	0.7	510	0	1	M
2	365	3	10	F	0.7	526	0	1	M
2	365	3	10	M	0.7	530	0	1	M
2	482	0	1	M	0.7	547	0	1	M
2	541	0	1	M	0.7	558	3	11	M
2	558	3	20	F	0.7	564	0	1	M
2	558	3	19	M	0.7	569	0	1	M
2	567	0	1	F	0.7	598	0	1	M
2	595	0	2	F	0.7	626	0	1	M
2	597	0	1	M	0.7	628	0	1	M
2	607	0	1	F	0.7	633	0	1	M
2	608	0	1	F	0.7	642	0	1	M
2	628	0	1	F	0.7	661	0	1	M
2	630	0	1	M	0.7	670	0	1	M
2	636	0	1	F	0.7	708	0	1	M
2	639	0	1	F	0.7	709	0	1	M
2	642	0	1	M	0.7	713	0	1	M
2	644	0	1	F	0.7	717	0	1	M
2	645	0	1	F	0.7	722	0	2	M
2	656	0	1	M	0.7	730	3	27	M
2	665	0	1	F	2	1	3	12	M
2	686	0	1	F	2	4	3	6	M
2	687	0	1	F	2	11	3	6	M
2	695	0	1	M	2	39	3	6	M
2	698	0	1	M	2	82	3	6	M
2	713	0	1	F	2	182	3	6	M
2	718	0	1	M	2	365	3	12	M
2	728	0	1	F	2	435	0	1	M
2	730	0	1	M	2	472	0	1	M
2	730	3	45	F	2	479	0	1	M
2	730	3	50	M	2	521	0	1	M
2	744	0	1	F	2	558	3	12	M

Supplemental Information—Formaldehyde (Inhalation)

Kerns et al study					Monticello et al study				
Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex	Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex
2	755	0	1	F	2	574	0	1	M
2	766	0	1	F	2	582	0	1	M
2	767	0	1	F	2	590	0	2	M
2	779	0	1	F	2	626	0	1	M
2	797	0	1	F	2	645	0	1	M
2	809	0	1	F	2	675	0	2	M
2	814	0	1	M	2	691	0	1	M
2	817	0	1	F	2	698	0	1	M
2	821	3	10	F	2	705	0	1	M
2	821	3	10	M	2	709	0	1	M
2	847	0	1	M	2	710	0	2	M
2	869	0	1	M	2	730	3	30	M
2	873	0	1	M	6	1	3	6	M
2	888	0	1	F	6	4	3	6	M
2	909	0	2	M	6	11	3	6	M
2	911	3	1	F	6	39	3	6	M
2	911	3	4	M	6	82	3	6	M
6	182	3	10	F	6	116	0	1	M
6	182	3	10	M	6	138	0	1	M
6	342	0	1	F	6	182	3	6	M
6	362	0	1	F	6	330	0	1	M
6	365	3	10	F	6	365	3	12	M
6	365	3	10	M	6	366	0	1	M
6	367	0	1	M	6	421	0	1	M
6	420	0	1	M	6	460	0	1	M
6	422	0	1	M	6	542	0	1	M
6	519	0	1	M	6	547	0	1	M
6	527	0	1	F	6	550	0	1	M
6	558	0	1	M	6	558	3	12	M
6	558	3	20	F	6	566	0	1	M
6	558	3	20	M	6	611	0	1	M
6	576	0	1	M	6	618	0	1	M
6	592	0	2	M	6	626	1	1	M

Supplemental Information—Formaldehyde (Inhalation)

Kerns et al study					Monticello et al study				
Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex	Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex
6	600	0	1	M	6	652	0	1	M
6	625	0	1	M	6	684	0	1	M
6	643	0	1	F	6	688	0	1	M
6	643	0	1	M	6	698	0	1	M
6	656	0	1	F	6	721	0	1	M
6	659	0	1	F	6	722	0	1	M
6	660	0	1	F	6	730	3	29	M
6	664	0	1	F	10	1	3	6	M
6	667	0	1	M	10	4	3	6	M
6	674	0	1	F	10	11	3	6	M
6	674	0	1	M	10	39	3	6	M
6	681	0	1	M	10	82	3	6	M
6	696	0	1	F	10	182	3	6	M
6	701	0	1	F	10	305	0	1	M
6	701	0	1	M	10	365	3	10	M
6	705	0	1	F	10	395	0	1	M
6	709	0	1	F	10	463	0	1	M
6	712	0	1	M	10	466	0	1	M
6	723	0	1	M	10	502	0	1	M
6	727	0	2	F	10	555	1	1	M
6	727	0	1	M	10	558	2	3	M
6	730	0	1	M	10	558	3	7	M
6	730	2	1	F	10	564	0	1	M
6	730	2	1	M	10	568	1	1	M
6	730	3	40	F	10	577	0	1	M
6	730	3	40	M	10	584	0	1	M
6	748	0	1	F	10	590	0	1	M
6	774	0	1	M	10	604	0	1	M
6	776	0	1	F	10	610	0	1	M
6	776	0	1	M	10	617	0	1	M
6	819	0	1	F	10	621	1	1	M
6	819	0	1	M	10	624	1	1	M
6	821	3	9	F	10	626	1	1	M

Supplemental Information—Formaldehyde (Inhalation)

Kerns et al study					Monticello et al study				
Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex	Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex
6	821	3	10	M	10	628	0	1	M
6	839	0	1	F	10	642	1	2	M
6	839	0	1	M	10	655	1	1	M
6	844	0	1	M	10	671	0	1	M
6	847	0	1	M	10	676	1	1	M
6	890	0	1	F	10	687	0	1	M
6	900	0	1	F	10	687	1	1	M
6	911	3	5	F	10	697	0	1	M
6	911	3	3	M	10	709	1	1	M
15	6	0	1	M	10	730	2	8	M
15	182	3	10	F	10	730	3	12	M
15	182	3	10	M	15	1	3	6	M
15	253	0	1	F	15	4	3	6	M
15	275	0	1	M	15	11	3	6	M
15	320	0	1	F	15	39	3	6	M
15	354	0	1	M	15	82	3	6	M
15	359	1	1	F	15	182	3	9	M
15	362	0	1	F	15	220	0	1	M
15	365	1	1	F	15	251	0	1	M
15	365	3	10	F	15	277	0	1	M
15	365	3	10	M	15	278	0	1	M
15	391	0	1	M	15	302	0	1	M
15	421	1	1	F	15	344	1	1	M
15	433	0	2	M	15	348	1	1	M
15	441	1	1	F	15	365	2	2	M
15	475	0	1	M	15	365	3	14	M
15	478	0	1	F	15	372	1	3	M
15	480	0	1	M	15	387	1	1	M
15	480	1	1	F	15	400	1	1	M
15	489	1	1	F	15	466	0	1	M
15	492	0	1	M	15	466	1	1	M
15	496	0	1	M	15	472	1	1	M
15	505	0	1	F	15	479	0	1	M

Supplemental Information—Formaldehyde (Inhalation)

Kerns et al study					Monticello et al study				
Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex	Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex
15	505	1	1	M	15	479	1	1	M
15	509	1	1	F	15	483	1	1	M
15	510	0	1	F	15	493	1	1	M
15	512	0	1	M	15	498	1	1	M
15	513	1	1	F	15	499	0	1	M
15	521	1	1	F	15	500	1	1	M
15	526	1	1	F	15	505	1	1	M
15	531	0	1	M	15	507	1	2	M
15	532	0	1	F	15	513	0	1	M
15	532	1	1	M	15	514	1	2	M
15	534	1	2	F	15	515	1	2	M
15	536	1	1	F	15	519	1	1	M
15	538	0	1	M	15	523	1	1	M
15	540	1	1	M	15	526	0	1	M
15	544	1	1	F	15	526	1	1	M
15	546	0	3	M	15	555	1	1	M
15	556	0	1	M	15	558	2	6	M
15	558	0	1	F	15	558	3	5	M
15	558	1	1	F	15	561	1	1	M
15	558	2	4	F	15	568	1	1	M
15	558	2	4	M	15	575	0	1	M
15	558	3	15	F	15	578	0	1	M
15	558	3	15	M	15	586	1	1	M
15	561	1	1	M	15	591	0	1	M
15	568	1	1	F	15	591	1	2	M
15	570	1	1	M	15	604	1	1	M
15	572	0	1	F	15	614	1	3	M
15	573	1	1	F	15	617	1	5	M
15	575	0	2	M	15	621	1	1	M
15	575	1	1	F	15	635	0	1	M
15	584	0	1	F	15	635	1	1	M
15	587	0	1	F	15	640	1	1	M
15	589	0	1	M	15	645	1	1	M

Supplemental Information—Formaldehyde (Inhalation)

Kerns et al study					Monticello et al study				
Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex	Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex
15	589	1	1	F	15	647	0	1	M
15	591	0	1	F	15	658	1	1	M
15	596	1	1	F	15	661	1	3	M
15	597	1	2	F	15	662	1	1	M
15	604	1	1	F	15	682	0	1	M
15	607	1	1	M	15	695	1	1	M
15	608	0	1	M	15	696	1	1	M
15	609	0	1	M	15	697	1	1	M
15	610	0	1	M	15	698	1	1	M
15	615	0	1	M	15	703	1	1	M
15	616	1	1	F	15	715	1	1	M
15	621	1	1	F	15	721	1	1	M
15	622	1	1	M	15	730	2	16	M
15	625	1	1	F	15	730	3	8	M
15	628	1	1	F					
15	630	0	1	F					
15	631	1	1	M					
15	632	0	1	F					
15	632	0	1	M					
15	632	1	1	M					
15	642	0	1	F					
15	643	1	1	M					
15	644	1	1	F					
15	645	0	1	M					
15	653	1	1	F					
15	655	1	1	M					
15	664	1	1	F					
15	666	1	1	M					
15	673	1	1	F					
15	674	0	1	M					
15	674	1	1	F					
15	680	0	1	F					
15	680	1	1	F					

Supplemental Information—Formaldehyde (Inhalation)

Kerns et al study					Monticello et al study				
Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex	Conc (ppm)	Days on study ^b	Tumor context ^c	# of animals	Sex
15	684	1	1	F					
15	687	0	2	M					
15	687	1	1	F					
15	689	0	1	M					
15	690	1	1	F					
15	694	1	1	M					
15	695	0	1	M					
15	697	1	1	F					
15	700	1	1	F					
15	706	1	1	F					
15	706	1	1	M					
15	707	0	2	F					
15	713	1	1	F					
15	716	0	1	M					
15	718	1	1	M					
15	719	0	1	M					
15	722	0	1	M					
15	722	1	1	M					
15	728	1	1	F					
15	728	1	2	M					
15	730	1	1	M					
15	730	2	7	F					
15	730	2	3	M					
15	730	3	7	F					
15	730	3	10	M					
15	751	1	1	M					
15	754	1	1	M					
15	768	1	1	M					
15	821	2	2	M					
15	821	3	3	M					

^aNTP historical controls used by Conolly et al. ([Conolly et al., 2003](#)) not included in this tabulation.

^bEPA reproduced the ages of the animals in ([Conolly et al., 2003](#)) for the two studies by adding 63 days, the age at beginning of exposure, to the days on study indicated in the CIIT dataset.

^c0- no tumor, non-sacrifice; 1- tumor, non-sacrifice; 2- tumor, sacrifice; 3- no tumor, sacrifice.

Table D-27. Hypothesis testing of parameter equivalence for modeling Monticello et al. (1996); Kerns et al. (1983) studies

Grouping of pooled Kerns & Monticello dose-response data	Best link function ^a	p-values					
		Sev1 a	Sev2 a	Sev1 b ₁	Sev2 b ₁	Sev1 b ₂	Sev2 b ₂
Study-Dose-Time-Response (C, I, F) ^b	Probit	0.06	0.99	0.71	0.99	--	--
Study-Dose-Time-Response (C, I, F)	CLogLog	0.06	0.99	0.07	0.99	0.78	0.74
Study-Dose-Time-Response (C, I-F) ^b	Probit	0.05	--	0.65	--	--	--
Study-Dose-Time-Response (C, I-F)	Probit	0.87	--	0.97	--	0.25	--
Study-Dose-Response (C, I, F)	Probit	--	--	0.67	--	--	--
Study-Dose-Response (C, I-F)	Probit	0.19	0.97	0.60	0.97	--	--

^aLowest AIC; Unrestricted Odds (dose and time parameters allowed to vary across severity categories).

^bTime data not considered in model.

Memo from CIIT Providing Updated Information on Kerns and Monticello studies and NTP Historical Data

For completeness, the CIIT memo related to this modeling ([Bermudez, 2004](#)) is included in its entirety below (note: converted from pdf, so there may be minor grammatical errors; the values in tables were confirmed).

[Memo Start]

Memo

To: File

From: E. Gross Bermudez

CC: F. Miller, Vice President for Research

R. Conolly, Senior Investigator

A. Jarabek, US EPA Visiting

Scientist

Date: June 15, 2004

Re: HCHO studies - Tumor Incidence

Supplemental Information—Formaldehyde (Inhalation)

The purpose of this memorandum is to document the data from the various formaldehyde publications sponsored by or conducted at the CIIT Centers for Health Research (CIIT) and to resolve some data discrepancies and arrive at a final data set that can be used for modeling. This memo includes the total CIIT formaldehyde data set (combined Kerns, Monticello, Everitt) that is available on CD and the information on CIITs use of the NTP control database.

Kerns Study (Table 4-7 in 1999 CIIT report)

Updating the data summary for the Kerns et al. (1983) study in the 1999 CIIT report on Page 4-20 to reflect all animals for which there are nasal slides and a consensus of pathology diagnoses, the tumor summary is as follows: 0 animals out of 237 animals at 0 ppm; 0 out of 239 at 2 ppm; 2 (1Male, 1Female) animals out of 235 at 6 ppm; 83 (31M, 52F) animals out of 225 at 15 ppm.

Concentration	Total Examined	scc Tumors	No Tumors
0	237	0	237
2	239	0	239
6	235	2	233
15	225	83	142

The total examined at all concentrations except the 6 ppm level was increased. These changes correct for what may have been misinterpretations of the incidence numbers presented in the original Kerns report (number of animals on study vs. number of animals examined) and/or limitations in data presented in the Kerns et al. (1983) publication.

Monticello Study (Table 7-3 in 1999 CIIT Report)

The following table replaces Table 7-3 in the 1999 CIIT report on Page 7-10 for the data summary of the Monticello et al. (1996) study. The only changes made are for 2 ppm rats where the number of noses examined was 96 not 90. The 90 was a typographical error as an additional 18 animals were examined by Jeff Everitt not 24.

Formaldehyde concentration (ppm)						
	0.0	0.7	2.0	6.01	9.93	14.94
Monticello et al. (1996) Number SCC	0	0	0	1	20	69
Number examined	90	90	96	90	90	147
Additional animals - CIIT (1999)	0	0	0	0	2	10

Supplemental Information—Formaldehyde (Inhalation)

Formaldehyde concentration (ppm)						
	0.0	0.7	2.0	6.01	9.93	14.94
Number SCC Number examined	14	17	18	18	13	14
Total number of SCC	0	0	0	1	22	79
Total number of animals examined	104	107	114	108	103	161

Summary Information

The following table provides a summary of the data for the two studies sponsored or performed by CIIT (Kerns et al., 1983 and Monticello et al., 1996).

Summary Table for Kerns and Monticello Studies Combined

Concentration	Total Examined	SCC Tumors	No Tumors
0	104	0	104
.7	107	0	107
2	114	0	114
6	108	1	107
10	103	22	81
15	161	79	82

Supplemental Information—Formaldehyde (Inhalation)

For control animals, 3,866 males and 3,818 females were used from the NTP historical data base. CIIT classified 13 of 15 NTP classified nasal tumors as nasal SCCs (based on examination by Kevin T. Morgan). The Kerns study examined 237 control animals and the Monticello study, 104 (90 originally and 14 added by Jeff Everitt). No control SCCs were reported in the Kerns or Monticello studies. The total of 8025 for controls and the total examined at other concentrations also includes animals that were very young (prior to the first tumor diagnosis), unscheduled animals and animals that were held for examination up to 6 months beyond the 24-month bioassay. The number of control animals from the CIIT Dataset (available on CD) represents animals for which slides of the nasal cavity are available and may include animals not reported in the Kerns or Monticello published incidence tables. This confirms 13 tumors out of 8025 control animals examined.

Because the biologically-based dose-response (BBDR) model used all of these data, including both bioassays, the additional evaluations by Dr. Jeff Everitt, and the animals that were held post-exposure, any other type of dose-response modeling approaches should attempt to address comparable data sets. As stated above it should be noted that some animals at all concentrations were examined at a very young age (Monticello and Kerns studies, early time points) and this needs to be taken into consideration when using these data.

These data are summarized in the following table.

**Summary Table for Data Used in Biologically-Based Dose Response (BBDR) Model
Developed by the CIIT Centers for Health Research (Conolly et al., 2003)**

Concentration	Total Examined	SCC Tumors	No Tumors
0	8025	13	8012
0.7	107	0	107
2.0	353	0	353
6.0	343	3	340
10.0	103	22	81
15	386	162	224

Conolly Modeling (Appendix Table 1 in 2003 Toxicological Sciences paper)

The time of death due to tumors of the thirteen (13) tumors from the control NTP data are listed in the following table. These dates were used in the BBDR model so that the dates essentially replace the "N/A" in the first column listed for the control animals in Appendix Table 1 of Conolly et al., (2003). The death dates of the survivor animals were not available and therefore are all estimated and entered as 104.4 weeks for these animals. This assumption was felt justified because of it being such a large data set that the loss of a few animals at other times during their lifetime would not unduly affect their estimate of lifetime nasal SCC probability.

Weeks from start of study	Number of rats with tumors	Cumulative probability
50	1	0.0001
85	1	0.0003
87.6	1	0.0004
91	1	0.0005
91.9	1	0.0007
93.7	1	0.0008
94.9	1	0.0009
96.6	1	0.0010
96.9	1	0.0012
97.6	1	0.0013
98.1	1	0.0014
102.3	1	0.0016
104.4	1	0.0017

[Memo End]

Time-to-tumor Modeling of Rat Nasal Tumor Incidence without Mechanistic Data

The impact of competing risks and interim sacrifices on the nasal tumor incidence needs to be considered in the dose-response analysis because of the strong influence of exposure on survival of animals in the bioassay. The simplest consideration of this factor is to adjust tumor incidences for early deaths according to Kaplan-Meier (K-M) survival estimates ([Kaplan and Meier, 1958](#)). This procedure adjusts the total number of animals at a given exposure level (the denominator) to allow for the possibility that some tumors may otherwise have developed in the animals that died early due to other causes. The Kaplan-Meier adjusted incidence (applied only at doses where tumors occur) is shown in Table D-28. The numbers at risk reflect all animals surviving one year on the study and is obtained by dividing the number with observed tumors by the K-M adjusted probability of tumor incidence.

Table D-28. Kaplan-Meier adjusted tumor incidence in the pooled F344 SCC data

Exposure level (ppm)	K-M adjusted incidence	Observed tumors/ number at risk ^a
0.0	0.0	0/242
0.7	0.0	0/70
2.0	0.0	0/254
6.0	0.0249	3/120
10.0	0.6144	22/36
15.0	0.8256	157/190

^a The denominator is K-M adjusted.

While dose-response modeling of the adjusted summary data in Table D-28 can be carried out, the individual animal data allows for a full time-to-tumor analysis. EPA used the multistage-Weibull (MSW) model ([Portier and Bailer, 1989](#); [Krewski et al., 1983](#); [Crouch, 1989](#)) to model the pooled data from the [Monticello et al. \(1996\)](#); [Kerns et al. \(1983\)](#) bioassays along with data from 94 animals that had not been previously examined ([Bermudez, 2004](#)). This model has the following form:

$$P(d) = 1 - \exp[-(\beta_0 + \beta_1 d + \beta_2 d^2 + \dots + \beta_k d^k) \times (t - t_0)^c] \quad (\text{D-18})$$

where $P(d)$ represents the lifetime risk (probability) of cancer at dose d (i.e., human equivalent exposure in this case); parameters $\beta_i \geq 0$, for $i = 0, 1, \dots, k$; t is the time at which the tumor was observed; and c is an estimated (fitted) parameter that characterizes the change in response with age. The parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death. By including the exact time of observation of the tumors, the model gives appropriate weight to the amount of time each animal was on study without a tumor and acknowledges earlier tumor incidence with increasing dose level.

A further consideration is the distinction between tumor types as being either fatal or incidental in order to adjust for competing risks. Incidental tumors are those tumors thought not to have caused the death of an animal (such as those observed during interim or terminal sacrifices), while fatal tumors are thought to have resulted in animal death. [Schlosser et al. \(2003\)](#) in their modeling of these same data note that the tumors arising in these studies were rapidly growing and quickly lethal and judged that the time to death from tumor would have been only slightly (on the order of weeks) longer than the actual sacrifice times of animals found with tumors. Therefore, it was considered reasonable to consider nasal tumors observed with early deaths to be fatal in the current analysis.

The dose-response analyses were conducted using the MSW module in EPA's Benchmark Dose software suite which is based on Weibull models drawn from ([Krewski et al., 1983](#)). Parameters were estimated using the method of maximum likelihood estimate (MLE). Specific multistage Weibull models were selected for the individual tumor types for each sex, based on the

values of the log-likelihoods according to the strategy used by ([U.S. EPA, 2002](#)). If twice the difference in log-likelihoods was less than a χ^2 with degrees of freedom equal to the difference in the number of stages included in the models being compared, the models were considered comparable, and the most parsimonious model (i.e., the lowest-stage model) was selected contingent on visual fits of the data as follows. Goodness of fit p-value was not provided by the software package. Plots of model fits of the parametric MSW model were visually compared with empirical nonparametric Kaplan-Meier ([Kaplan and Meier, 1958](#)) estimates fitted to the same data (Figure D-18, Figure D-19, Figure D-20). The empirical nonparametric model is designed to fit the data as “closely” as possible. Consequently, comparisons between the two fitted models provide a subjective assessment for goodness-of-fit of the multistage Weibull model to the data. Both the fitted MSW model and the fitted nonparametric model are displayed on the same graph. If a model with one more stage fitted the low-dose data better than the most parsimonious model, then the model with one higher stage was selected. Table D-29 provides the parameter values for the best fit (labeled model MSW1), and Table D-30 compares relevant parameters with other attempted fits (MSW2 and MSW3) to the data with the multistage Weibull model. The results in Table D-29 and Table D-30 used in conjunction with Figures D-18 through D-22 collectively represent EPA rationale for choosing model MSW1 as the best fit.

It was not possible to obtain model convergence allowing multiple β_i parameters to vary; reasonable fits were obtained when, through a process of trial and error, all the β_i except β_k were fixed at value zero for a MSW model of degree k. The parameter t_0 was estimated in models MSW2 and MSW3 by fitting the model to the time-tumor data; as seen from the Figures D-21 and D-22 and Table D-30, the fits in these cases were poor. The fit was decidedly superior when t_0 was fixed to be zero (model MSW1, see Figures D-18 through D-20 and Table D-30), a choice that is consistent with the assumption of rapidly fatal tumors. A sensitivity analysis of this assumption was also carried out by treating all tumors as incidental to the death of the affected animal. This yielded BMCLs and BMCs within 10% of estimates obtained under the rapidly fatal assumption; thus, tumor context does not appear to be a sensitive consideration for these data.

Supplemental Information—Formaldehyde (Inhalation)

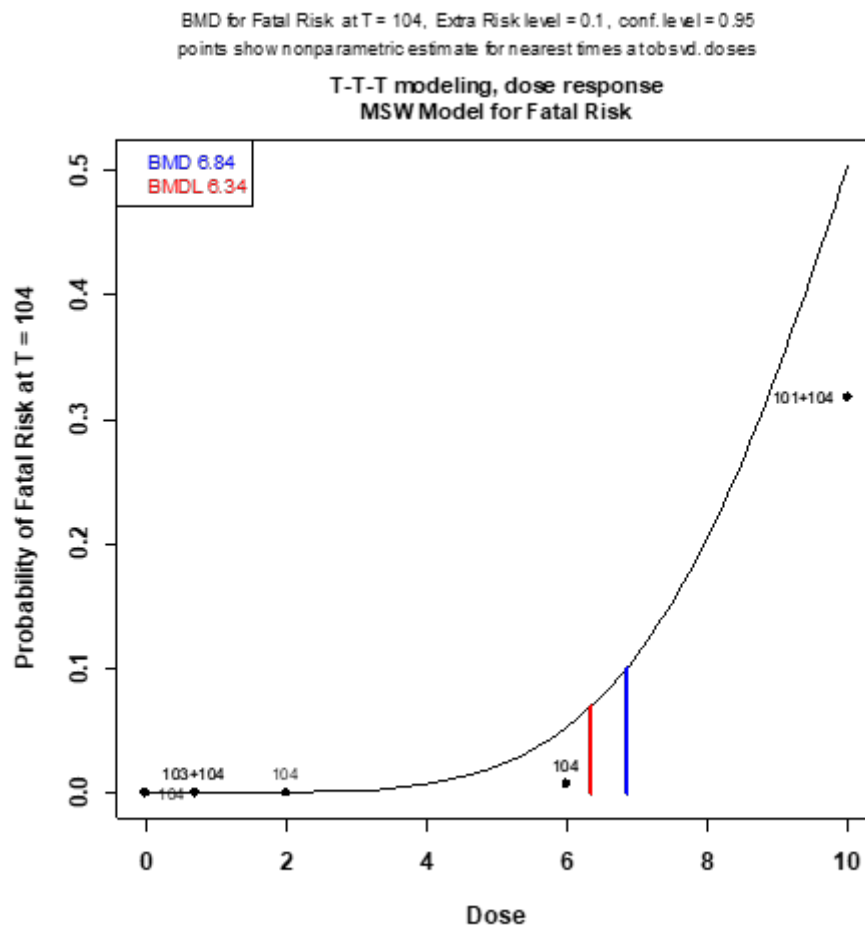


Figure D-18. Dose response for the best fitting multistage Weibull time-to-tumor model (Model MSW1) of pooled rat nasal tumor Plot is at the specified time risk estimates were derived for the benchmark dose calculation. Points compare the nonparametric estimate for the nearest times at the observed doses.

Supplemental Information—Formaldehyde (Inhalation)

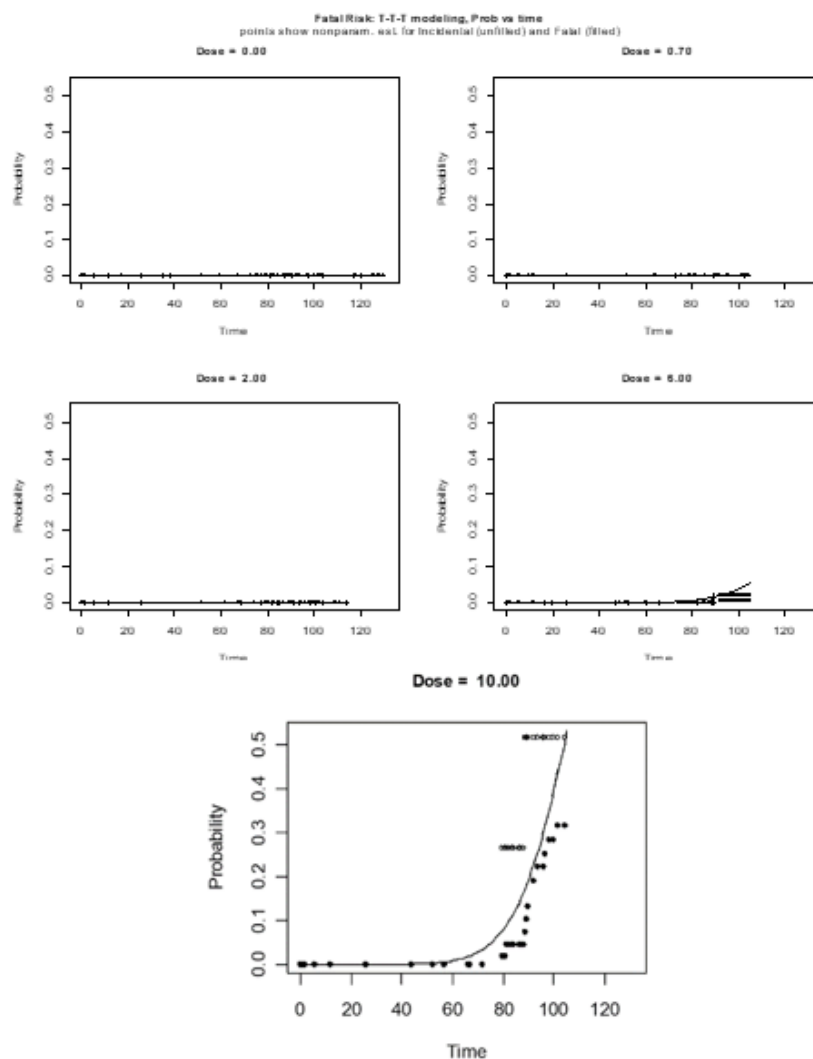


Figure D-19. Probability vs time for the best fitting MSW distribution calculated at each exposure concentration. Points compare with nonparametric estimate; incidental (unfilled), fatal (filled)

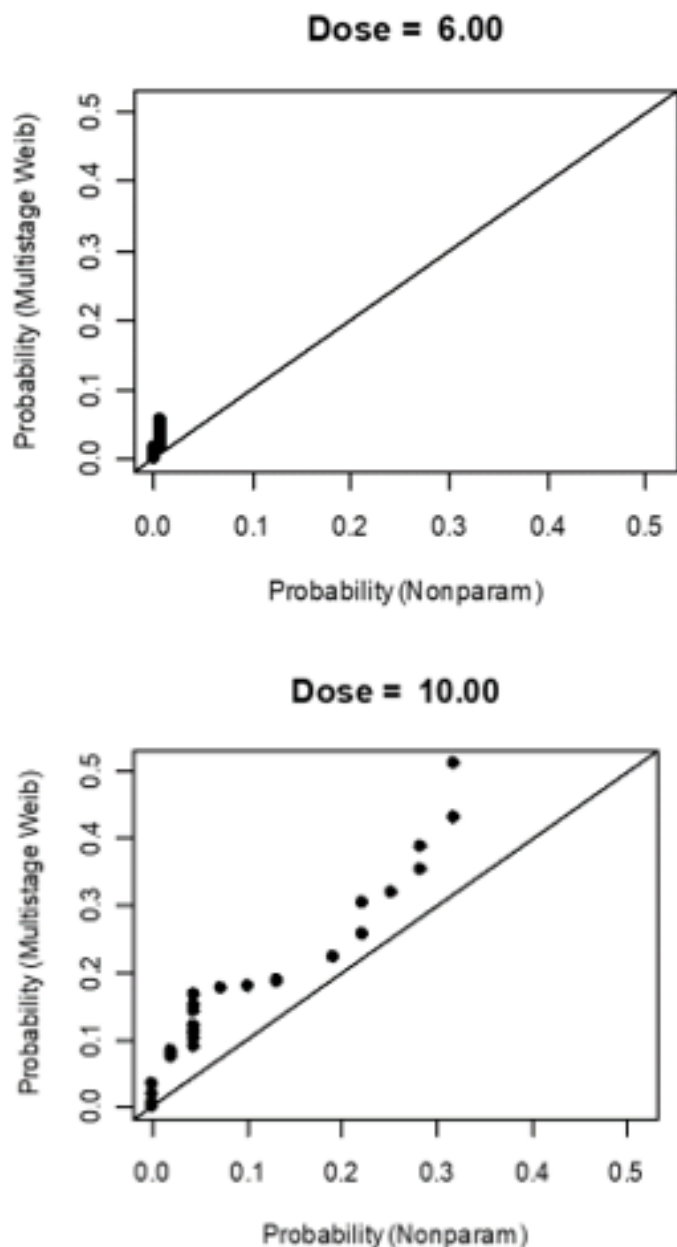


Figure D-20. Probabilities from best fitting MSW model plotted against probabilities from the nonparametric model. Plots are shown only for the 6 ppm and 10 ppm data. Each plot point represents the two probabilities at a given observation time. Straight line represents unit slope; goodness of fit can be assessed by how well plotted values align with this line.

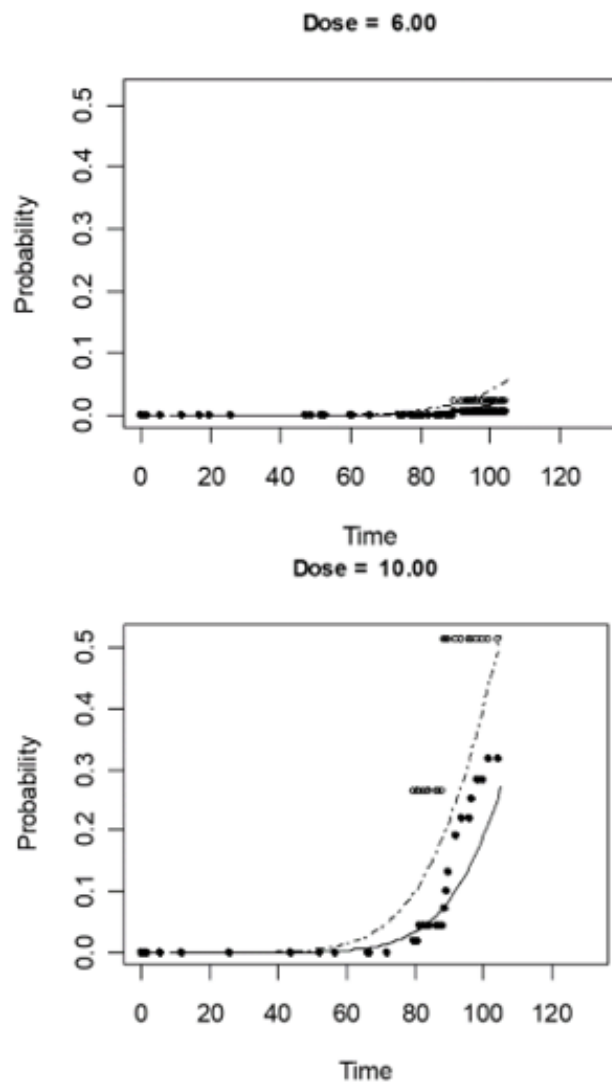


Figure D-21. Probability vs time for MSW distribution of model MSW2. Calculations are shown at exposure concentrations of 6 ppm and 10 ppm only for comparison purposes. Points represent nonparametric estimate for comparison; incidental (unfilled), fatal (filled).

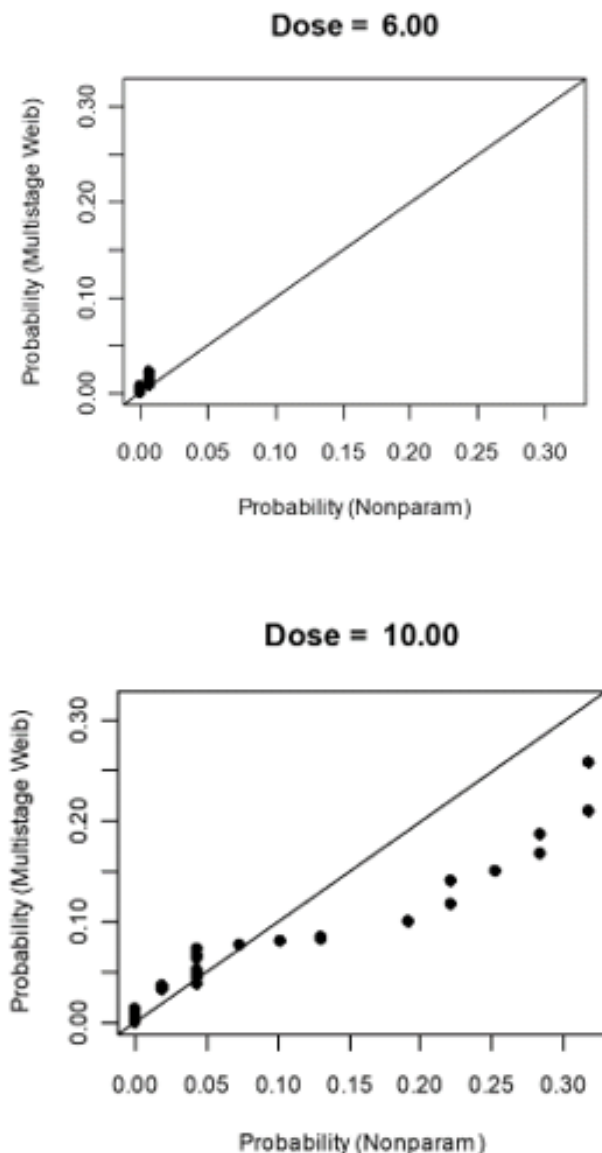


Figure D-22. Probabilities from MSW model MSW2 plotted against probabilities from the nonparametric model. Each plot point represents the two probabilities at a given observation time. Straight line represents unit slope; goodness of fit can be assessed by how well plotted values align with this line. Only the 6 ppm and 10 ppm data are plotted.

Table D-29. Multistage-Weibull model fit to combined Kerns and Monticello data, dropping high dose

```
=====
Multistage Weibull Model. (Version: 1.6.1; Date: 11/24/2009)
Solutions are obtained using donlp2-intv, (c) by P. Spellucci
=====

MSW model k=5 for Fatal Risk

The form of the probability function is:
P[response]=1-EXP{-(t-t_0)^c*
(beta_0+beta_1*dose^1+beta_2*dose^2+beta_3*dose^3+beta_4*dose^4+beta_5*dose^5
)}

The parameter betas are restricted to be positive

Dependent variable = CLASS
Independent variables = DOSE, TIME

Total number of observations = 222
Total number of records with missing values = 0
Total number of parameters in model = 8
Total number of specified parameters = 6
Degree of polynomial = 5

User specifies the following parameters:
    t_0      =          0
    beta_0 =          0
    beta_1 =          0
    beta_2 =          0
    beta_3 =          0
    beta_4 =          0

Maximum number of iterations = 64
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
      c      =          8.25
      t_0     =          0   Specified
      beta_0  =          0   Specified
      beta_1  =          0   Specified
      beta_2  =          0   Specified
      beta_3  =          0   Specified
      beta_4  =          0   Specified
      beta_5  = 1.61323e-022

Asymptotic Correlation Matrix of Parameter Estimates
( *** The model parameter(s)  t_0,beta_0,beta_1,beta_2,beta_3,beta_4
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix )

      c              beta_5
```


Supplemental Information—Formaldehyde (Inhalation)

```

c          1          -1
beta_5     -1          1

```

Parameter Estimates with 95.0% Wald Confidence Interval

Variable	Estimate	Std. Err.	Conf. Limits
c	8.11916	2.39978	3.41568, 12.8226
beta_5	2.94752e-022	3.25917e-021	-6.09311e-021, 6.68262e-021

Fitted Model	Log(likelihood)	# Param	AIC
	-90.3034	2	184.607

Data Summary

	C	F	I	U	Total
DOSE					
0	338	0	0	0	338
0.7	107	0	0	0	107
2	316	0	0	0	316
6	301	1	2	0	304
10	81	11	11	0	103

Minimum observation time for F tumor context = 79.3

Benchmark Dose Computation

```

Risk Response    =      Fatal
Risk Type        =      Extra
Specified effect =      0.1
Confidence level =      0.95

```

```

Time            =      104

```

```

BMD =      6.84417
BMDL =      6.34102
BMDU =      7.47507

```

Table D-30. Comparison of parameter estimates in Table D-29 with other Multistage-Weibull model fits to the same data

Model	Multistage degree k	# of est. params	Log-likelihood	AIC	c	t0	beta*
MSW 1 (best fit)	5	2	-90.3034	184.607	8.1192	Fixed at 0.0	2.9475e-022
MSW 2	5	3	-106.846	219.693	7.0753	11.8365	3.6714e-020
MSW 3	4	3	-110.048	226.095	6.9297	11.7798	5.9254e-019

*coefficient corresponding to the highest non-zero polynomial degree; other polynomial coefficients were set to zero.

Estimates from Experimental Animal Studies Using Biologically Based Dose-Response Modeling

Biologically based dose-response (BBDR) models were developed in a series of papers and in a health assessment report by scientists at the Chemical Industry Institutes of Toxicology (CIIT)

([Overton et al., 2001](#); [Kimbell et al., 2001b](#); [Kimbell and Subramaniam, 2001](#); [Conolly et al., 2000](#); [Conolly, 2002](#); [Conolly et al., 2003, 2004](#); [CIIT, 1999](#)) to interpret the tumor incidence observed in F344 rats in two long-term bioassays ([Monticello et al., 1996](#); [Kerns et al., 1983](#)) and extrapolate risk from rats to humans. The CIIT modeling and available data, and alternatives based on their original model were evaluated extensively for the purpose of this assessment and used in calculating the cancer potency. This section of the appendix separately addresses the BBDR models developed for the F344 rat and the human, and in each case: first provides clarifying details regarding the model, then summarizes all the issues evaluated, and finally provides detailed evaluations of key issues.

Model Structure and Calibration in ([Conolly et al., 2003, 2004](#))

In Conolly et al. ([2003](#)), tumor incidence data in the above long-term bioassays were modeled by using an approximation of the two-stage clonal growth model ([Moolgavkar et al., 1988](#)) and allowing formaldehyde to have directly mutagenic action. Conolly et al. ([2003](#)) combined these data with historical control data on 7,684 animals obtained from National Toxicology Program (NTP) bioassays. These models are based on the Moolgavkar, Venzon, and Knudson (MVK) stochastic two-stage model of cancer ([Moolgavkar and Venzon, 1979](#); [Moolgavkar and Knudson, 1981](#); [Moolgavkar et al., 1988](#)), which accounts for growth of a pool of normal cells, mutation of normal cells to initiated cells, clonal expansion and death of initiated cells, and mutation of initiated cells to fully malignant cells. The following notations are used in the rest of Appendix D.2.2:

- N cell, normal cell
- I cell, initiated cell
- LI, labeling index (number of labeled cells/(number labeled + unlabeled cells))
- ULLI, unit length labeling index (number labeled cells/length of basement membrane)
- N, number of normal cells that are eligible for progression to malignancy
- α_N , division rate of normal cells (hours⁻¹)
- μ_N , rate at which an initiated cell is formed by mutation of a normal cell (per cell division of normal cells)
- α_I , division rate of an initiated cell (hours⁻¹)
- β_I , death rate of an initiated cell (hours⁻¹)
- μ_I , rate at which a malignant cell is formed by mutation of an initiated cell (per cell division of initiated cells)

Cell replication rates and DPX concentrations are driven by local dose, which is formaldehyde flux to each region of nasal tissue expressed as pmol/mm²-hour and predicted by computational fluid dynamics (CFD) modeling using anatomically accurate representations of the nasal passages (see Appendix C.1.12). In the CIIT model, cell division and mutation is treated as a function of local flux. The spatial distribution of formaldehyde over the nasal lining was characterized by partitioning the nasal surface by formaldehyde flux to the tissue (rate of gas absorbed per unit surface area of the nasal lining), resulting in 20 “flux bins” with low bin numbers associated with low flux values. Each bin is comprised of elements of the nasal surface, which are

not necessarily contiguous, that receive a particular interval of formaldehyde flux per ppm of exposure concentration ([Kimbell et al., 2001b](#)). Because formaldehyde mass transfer is airflow-limited, flux is assumed to scale linearly with inhaled exposure concentration (ppm); accordingly, it is expressed in the CFD modeling in ([Kimbell et al., 2001b](#)) in terms of pmol/mm²-hour-ppm, and the spatial coordinates of elements comprising a particular flux bin are fixed for all exposure concentrations. Table D-31 tabulates the flux bins for the F344 rat. As seen from Table D-31, the nasal surface area attributed to a bin drop sharply with increasing flux bin numbers because there is a decreasing gradient of flux from proximal to distal regions of the nose.

Table D-31. Modeled formaldehyde flux on F344 rat nasal lining (Source: [Kimbell et al., 2001b](#))

Flux Bin	Flux/ppm ratio (pmol/mm ² -h-ppm)	Number of cells at risk in adult animals
1	5.08E+01	3.03E+07
2	1.90E+02	4.84E+06
3	3.25E+02	3.20E+06
4	4.55E+02	2.24E+06
5	5.88E+02	2.08E+06
6	7.18E+02	1.28E+06
7	8.58E+02	1.11E+06
8	9.78E+02	9.39E+05
9	1.11E+03	7.89E+05
10	1.25E+03	6.11E+05
11	1.37E+03	5.81E+05
12	1.51E+03	5.01E+05
13	1.64E+03	5.18E+05
14	1.76E+03	3.69E+05
15	1.90E+03	2.79E+05
16	2.02E+03	9.90E+04
17	2.16E+03	3.24E+04
18	2.28E+03	1.44E+04
19	2.40E+03	1.40E+03
20	2.56E+03	1.33E+03

Inputs to the model: The inputs to the two-stage cancer modeling consisted of results from other model predictions as well as empirical data. These included: regional uptake of formaldehyde in the respiratory tract predicted by using CFD modeling in the F344 rat and human ([Subramaniam et al., 1998](#); [Overton et al., 2001](#); [Kimbell et al., 2001b](#); [Kimbell and Subramaniam, 2001](#)) discussed

in Appendix A.2; concentrations of DPXs predicted by a PBPK model ([Conolly et al., 2000](#)) calibrated to fit the DPX data in F344 rat and rhesus monkey ([Casanova et al., 1991](#); [Casanova et al., 1994](#)) and subsequently scaled up to humans; and cell division rates for normal cells (α_N) inferred from labeling index data on rats exposed to formaldehyde ([Monticello et al., 1990](#); [Monticello et al., 1991](#); [Monticello et al., 1996](#)).

Calibration: The rat model in Conolly et al. (2003) involved six unknown statistical parameters that were estimated by fitting the model to the rat formaldehyde bioassay data shown in Table 2-20 of the main document ([Monticello et al., 1996](#); [Kerns et al., 1983](#)) plus historical data from several thousand control animals from all the rat bioassays conducted by the NTP. These NTP bioassays were conducted from 1976 through 1999 and included 7,684 animals with an incidence of 13 SCCs (i.e., 0.17% incidence). The resulting model predicts the probability of a nasal SCC in the F344 rat as a function of age and exposure to formaldehyde. The fit of the Conolly et al. (2003) model to the tumor incidence data is shown in Figure 5-5 of the main document.

Modeling formaldehyde's mutational action: Formaldehyde interacts with DNA to form DPXs. In Conolly et al. (2003), DPX formation is considered proportional to the intracellular dose of formaldehyde related to its directly mutagenic action. [Casanova et al. \(1989\)](#); [Casanova et al. \(1994\)](#) carried out two studies of DPX measurements in F344 rats. In the first study, rats were exposed to concentrations of 0.3, 0.7, 2, 6, and 10 ppm for 6 hours and DPX measurements were made over the whole respiratory mucosa of the rat, while in the second study, the exposure was to 0.7, 2, 6, or 15 ppm formaldehyde for 3 hours and measurements were made at “high” and “low” tumor sites. Conolly et al. (2000) used data from the second study to develop a PBPK model that predicted the time course of DPX concentrations as a function of regional formaldehyde flux (estimated in the CFD modeling and expressed as pmol/mm²-hour). In the two-stage clonal expansion model the mutation rate of normal and initiated cells were defined as the same linear function of DPX concentration as follows:

$$\mu_N = \mu_I = \mu_{N\text{basal}} + \text{KMU} \times \text{DPX} \quad (\text{D-19})$$

The unknown constants $\mu_{N\text{basal}}$ and KMU were estimated by fitting model predictions to the tumor bioassay data.

Use of labeling data: Cell replication rates in Conolly et al. (2003) were obtained by pooling labeling data from two phases of a labeling study in which male F344 rats were exposed to formaldehyde gas at similar concentrations (0, 0.7, 2.0, 6.0, 10.0, or 15.0 ppm). The first phase employed injection labeling with a 2-hour pulse labeling time, and animals were exposed to formaldehyde for early exposure periods of 1, 4, and 9 days and 6 weeks ([Monticello et al., 1991](#)). The second phase used osmotic minipumps for labeling with a 120-hour labeling time to quantify labeling in animals exposed for 13, 26, 52, and 78 weeks ([Monticello et al., 1996](#)). The combined pulse and continuous labeling data were expressed as one exposure time-weighted average (TWA)

over all sites for each exposure concentration. α_N was calculated from these labeling data by using an approximation from Moolgavkar and Luebeck (1992). A dose-response curve for normal cell replication rates (i.e., α_N as a function of formaldehyde flux) was then calculated as shown in Figure D-23.

Upward extrapolation of normal cell division rates: The extensive labeling data collected by (Monticello et al., 1991; Monticello et al., 1996) present an opportunity to use precursor data in assessing cancer risk. However, these empirical data were used to determine $\alpha_N(\text{flux})$ only for the lower flux range, 0–9,340 pmol/mm²-hour [see Subramaniam et al. (2008) for the reasons], as shown by the solid line in Figure D-23, whereas the highest computed flux at 15.0 ppm exposure was 39,300 pmol/mm²-hour. Therefore, Conolly et al. (2003) introduced an adjustable parameter, α_{max} , that represented the value of $\alpha_N(\text{flux})$ at the maximum flux of 39,300 pmol/mm²-hour. α_{max} was estimated by maximizing the likelihood of the two-stage model fit to the tumor incidence data. For 9,340 < flux ≤ 39,300 pmol/mm²-hour, $\alpha_N(\text{flux})$ was determined by linear interpolation from $\alpha_N(9,340)$ to α_{max} , as shown by the dashed line in Figure D-23.

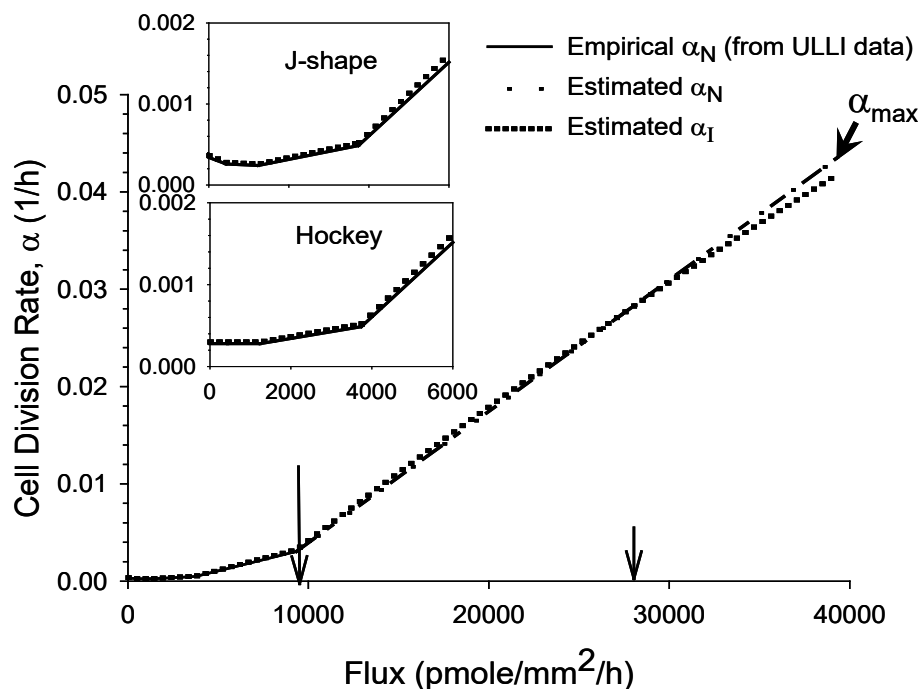


Figure D-23. Dose response of normal (α_N) and initiated (α_I) cell division rate in Conolly et al. (2003).

Note: Empirically derived values of α_N (TWA over six sites) from Table 1 in Conolly et al. (2003) and optimized parameter values from their Table 4 were used. The main panel is for the J-shaped dose response. Insets show J-shaped and hockey-stick shaped representations at the low end of the flux range. The long arrow denotes the upper end of the flux range for which the empirical unit-length labeling data are available for use in the clonal growth model. α_{max} is the value of α_N at the maximum formaldehyde flux delivered at 15 ppm exposure and estimated by optimizing model fit to the tumor incidence data. $\alpha_I < \alpha_N$ for flux greater than the value indicated by the small vertical arrow. (Conolly et al., 2003, 2004) assumed $\beta_I = \alpha_N$ at all flux values.

Source: Subramaniam et al. (2008).

Division and death rates of initiated cells: The pool of cells used for obtaining the LI data in (Monticello et al., 1991; Monticello et al., 1996) consists of largely normal cells, and it may be expected that there would be increasing numbers of initiated cells at higher exposure concentrations. Because the division rates of initiated cells in the nasal epithelium, α_i , either background or formaldehyde exposed, could not be inferred from the available empirical data, Conolly et al. (2003) assumed a two-parameter function to link α_I to α_N

$$\alpha_I = \alpha_N \times \{\text{multb} - \text{multc} \times \max[\alpha_N - \alpha_{N(\text{basal})}, 0]\} \quad (\text{D-20})$$

where $\alpha_N \equiv \alpha_N(\text{flux})$, $\alpha_{N(\text{basal})}$ is the estimated average cell division rate in unexposed normal cells, and multb and multc are unknown parameters estimated by likelihood optimization against the

tumor data.³³ The value of $\alpha_{N(\text{basal})}$ was equal to 3.39×10^{-4} hours⁻¹ as determined by Conolly et al. (2003) from the raw averaged unit length labeling index data. The ratio $\alpha_I:\alpha_N$ decreases with flux approximately from 1.07 to 0.96 over the flux range used in the modeling (see Figure 6 in (Subramaniam et al., 2008)).

Death rates of Initiated cells (β_I) are assumed to equal the division rates of normal cells (α_N) for all formaldehyde flux values, that is

$$\beta_I(\text{flux}) = \alpha_N(\text{flux}) \quad (\text{D-21})$$

No biological justification for these assumed relationships was provided by the authors. Conolly et al. (2003) stated that this formulation for α_I and β_I provided the best fit of the model to the tumor data.

Structure of the CIIT human model: Subsequent to the BBDR model for modeling rat cancer, Conolly et al. (2004) developed a corresponding model for humans for the purpose of extrapolating the nasal cancer risk estimated by the rat model to humans. Also, rather than considering only nasal tumors (as in the rat model), the human extrapolation model was used to predict the risk of all human respiratory tumors. The human extrapolation model is conceptually very similar to the rat model, and is based on an anatomically realistic representation of the human nasal passages in a single individual and an idealized representation of the LRT. Local formaldehyde flux to the tissue is estimated by a CFD model for humans (Subramaniam et al., 1998; Overton et al., 2001; Kimbell et al., 2001b). However, the model does not incorporate any data on human responses to formaldehyde exposure.

Rates of cell division and cell death are, with a minor modification, assumed to be the same in humans as in rats. The concentration of formaldehyde-induced DPXs in humans is estimated by scaling up from values obtained from experiments in the F344 rat and rhesus monkey.

The statistical parameters for the human model are either estimated by fitting the model to the human background data, assumed to have the same value as obtained in the rat model, or, in one case, fixed at a value suggested by the epidemiologic literature. The delay, D , is fixed at 3.5 years, based on a fit to the incidence of lung cancer in a cohort of British doctors (Doll and Peto, 1978). The two other parameters in the rat model that affect the background rate of cancer (multb and μ_{basal}) are estimated by fitting to U.S. cancer incidence or mortality data. These parameters affect the baseline values for the human α_I , μ_N , and μ_I . Because α_{max} , multfc , and KMU do not affect the background cancer rate, they cannot be estimated from the (baseline) U.S. cancer incidence rates. Therefore, in (Conolly et al., 2003, 2004), α_{max} and multfc are assumed to have the same

³³Multb and multc were equal to 1.072 and 2.583, respectively (J-shaped α_N), and 1.070 and 2.515, respectively (hockey-stick shaped α_N).

values in humans as in rats, and the human value for KMU is obtained by assuming that the ratio $KMU:\mu_{\text{basal}}$ is invariant across species. Thus,

$$KMU_{(human)} = KMU_{(rat)} \times \frac{\mu_{Nbasal(human)}}{\mu_{Nbasal(rat)}} \quad (D-22)$$

Evaluation of Conolly et al. (2003) Modeling of Nasal Cancer in the F344 Rat and Alternative Implementations

Table 5-39 in the dose-response section of the main document listed various issues that were evaluated by EPA pertaining to the BBDR modeling. An overview of that evaluation is first provided here, following which only the following four major issues are further elaborated: physiologically based pharmacokinetic modeling of DPXs, use of historical controls, the uncertainty and variability in the dose response for normal cell-replication rates, and sensitivity of model results to uncertainty in the kinetics of initiated cells.

Summary of Issues Evaluated in the Rat BBDR Modeling

Table D-32 summarizes model uncertainties and their impact as evaluated by EPA and points the reader to sections of this document or published manuscripts ([Subramaniam et al., 2007](#); [Subramaniam et al., 2008](#); [Crump et al., 2008](#)) where key uncertainties are discussed in more detail. The results in Subramaniam et al. (2007) and Crump et al. (2008) have been debated further in the literature ([Crump et al., 2009](#); [Conolly et al., 2009](#)). Other alternatives to the CIIT biological modeling (but based on that original model) are also further explored and evaluated below.

Table D-32. Evaluation of assumptions and uncertainties in the CIIT model for nasal tumors in the F344 rat

	Assumptions, approach, and characterization of input data in model ^a	Rationale for assumption/ approach	EPA evaluation	Further elaboration of evaluation
1	Steady-state flux estimates are not affected by airway and tissue reconfiguration due to long-term dosing.	Histopathologic changes not likely to be rate-limiting factors in dosimetry.	1) Thickening of epithelium and squamous metaplasia occurring at later times for the higher dose (Kimbell et al., 1997a) will reduce tissue flux. Not incorporated in model. 2) These effects will push regions of higher flux to more posterior regions of respiratory tract. Likely to affect calibration of rat model. Uncertainty not evaluated quantitatively. 3) Calibration of PBPK model for DPXs was seen to be highly sensitive to tissue thickness.	Subramaniam et al. (2008); Cohen Hubal et al. (1997); Klein et al. (2011)

Supplemental Information—Formaldehyde (Inhalation)

	Assumptions, approach, and characterization of input data in model ^a	Rationale for assumption/ approach	EPA evaluation	Further elaboration of evaluation
2	DPX is dose surrogate for formaldehyde's mutagenic potential. DPX clearance is rapid and complete in 18 hrs.	Casanova et al. (1994).	Half-life for DPX clearance in in vitro experiments on transformed cell lines was 7 times longer than estimated by (Conolly et al., 2003, 2004) and perhaps 14 times longer with normal (nontransformed) human cells. In vivo studies Lai (2016) indicate a slow repair of DPX. Some DPX accumulation is therefore likely. However, model calibration and dose response in rat was insensitive to this uncertainty.	Quievryn and Zhitkovich (2000); Subramaniam et al. (2007); D.2.2
3	Formaldehyde's mutagenic action takes place only while DPX's are in place.		DNA lesions may remain after DPX repair and incomplete repair of DPX can lead to mutations (Barker et al., 2005). There is some potential for formaldehyde-induced mutation after DPX clearance. Thus, it is possible that formaldehyde mutagenicity may be underrepresented in model. Could not quantitatively evaluate uncertainty (no data on clearance of secondary lesions).	Subramaniam et al. (2008);
4	Hoogenveen et al. (1999) solution method, which is valid only for time-independent parameters, is accurate enough.	Errors due to this assumption thought to be significant only at high concentration and not at human exposures.	EPA implemented a solution method valid for time-dependent parameters. Results did not differ significantly from those obtained assuming Hoogenveen et al. (1999) solutions. However, impact was not evaluated for the case where cell replication rates vary in time.	Crump et al. (2005); Subramaniam et al. (2007)
5	All observed SCC tumors are rapidly fatal; none are incidental tumors.	Death is expected to occur typically within 1–2 weeks of observed tumor (personal communication with R. Conolly).	1) Overall, assumption does not impact model calibration or prediction. 2) However, because 57 animals were observed to have tumors at interim sacrifice times, EPA implementation of this model distinguished between incidental and fatal tumors. Time lag between observable tumor and time of death was significant compared to time lag between first malignant cell and observable tumor.	Subramaniam et al. (2007)

Supplemental Information—Formaldehyde (Inhalation)

	Assumptions, approach, and characterization of input data in model^a	Rationale for assumption/ approach	EPA evaluation	Further elaboration of evaluation
6	Historical controls from entire NTP database were lumped with concurrent controls in studies.	Large number of control animals (7,684). Intercurrent mortality was not expected to be substantial.	1) Tumor incidence in “all NTP” 10-fold higher than in “all inhalation NTP” controls. Including all NTP controls is considered inappropriate. 2) Low-dose-response curve is very sensitive to use of historical controls. 3) Large impact on parametrizations and predictions from corresponding human extrapolation model.	Subramaniam et al. (2007); Crump et al. (2008); D.2.2; Table D-35; Table D-40; Figure D-38
7a	LI was derived from experimentally measured ULLI.	Derived from correlating ULLI to LI measured in same experiment.	Significant variation in number of cells per unit length of basement membrane. Spread in ULLI/LI $\approx$ 25%. Impact on risk not evaluated.	Subramaniam et al. (2008)
7b	Pulse and continuous labeling data were combined in deriving α_N from LI.	All continuous LI values were normalized by mean ratio of pulse to continuous LI for controls.	Formula used for deriving α_N from LI is not applicable for pulse labeling data. Pulse labeling is measure of number of cells in S-phase, not of their recruitment rate into S-phase; not enough information to derive α_N from pulse data. Impact on risk predictions could not be evaluated.	Subramaniam et al. (2008); D.2.2
7c	To construct dose response for α_N , labeling data were weighted by exposure time (t) and averaged over all nasal sites (TWA). For a given exposure concentration, flux was then averaged over all nasal sites.	Site-to-site variation in LI was large and did not vary consistently with flux. No reasonable approach was available for extrapolating observed time variation in labeling in rats to humans.	1) TWA assigns low weight to early time LI values, but α_N for early time (t) is very important to the cancer process. Because pulse ULLI was used for $t < 13$ wks, impact of these ULLIs on risk could not be evaluated. 2) Time dependence in α_N derived from continuous ULLI does not significantly impact model predictions. 3) Site-to-site variation of α_N is at least 10-fold and has major impact on model calibration. Variation in tumor incidence data across sites is 10-fold. 4) Large differences in number of cells across nasal sites, so averaging over sites is problematic. 5) TWA is also problematic because histologic changes, thickening of epithelium and metaplasia occur at later times for the higher dose and would affect replication rate.	Subramaniam et al. (2008); D.2.2, Table D-40, Figures D-21 to D-33

Supplemental Information—Formaldehyde (Inhalation)

	Assumptions, approach, and characterization of input data in model ^a	Rationale for assumption/ approach	EPA evaluation	Further elaboration of evaluation
7d	TWA α_N (flux) rises above baseline levels only at cytolethal dose. Above such dose, α_N (flux) rises sharply due to regenerative proliferation.	Variability in α_N (flux) is partly represented by also considering hockey-stick (threshold in dose) when TWA indicates J-shaped (inhibition of cell division) description of α_N (flux).	1) Uncertainty and variability in α_N were quantitatively evaluated to be large. In addition, there are several qualitative uncertainties in characterization of α_N (flux) from LI. 2) Several dose-response shapes, including a monotonic increasing curve without a threshold, were considered in order to adequately describe highly dispersed cell replication data. This has substantial impact on low dose risk.	Subramaniam et al. (2008); D.2.2, Figures D-21 to D-33, Figure D-42.
8a	Dose response for α_I was obtained from α_N , assuming ratio ($\alpha_I:\alpha_N$) to be a two-parameter function of flux (equation D-20). Parameters were estimated by optimizing model predictions against tumor incidence data.	($\alpha_I:\alpha_N$) was >1.0 in line with the notion of I cells possessing a growth advantage over N cells. Assumption satisfies Occam's razor principle (Conolly et al., 2009).	1) $\alpha_I:\alpha_N$ in CIIT modeling is <1.0 (growth disadvantage) for higher flux values and is >1.0 only at lower end of flux range in model (Figure D-23). 2) Because there are no data to inform α_I , sensitivity of risk estimates to various functional forms was evaluated. Risk estimates for the rat were extremely sensitive to alternate biologically plausible assumptions for α_I (flux) and varied by many orders of magnitude below concentrations with observable tumors, including values lower than baseline risk. All these models described tumor incidence data and cell replication and DPX data equally well.	Subramaniam et al. (2008); Crump et al. (2008); Crump et al. (2009); D.2.2, Figures D-36, D-37; Tables D-37, D-38.
8b	Death rate of I cells is assumed equal to division rate of N cells i.e., β_I (flux) = α_N (flux). (equation D-21)	Based on homeostasis ($\alpha_N = \beta_N$) and assumption that formaldehyde is equally cytotoxic to N cells and I cells. Assumption satisfies Occam's razor principle (Conolly et al., 2009).	1) In general, data indicate I cells are more resistant to cytolethality and that ADH3 clearance capacity is greater in transformed cells. Therefore, $\beta_I = \alpha_N$ is a tenuous model assumption. 2) Alternate assumption, β_I proportional to α_I , was examined. Risk estimates were extremely sensitive to assumptions on β_I .	Subramaniam et al. (2008); Crump et al. (Crump et al., 2009); Crump et al. (2008); D.2.2, Figures D-36, D-37; Table D-37.

^a(Conolly et al., 2003, 2004).

Given the scope of issues to examine, the evaluation of the BBDR modeling as presented in Conolly et al. (2003), and in alternative approaches considered by EPA, proceeded in stages. First, the dosimetric models for formaldehyde flux and DPXs were evaluated. Confidence in the CFD modeling of formaldehyde flux has been assessed in the toxicokinetic modeling section earlier, and is not repeated here. The evaluation of PBPK models for predicting DPXs is presented below.

Second, the [Hoogenveen et al. \(1999\)](#) solution was replaced by one that is valid for a model with time-varying parameters [[Crump et al. \(2005\)](#)], and tumors found at scheduled sacrifices were assumed to be incidental rather than fatal (see Table D-32 and Subramaniam et al. ([2007](#))). Third, PBPK model-predicted weekly averaged solutions for DPX concentration levels were used instead of hourly varying solutions (see Figure 1 and Appendix A in ([Subramaniam et al., 2007](#))). The log-likelihood values and tumor probabilities remained essentially unchanged. Upon quantitative evaluation, these factors, although important from a methodological point of view, were not found to be major determinants of either calibration or prediction of the model for the F344 rat data ([Subramaniam et al., 2007](#)). EPA evaluation first attempted to reproduce the Conolly et al. ([2003](#)) results under similar conditions and assumptions, including the assumption that tumors were rapidly fatal. Figure 5-5 of the main document shows the results from Conolly et al. ([2003](#)) and the predicted probabilities from Subramaniam et al. ([2007](#)) (source code made available by Dr. Conolly). These are compared with the best-fitting model and plotted against the Kaplan-Meier (KM) probabilities. Although the results are largely similar, there are some residual differences, and these are detailed in Subramaniam et al. ([2007](#)).

Following Georgieva et al. ([2003](#)), Subramaniam et al. ([2007](#)) used the DPX clearance rate constant obtained from in vitro data instead of the assumption in Conolly et al. ([2003](#)) that all DPXs cleared within 18 hours ([Subramaniam et al., 2007](#)). With this revision, weekly average DPX concentrations were larger than those in Conolly et al. ([2003](#)) by essentially a constant ratio equal to 4.21 (range of 4.12–4.36) when averaged over flux bin and exposure concentrations. Cancer model fits to the rat tumor incidence data using the two sets of DPX concentrations (everything else remaining the same) provided very similar parameter estimates, except that the parameter KMU_{rat} in equation D-19 was 4.23 times larger with the Conolly et al. ([2003](#)) DPX concentrations. In other words, the product $KMU \times DPX$ remained substantially unchanged. However, it is important to note that the different clearance rate does significantly impact the scale-up of the two-stage clonal growth model to the human because the parameter KMU_{human} is not estimated separately but related to KMU_{rat} (see equation D-22).

After making the above modifications, the impact of the other uncertainties in Table D-32 were examined; only three uncertainties had large impacts on the modeling of the F344 rat data. These uncertainties and the evaluation of the PBPK modeling of DPX will be discussed in more detail below:

- 1) evaluation and model selection of PBPK models for DPX,
- 2) use of historical controls,
- 3) uncertainty and variability in characterizing cell replication rates from the labeling data, and
- 4) uncertainty in model specification of initiated cell kinetics.

Physiologically based pharmacokinetic models for DPX: evaluation and model selection

The CFD modeling discussed in the toxicokinetics section models the transport of formaldehyde through the air phase to the tissue lining on the respiratory tract. While these calculations involved the specification of boundary conditions that appropriately characterize the air-tissue interface, the internal dose of formaldehyde and its reaction with tissue constituents was not explicitly modeled. Several physiologically based pharmacokinetic (PBPK) models have been developed to describe the disposition of formaldehyde in the tissue accounting for formaldehyde reaction via saturable and first order pathways that include the formation and, in some models clearance, of DNA protein cross links (DPX) formed by formaldehyde. These models relied wholly or partly on various experimental measurements of DPX in the upper respiratory tract of the F344 rat and rhesus monkey and in the lower respiratory tract of the rhesus monkey ([Casanova et al., 1989](#); [Casanova et al., 1991](#); [Casanova et al., 1994](#)), which were discussed earlier in Appendix C.1. The measurements, and subsequently the models that were based upon these data, allowed the use of formaldehyde-DPX as an internal dosimeter of inhaled formaldehyde, in particular, as a surrogate for the molecular dose associated with formaldehyde's mutagenic potential. These models are tabulated below in Table D-33.

Table D-33. PBPK models for formaldehyde-DPX

Model	Dpx data	Animal species	Human extrapolation model	Compartments and pathways	Includes air-phase formaldehyde flux?
Casanova et al. (1991)	Casanova et al. (1989); 6-hr exp; 0.3, 0.7, 2.0, 6.0, 10 ppm Casanova et al. (1991); 6-hr exp; 0.7, 2.0, 6.0 ppm	F344 rat Rhesus monkey	No	Single well-stirred compartment. Saturable & 1 st order metabolism, 1 st order DPX formation but not clearance.	No
Heck & Casanova (1994)	Casanova et al. (1994); 0.7, 2, 6, 15 ppm preexposed + naïve groups	F344 rat	No	Similar to Casanova et al. (1991). Included effects of preexposure, induction of hyperplasia at conc > 6 ppm.	No
Cohen Hubal et al. (1997)	Casanova et al. (1989) above + Casanova (1994); 3-hr exp; 0.7, 2.0, 6.0, 15 ppm	F344 rat	No	Casanova (1991) model+air-phase transport+ 1 st order DPX clearance. Predicted DPX in a more localized region based on model calibrated over whole nose	Yes (Kimbell et al., 1997a)

Supplemental Information—Formaldehyde (Inhalation)

Model	Dpx data	Animal species	Human extrapolation model	Compartments and pathways	Includes air-phase formaldehyde flux?
Conolly et al. (2000)	Casanova et al. (1989) above + Casanova (1994); 3-hr exp, 0.7, 2.0, 6.0, 15 ppm Casanova et al. (1991); 6-hr exp; 0.7, 2.0, 6.0 ppm	F344 rat Rhesus monkey	Yes	Similar to Cohen Hubal et al. (1997). Derived allometric rule based on rat and rhesus model to develop human extrapolation model	Yes (Kimbell et al., 2001b)
Georgieva et al. (2003)	Casanova et al. (1989) above + Casanova (1994) 3 hr exp, 0.7, 2.0, 6.0, 15 ppm	F344 rat	No	Multilayer tissue compartment, epithelia of varying thickness. Saturable & 1 st order metabolism, 1 st order DPX formation & clearance, clearance rate derived from in vitro data	Yes, (Kimbell et al., 2001b)
Franks et al. (2005)	Did not use data on DPX or formaldehyde levels for calibration. Parameter values from other models were used.	Model developed for humans		Continuous distribution of formaldehyde across mucous, epithelial & blood perfused submucosal layers; diffusional transport of formaldehyde through mucous layer; Saturable & 1 st order metabolism, 1 st order DPX formation but not clearance. Model evaluated systemic transport of formaldehyde.	No
Subramaniam et al. (2007)	Casanova et al. (1989) above + Casanova (1994) 3 hr exp, 0.7, 2.0, 6.0, 15 ppm.	F344 rat	No	Saturable & 1 st order metabolism, 1 st order DPX formation & clearance, clearance rate derived from in vitro data	Yes, (Kimbell et al., 2001b)

In addition, Klein et al. (2011) used Conolly et al. (2000) as a case study to demonstrate approaches for uncertainty analyses of PBPK modeling for situations involving limited time course data. Of the models in Table D-33, clearance of DPX by repair processes was not considered in Casanova et al. (1991), Heck and Casanova (1994) and Franks et al. (2005), and only Conolly et al. (2000) extended their animal PBPK model to develop a corresponding model for the human. The Conolly et al. (2000) modeling presents other features that are useful in the context of modeling formaldehyde dose response. Their PBPK modeling of DPX kinetics explicitly incorporates regional formaldehyde dosimetry in the nasal lining by using results from CFD modeling of airflow and gas

uptake. Furthermore, results from their models were used as input to biologically based cancer dose-response (BBDR) modeling developed by the same authors. Because of these reasons, EPA evaluated the Conolly et al. (2000) PBPK effort, following which it was modified (see Appendix A in Subramaniam et al. (2007)) and used in EPA's dose-response assessment. The Conolly et al. (2000) model is first described below.

In earlier risk assessment efforts by Hernandez et al. (1994) and Casanova et al. (1991), the average DPX concentration was considered a surrogate tissue dose metric for the area-under-the-curve (AUC) of the reactive formaldehyde species. Conolly et al. (2003) assigned a more specific role for DPXs, treating local DPX concentration as a dose surrogate indicative of the intercellular concentration of formaldehyde leading to formaldehyde-induced mutations. These authors indicated that it was not known whether DPXs directly induced mutations (Merk and Speit, 1998; Conolly et al., 2003). The Conolly et al. (2000) model consists of a single well-mixed compartment for the nasal lining as follows:

- 1) Formaldehyde flux to a given region of the nasal lining is provided as input to the modeling and is obtained in turn as the result of a CFD model. This flux is defined as the amount of formaldehyde delivered to the nasal lining per unit time per unit area per ppm of concentration in the air in a direction transverse to the airflow. It is locally defined as a function of location in the nose and the inspiratory flow rate and is linear with exposure concentration.
- 2) The clearance of formaldehyde from the tissue is modeled as a saturable pathway representing enzymatic metabolism of formaldehyde primarily by formaldehyde dehydrogenase (involving Michaelis-Menten parameters V_{max} and K_m); a separate first-order pathway, which is assumed to represent the intrinsic reactivity of formaldehyde with tissue constituents (rate constant k_f); and a first-order binding to DNA that leads to DPX formation (rate constant k_b).
- 3) The clearance or repair of DPX is modeled as a first order process (rate constant k_{loss}).

DPX concentrations were estimated from a study by Casanova et al. (1994) in which rats were exposed 6 hours/day, 5 days/week, plus 4 days for 11 weeks to filtered air (naïve) or to 0.7, 2, 6, or 15 ppm (0.9, 2.5, 7.4, or 18 mg/m³) formaldehyde (preexposed). On the 5th day of the 12th week, the rats were then exposed for 3 hours to 0, 0.7, 2, 6, or 15 ppm ¹⁴C-labeled formaldehyde (with preexposed animals exposed to the same concentration as during the preceding 12 weeks and 4 days). The animals were sacrificed and DPX concentrations determined at two sites in the nasal mucosa. Conolly et al. (2000) used these naïve rat data to develop a PBPK model that predicted the time-course of DPX concentrations as a function of formaldehyde flux at these sites.³⁴

Casanova et al. (1994) observed that the DPX concentrations measured in the preexposed animals (exposed for 11.5 weeks) were not significantly higher than those in naïve (air-exposed

³⁴Subramaniam et al. (2007) who also used the same data verified that they were on naïve rats; however, Conolly et al. (2000) state that they used data on preexposed rats.

control) animals in which there was no significant DPX accumulation. This was interpreted to mean that DPX repair is rapid enough to completely eliminate the DPX formed in a single 6-hour exposure by the beginning of the next day. Based on this observation, Conolly et al. (2000) assumed a value of $6.5 \times 10^{-3} \text{ minute}^{-1}$ for *kloss*, the first-order rate constant for the clearance (repair) of DPXs, such that the DPXs predicted at the end of a 6-hour exposure to 15 ppm were reduced to exactly the detection limit for DPXs in 18 hours.

Uncertainties in PBPK Modeling of the Rat and Rhesus DPX Data

The above assumption of rapid DPX repair in Conolly et al. (2000) appears to be questionable on three grounds. First, in vitro data from three human cell lines indicated a much slower clearance, with an average *kloss* of $9.24 \times 10^{-4} \text{ minute}^{-1}$ (Quievryn and Zhitkovich, 2000). While the in vitro data can be uncertain because these cells were transformed and immortalized, it appears that DPX repair in normal cells would be even slower. When nontransformed freshly purified human peripheral lymphocytes were used instead, the half-life for DPX repair was about 50% longer than in the cultured cells (Quievryn and Zhitkovich, 2000). Later in vivo studies by Lai (2016) indicated an even more substantially slower repair. In rats exposed to 2.5 mg/m³ formaldehyde for 28 days, 87% of the exposure-induced DPX were retained in the nasal tissues at 1-week postexposure.

Second, Subramaniam et al. (2007) reexamined the Casanova et al. (1994) data for their PBPK modeling and concluded that the experimental results in Casanova et al. (1994) were consistent with the smaller experimental value of *kloss* indicated by the Quievryn and Zhitkovich (2000) data. Subramaniam et al. (2007) found a significantly decreased ($\approx 40\%$) level of DPXs in the high tumor regions of preexposed animals relative to naïve animals at 6 and 15 ppm. This was accompanied by a substantial increase in weight of the tissues dissected from those regions indicating a thickening of the tissues as is to be expected from metaplastic transformation of normal tissue to the squamous type due to formaldehyde toxicity. However, after testing the outcome of changing the tissue thickness in the PBPK model for DPXs, it was apparent to these authors that such a change alone could not account for the dramatic reduction in DPX levels after preexposure, even with the higher value of *kloss* used by Conolly et al. (2000). Because *Vmax* was found to be very sensitive to tissue thickness (as also noted by others; (Klein et al., 2011; Georgieva et al., 2003; Conolly et al., 2000)), Subramaniam et al. (2007) increased the value of *Vmax* with exposure (in a tissue region- and dose-specific manner) and found that it was possible to explain the naïve versus preexposed data of Casanova et al. (1994) with the 7-fold lower value of *kloss*. This was consistent with the hypothesis of either an induction in the activity of enzymes that remove formaldehyde (aldehyde- and formaldehyde dehydrogenase) or other changes in the biochemical properties of highly exposed tissue.

Third, the value for *kloss* used by Conolly et al. (2000) was inferred indirectly from measurements made at only two time points where significant changes in the tissue had occurred. On account of these reasons, Subramaniam et al. (2007) considered the use of the lower value for

*k*loss from in vitro observations to be more appropriate. The same lower value of *k*loss was also used by Georgieva et al. (2003). Consequently, Subramaniam et al. (2007) reimplemented and reoptimized the Conolly et al. (2000) model with this modification and obtained a good fit to the acute DPX data. The reimplemented model is used in this assessment (see Table D-34 for DPX concentrations predicted by the model). Both models provide good similar fits to the DPX data gathered from different regions of the nose immediately after single 3.0-hour and 6.0-hour acute exposures.

Table D-34. Comparison of (Conolly et al., 2000) and (Subramaniam et al., 2007) DPX concentrations, averaged over 1 week (pmol/mm³)

Flux Bin	Conolly					Subramaniam				
	0.7 ppm	2 ppm	6 ppm	10 ppm	15 ppm	0.7 ppm	2 ppm	6 ppm	10 ppm	15 ppm
1	8.08E-05	2.35E-04	7.26E-04	1.24E-03	1.96E-03	3.35E-04	9.74E-04	3.02E-03	5.17E-03	8.19E-03
2	3.07E-04	9.22E-04	3.17E-03	6.12E-03	1.16E-02	1.27E-03	3.83E-03	1.33E-02	2.58E-02	4.96E-02
3	5.34E-04	1.66E-03	6.46E-03	1.46E-02	3.47E-02	2.22E-03	6.90E-03	2.73E-02	6.27E-02	1.51E-01
4	7.61E-04	2.44E-03	1.09E-02	2.94E-02	7.64E-02	3.17E-03	1.02E-02	4.65E-02	1.28E-01	3.30E-01
5	9.99E-04	3.32E-03	1.74E-02	5.35E-02	1.31E-01	4.16E-03	1.39E-02	7.51E-02	2.33E-01	5.56E-01
6	1.24E-03	4.28E-03	2.65E-02	8.45E-02	1.88E-01	5.16E-03	1.79E-02	1.15E-01	3.64E-01	7.93E-01
7	1.51E-03	5.43E-03	3.99E-02	1.23E-01	2.52E-01	6.29E-03	2.28E-02	1.74E-01	5.23E-01	1.06E+00
8	1.75E-03	6.53E-03	5.44E-02	1.57E-01	3.07E-01	7.28E-03	2.75E-02	2.36E-01	6.66E-01	1.28E+00
9	2.01E-03	7.86E-03	7.27E-02	1.96E-01	3.68E-01	8.41E-03	3.32E-02	3.14E-01	8.26E-01	1.53E+00
10	2.30E-03	9.42E-03	9.39E-02	2.37E-01	4.32E-01	9.62E-03	3.99E-02	4.03E-01	9.95E-01	1.79E+00
11	2.59E-03	1.11E-02	1.15E-01	2.76E-01	4.93E-01	1.08E-02	4.72E-02	4.92E-01	1.16E+00	2.04E+00
12	2.89E-03	1.30E-02	1.38E-01	3.17E-01	5.55E-01	1.21E-02	5.56E-02	5.87E-01	1.33E+00	2.30E+00
13	3.19E-03	1.51E-02	1.61E-01	3.57E-01	6.16E-01	1.34E-02	6.49E-02	6.81E-01	1.49E+00	2.55E+00
14	3.50E-03	1.75E-02	1.84E-01	3.97E-01	6.76E-01	1.47E-02	7.52E-02	7.75E-01	1.65E+00	2.80E+00
15	3.83E-03	2.02E-02	2.08E-01	4.38E-01	7.38E-01	1.61E-02	8.72E-02	8.73E-01	1.82E+00	3.05E+00
16	4.15E-03	2.31E-02	2.30E-01	4.76E-01	7.97E-01	1.75E-02	9.98E-02	9.66E-01	1.98E+00	3.29E+00
17	4.53E-03	2.67E-02	2.56E-01	5.20E-01	8.63E-01	1.91E-02	1.16E-01	1.07E+00	2.16E+00	3.56E+00
18	4.89E-03	3.03E-02	2.79E-01	5.58E-01	9.21E-01	2.06E-02	1.31E-01	1.17E+00	2.31E+00	3.80E+00
19	5.24E-03	3.39E-02	3.00E-01	5.94E-01	9.76E-01	2.20E-02	1.47E-01	1.25E+00	2.46E+00	4.03E+00
20	5.75E-03	3.97E-02	3.31E-01	6.46E-01	1.05E+00	2.42E-02	1.72E-01	1.38E+00	2.68E+00	4.35E+00

Sensitivity to use of historical controls

Use of historical controls: Conolly et al. (2003) combined the historical controls arising from the entire NTP database of bioassays. Tumor and survival rates in control groups from different NTP studies are known to vary due to genetic drift in animals over time and differences in laboratory procedures, such as diet, housing, and pathological procedures (Rao et al., 1987; Haseman, 1995). In order to minimize extra variability when historical control data are used, the current NTP practice is to limit the historical control data, as far as possible, to studies involving the same route of exposure and to use historical control data from the most recent studies (Peddada and Kissling, 2006).

Bickis and Krewski ([1989](#)) analyzed 49 NTP long-term rodent cancer bioassays and found a large difference in determinations of carcinogenicity, depending on the use of historical controls with concurrent control animals. The historical controls used in the CIIT modeling controls came from different rat colonies and from experiments conducted in different laboratories over a wide span of years, so it is clearly problematic to assume that background rates in these historical control animals are the same as those in the concurrent control group. There are considerable differences among the background tumor rates of SCCs in all NTP controls (13/7,684 = 0.0017), NTP inhalation controls (1/4,551 = 0.0002), and concurrent controls (0/341 = 0.0). The rate in all NTP controls is significantly higher than that in NTP inhalation controls ($p = 0.01$, Fisher's exact test). Given these differences, the inclusion of any type of historical controls is problematic and is thought to have limited value if these factors are not controlled for ([Haseman, 1995](#)).

Influence of historical controls on model calibration and on human model: To investigate the effect of including historical controls in the CIIT model, the analyses in Subramaniam et al. ([2007](#)) were conducted by using the following sets of data for controls (the fraction of animals with SCCs is denoted in parentheses): (a) only concurrent controls (0/341), (b) concurrent controls plus all the NTP historical control data used by ([Conolly et al., 2003](#)) (13/8,031), (c) concurrent controls plus data from historical controls obtained from NTP inhalation studies (1/4,949) ([National Toxicology Program \(NTP\), 2005](#)).³⁵

The results of the evaluation are shown in Table D-35. For these analyses, the same normal cell replication rates and the same relationship, equations D-20 & D-21, between initiated cell and normal cell replication rates as used in Conolly et al. ([2003](#)) were used. In all cases, weekly averaged values of DPX concentrations were used. Model fits to the tumor incidence data were similar in all cases to that shown in Figure 5-5 [see Subramaniam et al. ([2007](#)) for a more complete discussion]. The biggest influence of the control data was seen to be on the estimated basal mutation rate in rats, $\mu_{Nbasal(rat)}$, which, in turn, influences the estimated mutation effect in humans through equation D-22. α_{max} was also seen to be a sensitive parameter and is discussed later. See Subramaniam et al. ([2007](#)) for other parameters in the calibration.

Table D-35. Influence of control data in modeling formaldehyde-induced cancer in the F344 rat

Case	A	D	B	E	C	F
Control animals (combined with	All NTP historical ^a	All NTP historical ^a	NTP inhalation historical ^a	NTP inhalation historical ^a	Concurrent only ^a	Concurrent only ^a

³⁵Three animals in the inhalation historical controls were diagnosed with nasal SCC. Of these, two of the tumors were determined to have originated in tissues other than the nasal cavity upon further review (Dr. Kevin Morgan and Ms. Betsy Gross Bermudez, personal communication). These two tumors, therefore, were not included on the advice of Dr. Morgan. See Subramaniam et al. ([2007](#)) for more details.

Supplemental Information—Formaldehyde (Inhalation)

Case	A	D	B	E	C	F
concurrent controls)						
Cell replication dose response	J shape	Hockey stick	J shape	Hockey stick	J shape	Hockey stick
Log-likelihood	-1,692.65	-1,693.68	-1,493.21	-1,493.35	-1,474.29	-1,474.29
$\mu_{N_{\text{basal}}}$	1.87×10^{-6}	2.12×10^{-6}	7.32×10^{-7}	9.32×10^{-7}	0.0	0.0
KMU	1.12×10^{-7}	0.0	6.84×10^{-7}	6.18×10^{-7}	1.20×10^{-6}	1.20×10^{-6}
KMU: $\mu_{N_{\text{basal}}}$	0.06 (0.0, 0.40)	0.0 (0.0, 0.25)	0.94 (0.26, 6.20)	0.66 (0.2, 5.20)	∞ (0.42, ∞)	∞ (0.41, ∞)
α_{max}	0.045 (0.029, 0.045)	0.045 (0.029, 0.045)	0.045 (0.026, 0.045)	0.045 (0.027, 0.045)	0.045 (0.027, 0.045)	0.045 (0.027, 0.045)

^aValues in parentheses denote lower and upper 90% confidence bounds.

Source: Adapted from Subramaniam et al. (2007).

The ratio KMU: $\mu_{N_{\text{basal}}}$ is of particular interest because extrapolation to human in Conolly et al. (2004) assumed its invariance as given by equation B-15. Now, $\mu_{N_{\text{basal}}}$ in the human is estimated independently by fitting a scaled-up version of the two-stage model to human baseline rates of tumor incidence. Thus, a decrease in the value of $\mu_{N_{\text{basal}}}$ estimated in the rat modeling increases the formaldehyde-induced mutational effect in the human.

The MLE of KMU_{rat}: $\mu_{N_{\text{basal}}(\text{rat})}$ is zero in (Conolly et al., 2003). However, in the various cases examined in Subramaniam et al. (2007) it takes a range of values from 0 to 0.9 mm³/pmol and undefined (or infinite, when $\mu_{N_{\text{basal}}} = 0$). The 95% upper confidence bound on this ratio ranges from 0.25–6.2 [these values would be four times larger had the Conolly et al. (2003) DPX concentrations been used] to infinite. Thus, the extrapolation to human risk by using the approach in Conolly et al. (2004) becomes particularly problematic when only concurrent controls are used, because then the mutational contribution to formaldehyde-induced risk in humans becomes unbounded. This issue will be discussed again toward the end of the discussion on historical controls.

It may be noted, however, that absence of tumors in the limited number of concurrent animals does not imply that the calculation will necessarily predict a zero background probability of tumor (i.e., a parameter estimate of $\mu_{N_{\text{basal}}} = 0$). Nonetheless, when $\mu_{N_{\text{basal}}} = 0$, an upper bound for $\mu_{N_{\text{basal}}}$ using the concurrent controls could be inferred. Accordingly, the 90% statistical lower confidence bound on the ratio KMU: $\mu_{N_{\text{basal}}}$ is also reported in Table D-35. Such a value would of course provide a lower bound on risk by using this model and, therefore, would not be conservative.

Conolly et al. (2003) estimated KMU to be zero for both their hockey-stick and J-shaped dose-response models for cell replication. However, the estimate for the coefficient KMU [obtained using the solution of Crump et al. (2005)] is zero only for the case of the model with the hockey-stick curve for cell replication and with control data as used by Conolly et al. (2003). It is positive in all other cases and statistically significantly so in all cases in which either NTP inhalation control

data or concurrent controls were used. With concurrent controls only and the J-shaped cell replication model, the MLE estimate for KMU (1.2×10^{-6}) is larger than the statistical upper bound obtained by Conolly et al. (2003) (8.2×10^{-7}). The estimate would be about 4.2 times larger had the Conolly et al. (2003) DPX model been used.

Influence of historical controls on dose-response curve: Subramaniam et al. (2007) showed that inclusion of historical controls had a strong impact on the tumor probability curve below the range of exposures over which tumors were observed in the formaldehyde bioassays. As shown there, the MLE probabilities for occurrence of a fatal tumor at exposure concentrations below 6 ppm were roughly an order of magnitude higher when all the NTP historical controls were used, compared with MLE probabilities predicted when historical controls were drawn only from inhalation bioassays, and many orders of magnitude higher than MLE probabilities predicted when only concurrent controls were used in the analysis. (Note that this comparison should not be inferred to apply to upper bound risk estimates because there were many fewer concurrent than historical controls, so error bounds could be much larger in the case where concurrent controls were used.)

However, as shown by these authors, model fits to the tumor data in the 6–15 ppm exposure concentration range were qualitatively indifferent to which of these control data sets was used. This observation emphasizes the statistical aspect of the CIIT modeling—that significant interplay among the various adjustable parameters allows the model to achieve a good fit to the tumor incidence data independent of the control data used. On the other hand, the results in Subramaniam et al. (2007) show that changes in the control data affect parameter KMU, resulting in significantly different tumor predictions at lower exposure concentrations. Therefore, the strong influence of using all the NTP historical controls on the low-dose region of the time-to-tumor curves presented in Subramaniam et al. (2007) suggests that large uncertainties may arise in extrapolating to both human and rat (in the low-dose region) from such considerations alone.

A crucial point needs to be noted with regard to the use of inhalation NTP historical controls (i.e., cases B and E) in the two-stage clonal growth modeling. The single relevant tumor in the NTP inhalation studies came from the very first NTP inhalation study, dated 1976, and the animals in this study were from Hazelton Laboratories, whereas the concurrent animals were all from Charles River Laboratories. Similar problems arise with inclusion of several other NTP inhalation studies. As mentioned before, genetic and other time-related variation can lead to different tumor and survival rates, and in general it is recommended that use of historical controls be restricted to the same kind of bioassays and to studies within a 5–7-year span of the concurrent animals (Peddada et al., 2007). Thus, it is problematic to assume that the tumor in the 1976 NTP study is representative of the risk of SCCs in the formaldehyde bioassays. Even if it were appropriate to consider the 1976 study, this leads to the unstable situation in which the only piece of data that might keep the model predictions of human risk bounded is a single tumor found among several thousand rats from NTP

bioassays ([Crump et al., 2008](#)). In summary, although it can be argued that the rate of SCCs among the controls in the rat bioassay is probably not zero, it is also problematic to assume that this rate can be adequately represented by the background rate in NTP historical controls or even in NTP inhalation historical controls.

Effect of historical controls on modeling inferences regarding mode-of-action: Subramaniam et al. ([2007](#)) also examined the contribution of the DPX component (which represents the directly mutagenic potential of formaldehyde in the model) to the calculated tumor probability, choosing for their case study the optimized models that use the NTP inhalation control data. In the range of exposures where tumors were observed (6.0–15.0 ppm), the DPX term was found to be responsible for 58–74% of the added tumor probability. Below 6.0 ppm the estimated DPX contribution was extremely sensitive to whether the hockey-stick shape or J-shaped was used to characterize the dose response for cell replication, and varied between 2% and 80%.

Several formaldehyde risk assessment efforts and papers have argued based on the CIIT BBDR cancer modeling that the direct mutations induced by formaldehyde are relatively irrelevant compared to the importance of cytotoxicity-induced cell proliferation in explaining the observed tumorigenicity in rodent bioassays ([Slikker et al., 2004](#); [Conolly et al., 2004](#); [Bogdanffy et al., 1999](#); [Bogdanffy et al., 2001](#)). The results from Subramaniam et al. ([2007](#)) in the above paragraph indicate that the CIIT modeling neither supports an inference that cytotoxicity-driven regenerative cell proliferation is the sole driver of formaldehyde-induced carcinogenicity nor an inference that a significant contribution of mutagenicity at lower doses is unlikely.

Characterization of uncertainty-variability in cell replication rates

Dose-response for normal cell division rate as used in model: ([Monticello et al., 1991](#); [Monticello et al., 1996](#)) used unit length labeling index (ULLI) to quantify cell replication within the respiratory epithelium. ULLI is a ratio between a count of labeled cells and the corresponding length (in millimeters) of basal membrane examined, whereas the per-cell labeling index (LI) is the ratio of labeled cells to all epithelial cells, in this case, along some length of basal membrane and its associated layer of epithelial cells. [Monticello et al. \(1991\)](#); [Monticello et al. \(1996\)](#) published ULLI values averaged over replicate animals for each combination of exposure concentration, exposure time, and nasal site. These values are plotted in Figure D-24.

To use the ULLI data in clonal growth modeling, ULLI needed to be related to LI, and thereby to cell replication rate (α_N) of normal cells. Conolly et al. ([2003](#)) adopted the following procedure in using these values:

- 1) The injection labeled ULLI data were first normalized by the ratio of the average minipump ULLI for controls to the average injection labeled ULLI for controls.

- 2) Next, these ULLI average values were weighted by the exposure times in ([Monticello et al., 1991](#); [Monticello et al., 1996](#)) and averaged over the nasal sites. Thus, the data were combined into one TWA for each exposure concentration.
- 3) LI was linearly related to the measured ULLI by using data from a different experiment ([Monticello et al., 1990](#)) where both quantities had been measured for two sites in the nose.
- 4) Cell replication rates of normal cells (α_N) were then calculated as $\alpha_N = (-0.5/t)\log(1 - LI)$ ([Moolgavkar and Luebeck, 1992](#)), where LI is the labeling index and t is the period of labeling.
- 5) This was repeated for each exposure concentration of formaldehyde, resulting in one value of α_N for each exposure concentration.
- 6) Correspondingly, for a given exposure concentration, the steady-state formaldehyde flux into tissue, computed by CFD modeling, was averaged over all nasal sites. Thus, the $\alpha_N(\text{flux})$ constructed by Conolly et al. ([2003](#)) consisted of a single α_N and a single average flux for each of six exposures.

This yielded a J-shaped dose-response curve for cell replication (when viewed on a nontransformed scale for α_N), as shown in Figure D-25 for the full range of flux values used in their modeling. The authors also considered a hockey-stick threshold representation of their J-shaped curve for α_N in order to make a health-protective choice, and the differences between the two can be seen from the insets in the Figure. In these curves, the cell replication rate is less than or the same as the baseline cell replication rate at low formaldehyde flux values. The shape of the dose-response curve for cell replication as characterized in Conolly et al. ([2003](#)) is seen as representing regenerative cell proliferation secondary to the cytotoxicity of formaldehyde ([Conolly, 2002](#)). Considerable uncertainty and variability, both quantitative and qualitative, exist in the use and interpretation of these labeling data for characterizing a dose response for cell replication rates. The primary issues are discussed here. Unlike the preceding sections, these have largely not been published elsewhere, so more details are provided.

Supplemental Information—Formaldehyde (Inhalation)

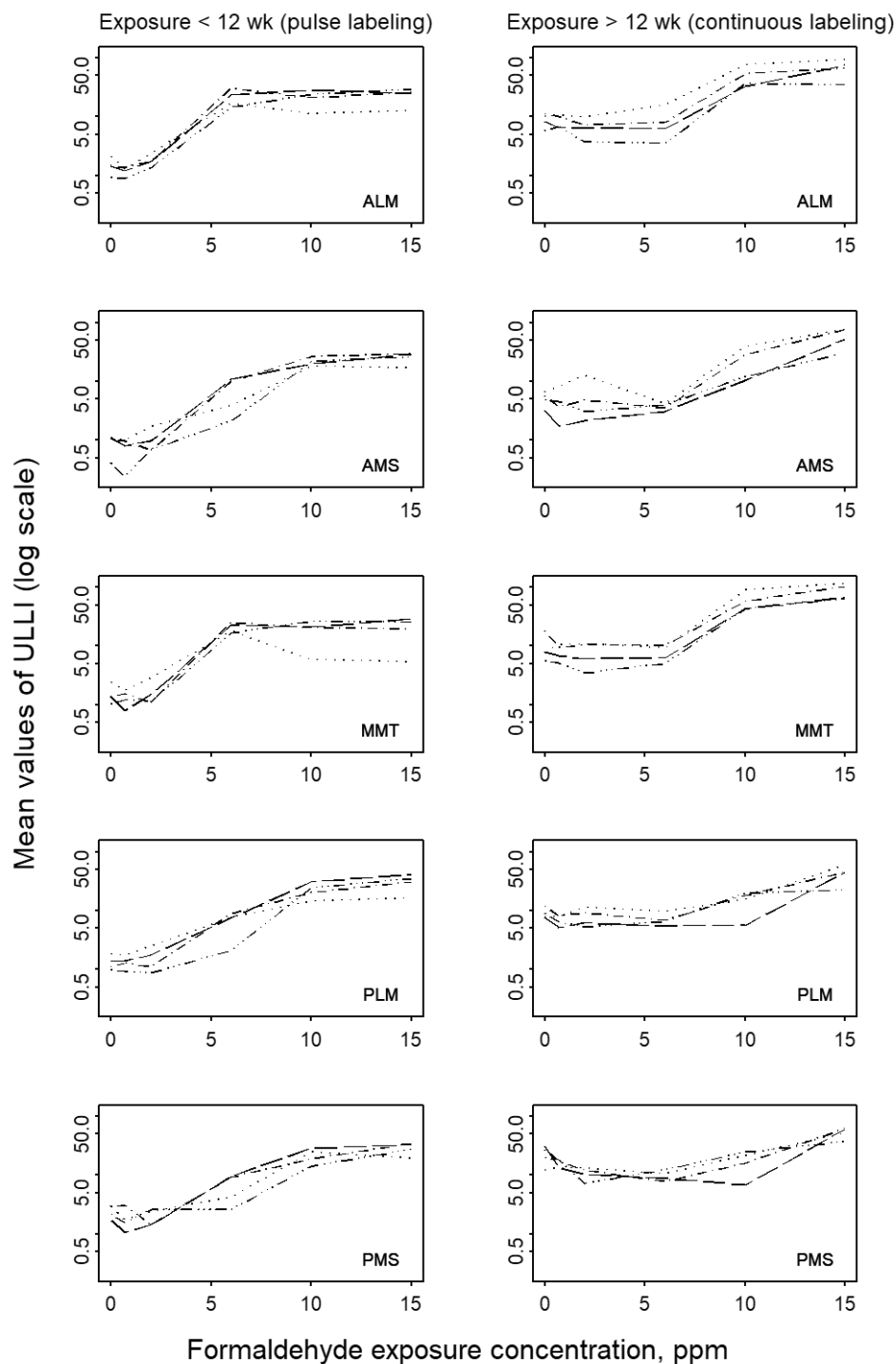


Figure D-24. ULLI data for pulse and continuous labeling studies.

Note: Data are from pulse labeling study, left-hand side, at 1–42 days of exposure and from the continuous-labeling study, right-hand side, at 13–78 weeks of exposure for five nasal sites ALM, AMS, MMT, PLM, and posterior mid septum [PMS]). Within each graph, lines with more breaks correspond to shorter exposure times. Data source: ([Monticello et al., 1991](#); [Monticello et al., 1996](#)).

Time variability in labeling data:

Short-time exposure effects on cell replication: Figure D-24 shows the site and time variation in the raw unit-length labeling index (ULLI) data for 1 day to 78 weeks of exposure duration. The dose-response for ULLI is quite different between the “early time” (left panel) and “later time” (right panel) and these early time effects may be quite important to the cancer modeling. At the earliest times in the left panel, the data show an increased trend in labeling at 2 ppm for the sites anterior lateral meatus (ALM), anterior medial septum (AMS), posterior lateral meatus (PLM), and medial maxilloturbinate (MMT) relative to control.

The early times would be important if, say, repeated episodic exposures were considered, where adequate time has not elapsed for adaptive effects to take place. Such an exposure scenario may be the norm in the human context. In the cancer modeling in Conolly et al. (2003), because the LI was weighted by exposure time, the contribution of the early time labeling data is minimized.

Uncertainty due to combining pulse and continuous labeled data: The formula used for obtaining α_N from LI in Conolly et al. (2003) was due to Moolgavkar and Luebeck (1992) who derived this formula for continuous LI, cautioning that it is not applicable for pulse labeled data because it is unclear as to how long the (pulsed) label is available in the tissue. However, Conolly et al. (2003) applied this formula to the injection (pulse) labeled data also. Such an application is problematic because it takes about 4-8 hours of labeling for the relation between labeling index and the rate of cell proliferation to stabilize (see for example the data in Novak and Fedoroff (1996)). Therefore, there is concern that the 2-hour pulse labeled data will represent the pool of cells in S-phase rather than inform the rate at which cells are recruited to that pool. Furthermore (and perhaps on account of this), the baseline values of α_N calculated by Conolly et al. (2003) from both data sets differ considerably. Consequently, it is not evident that adjustment by a multiplier can produce a reasonably correct estimate of labeling index and cell division rate for the pulse-label data of Monticello et al. (1991) that is comparable to those from the continuous-label data of Monticello et al. (1996), especially not for purposes of combining and jointly analyzing the resulting estimates of cell proliferation rates. As such, we are not aware of any reasonable manner to derive cell replication rates from these pulse data without acquisition of data at additional time points. Because of these problems in incorporating the pulse-labeled data, further quantitative analysis of cell replication rates is restricted in this document to the continuous labeled data (Monticello et al., 1996), which do not include measurements made before 13 weeks of exposure.

Site and time variability in derived cell replication rate

In the remainder of this section, the factors that are considered in order to represent the uncertainty and variability in the cell replication data when developing alternate dose-response curves for $\alpha_N(\text{flux})$ will be elaborated.

The ULLI data for individual animals were provided by CIIT, which were transformed to LI values using the linear relationship from step 3 above. For these replicate data, cell replication rates

of normal cells (α_N) were then calculated as $\alpha_N = (-0.5/t)\log(1 - LI)$ as in Step 4. Figure D-25 (adapted from ([Subramaniam et al., 2008](#))) shows the variability in α_N due to replicated animals, exposure times, and nasal sites in the continuous labeled data obtained by Monticello et al. ([1996](#)). In this figure, $\log \alpha_N$ versus site-specific flux are plotted for six sites and four exposure times for four to six replicate animals in each case. (The mean ULLI over these replicates were shown in Figure D-24 for each site and time as a function of exposure concentration.) It needs to be noted that these nasal sites differ considerably in the number of cells estimated at these locations as shown in Table D-36. Each point in Figure D-25 represents data from a single site for a single animal at a given time. For comparison, the time weighted and site averaged $\alpha_N(\text{flux})$ in Conolly et al. ([2003](#)) is also plotted in this figure at their averaged flux values (filled red circles). For flux $>9,340 \text{ pmol/mm}^2\text{-hour}$, Conolly et al. ([2003](#)) extrapolated this empirically derived $\alpha_N(\text{flux})$ by using a scheme discussed in the section on *Model Structure and Calibration* in Appendix D.2.2. The curves shown connecting the filled circles in the figure represent their linear interpolation (long dashes) among the six points. Their linear extrapolation for flux value $>9,340 \text{ pmol/mm}^2\text{-hour}$ is also shown (short dashes). Note that the linear interpolation and extrapolation are shown transformed to a logarithmic scale in this plot.

As discussed, the raw labeling data plotted in Figure D-24 indicates considerable temporal variability. In Figure D-26 & Figure D-27, fitted dose-response curves showing $\log_{10}(\alpha_N)$ versus flux with simultaneous confidence limits separately for each time point for two of the largest sites in Table D-36 (ALM and PLM) are plotted for the continuous labeled data. Note that flux levels are different at each site. Simple polynomial models in flux (as a continuous predictor), with time included as a factor (i.e., a class or indicator variable, τ_i representing the effect of the i^{th} time) were used as follows:

$$\log(\alpha_N) = a + b \times \text{flux} + c \times \text{flux}^2 + d \times \text{flux}^3 + \tau_i \quad (\text{D-23})$$

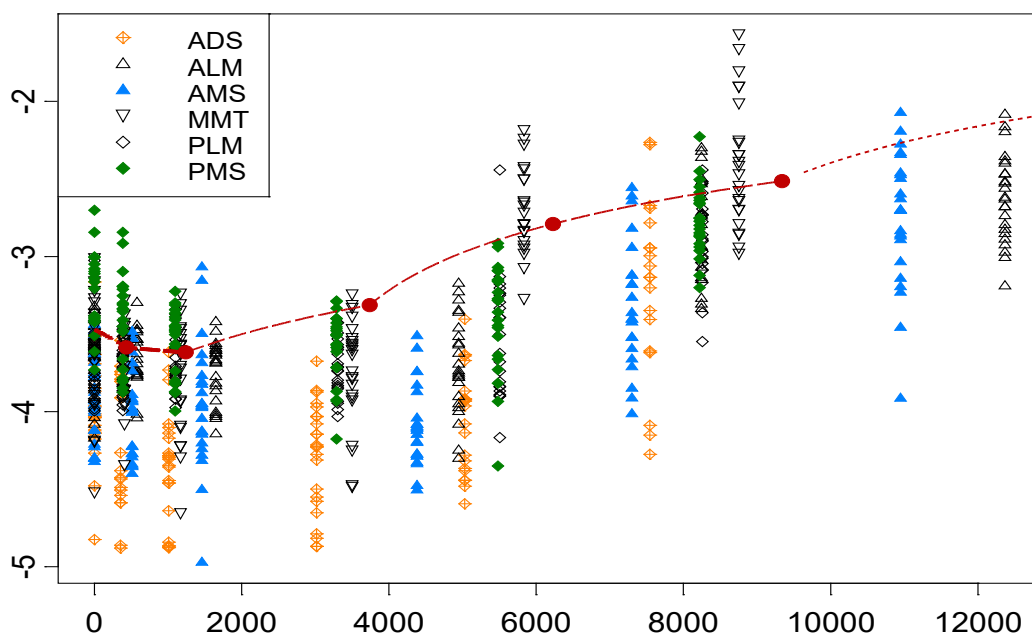


Figure D-25. Logarithm of normal cell replication rate α_N versus formaldehyde flux (in units of pmol/mm²-hour) for the F344 rat nasal epithelium.

Note: Values were derived from continuous unit length labeled data obtained by Monticello et al. (1996) for four to six individual animals at all six nasal sites (legend, sites as denoted in original paper) and four exposure durations (13, 26, 52, 78 weeks). Each point represents a measurement for one rat, at one nasal site, and at a given exposure time. Filled red circles: $\alpha_N(\text{flux})$ used in Conolly et al. (2003) plotted at their averaged flux values (see text for details). Long dashed red lines: their linear interpolation among points. Short dashed red line: their linear extrapolation for flux values 9,340 to 39,300 pmol/mm²-hr (full extrapolation not shown; see Figure D-23 for full range). Linear interpolation/extrapolation is shown with y-axis transformed to logarithmic scale.

Source: Subramaniam et al. (2008).

Table D-36. Variation in number of cells across nasal sites in the F344 rat

Nasal site	No. of cells
Anterior lateral meatus	976,000
Posterior lateral meatus	508,000
Anterior mid septum	184,000
Posterior mid septum	190,000
Anterior dorsal septum	128,000
Anterior medial maxilloturbinate	104,000

Note: Mean number of cells in each side of the nose of control animals.

Source: Monticello et al. (1996).

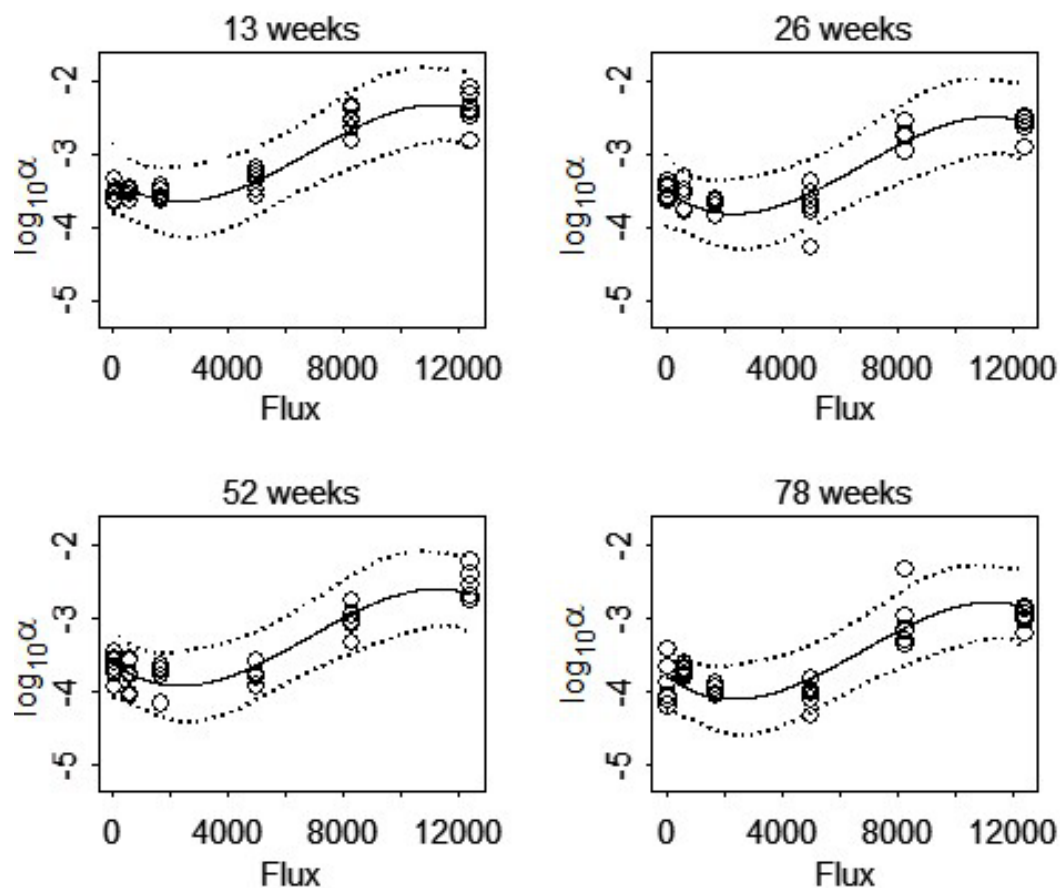


Figure D-26. Logarithm of normal cell replication rate versus formaldehyde flux with simultaneous confidence limits for the ALM.

Source: Subramaniam et al. (2008).

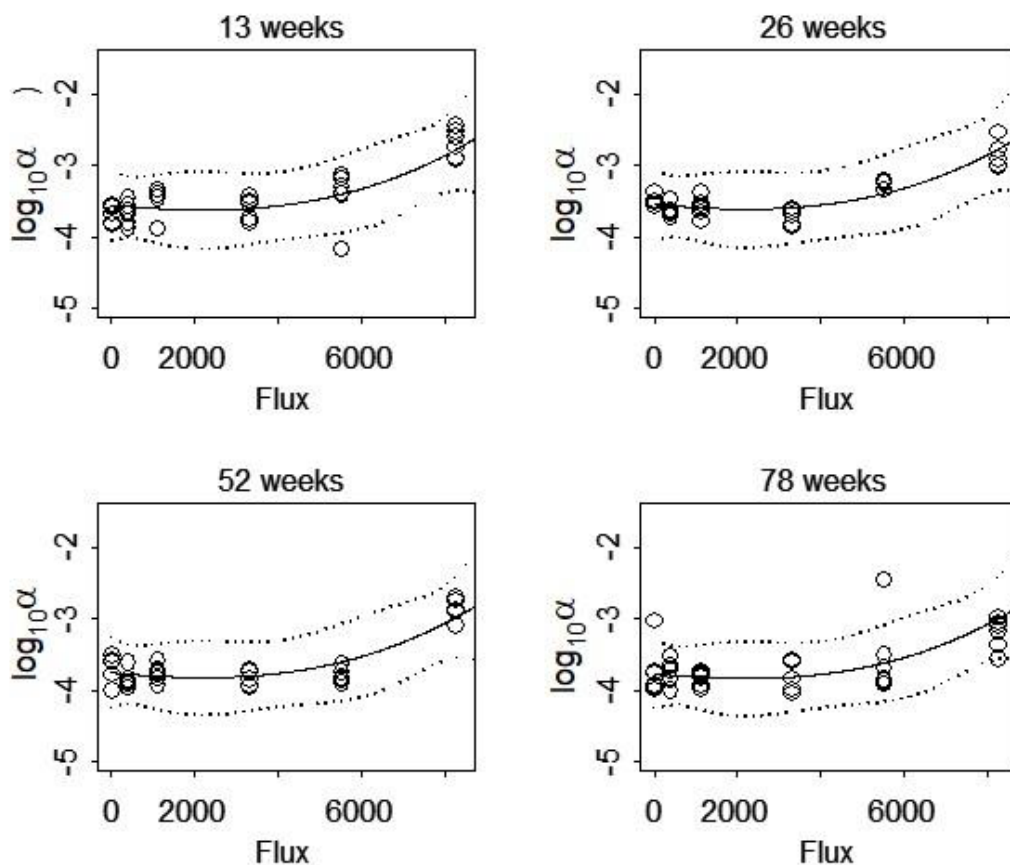


Figure D-27. Logarithm of normal cell replication rate versus formaldehyde flux with simultaneous confidence limits for the PLM.

Source: Subramaniam et al. (2008).

The variability considered is that among animals and any measurement error as well as any other design-related components of error. Simultaneous 95% confidence limits for $\log(\alpha_N)$ were produced using Scheffe's method (Snedecor and Cochran, 1980). These 95% confidence limits span a range of 0.96 in $\log_{10}(\alpha_N)$, or nearly a 10-fold range in median α_N . There is additional dispersion in these data that does not appear in Figures D-25 to D-27 for α_N , derived using the mean value of ULLI:LI; due to variation in the number of cells per mm basement membrane, the ratio of ULLI:LI had a spread of approximately $\pm 25\%$ (0.45 to 0.71, mean 0.60) among the eight observations considered in Monticello et al. (1990). Thus:

- 1) As suggested by Table D-36, and Figures D-26 and D-27, the shape of $\alpha_N(\text{flux})$ in Conolly et al. (2003) is likely to be very sensitive to how α_N is weighted and averaged over site and time.

- 2) Averaging of sites could significantly affect model calibration because of substantial nonlinearity in model dependence on α_N at the 10 and 15 ppm doses associated with high cancer incidence.
- 3) Monticello et al. (1996) found a high correlation between tumor rate and the ULLI weighted by the number of cells at a site. Therefore, considering these factors while regressing α_N against tissue dose would be important in the context of site differences in tumor response.
- 4) Histologic changes and thickening occur in the nasal epithelium over time in the higher dose groups (Morgan, 1997), factors that are likely to affect estimates of local formaldehyde flux, uptake, and replication rates (Subramaniam et al., 2008).

It is clear from Figures D-25, D-26 and D-27 that the time dependence in cell replication is significant. It would also be useful to examine if this time dependence affects the results of the time-to-tumor modeling and if early temporal changes in replication rate are important to consider because of the generally cumulative nature of cancer risk. The time window over which formaldehyde-induced cancer risk is most influenced is not known, but the time weighting used by Conolly et al. (2003) assigns a relatively low weight to labeling observed at early times compared with those observed at later time points. Finally, initiated cells are likely to be replicating at higher rates than normal cells as evidenced in several studies on premalignant lesions (Rotstein et al., 1986; Dragan et al., 1995; Coste et al., 1996). Therefore, LI data as an estimator of normal cell replication rate would be most reliable at early times when the mix of cells sampled include fewer preneoplastic or neoplastic cells.

Given the above uncertainties and variability not characterized in CIIT (CIIT, 1999) or in Conolly et al. (2003), it is clear that many alternatives can be developed for the dose-response for cell replication [$\alpha_N(\text{flux})$], including curves consistent with a monotonically increasing trend (also see discussion of the cell replication data from Meng et al. (2010) surrounding Figure D-42). It is important to examine whether additional dose-response curves that fit the cell replication data reasonably well have an impact on estimated risk. Such sensitivity analyses are carried out in the sections that follow.

Alternate dose-response curves for cell replication

In conjunction with the other uncertainties, mainly the use of control data and alternative model structures for initiated cell kinetics, the number of plausible clonal growth models to be exercised soon require a prohibitively large investment of time. Therefore, detailed analyses were restricted to a select set of biologically plausible choices of curves for $\alpha_N(\text{flux})$, which would allow the identification of a range of plausible risk estimates (MLEs and statistical bounds).

Six alternative equations for α_N were developed by regression analysis of the Monticello et al. (1996) ULLI data. The replicate data corresponding to the summary data presented in this paper were kindly provided to EPA by CIIT for further analyses. In each of these equations, α_N is expressed as a function of formaldehyde flux to nasal tissue (pmol/mm²-hour) and, in the case of

equation D-29 that explored time-dependence, the duration of exposure to formaldehyde in weeks. All the graphs use flux/10,000 for the x-axis, and the y-axis expresses $\log_{10} \alpha_N$.

One source of uncertainty in the cell replication dose response in Conolly et al. (2003) is the large value of $\alpha_{\max}$ (the cell replication rate corresponding to the upper end of the flux range at 15 ppm exposure) in the upward extrapolation from the empirically determined $\alpha_N(\text{flux})$ (see Figure D-23 and surrounding text). The optimal value of $\alpha_{\max}$, indicated by the thick short arrow in Figure D-23, was found by Conolly et al. (2003) to be 0.0435 hour⁻¹. As noted by the authors, an argument in support of this value is that it corresponds to the inverse of the fastest cell cycle times found in the literature. Because the model treats the induced replication rates as being time invariant, this means that cells in the high-flux region(s) divide at the highest cell turnover rate ever observed throughout most of an animal's life. This does not seem to be biologically plausible (Subramaniam et al., 2008).

Our analysis found that a 20% increase or decrease in the estimated value for $\alpha_{\max}$ degraded the fit to the tumor incidence data considerably. Because of the interplay among the parameters estimated by optimization, this sensitivity of the model to $\alpha_{\max}$ indicates that it is necessary to examine if other plausible values of $\alpha_{\max}$ are also indicated by the data and to what extent low dose estimates of risk are influenced by the uncertainty in its value. The need for such an analysis is also indicated by Figure D-23. The value of $\alpha_{\max}$ ($\log_{10} \alpha_{\max} = -1.37$) in Conolly et al. (2003) is roughly an order of magnitude greater than the highest empirical values of $\alpha_N(\text{flux})$ derived from the observed labeling index data corresponding to the flux denoted by the long arrow in Figure D-23. If the data pooled over all sites and times are to be used for $\alpha_N(\text{flux})$, then, based solely on the trend in $\alpha_N(\text{flux})$ in Figure D-23, it appears unlikely that $\alpha_N(\text{flux})$ could increase up to this value of $\alpha_{\max}$. Visually, these empirically derived data in Figure D-25 collectively suggest that α_N versus flux could be leveling off rather than increasing 10-fold. Therefore, as an alternative to the approach taken in Conolly et al. (2003) of estimating $\alpha_{\max}$ via likelihood optimization against the tumor data, regressions of the empirical cell replication data in Figure D-25 were used to extrapolate $\alpha_N(\text{flux})$ outside the range of observation (recognizing the uncertainty and model dependence that still results from extrapolating well outside the range of observed data).

In fitting dose-response curves to the cell replication data, a functional form was used that was flexible to allow a variety of monotonic and nonmonotonic shapes, with a parameter that determined the asymptotic behavior of the dose-response function. This allowed the extrapolation of $\alpha_N(\text{flux})$ to higher flux levels by only relying on the empirical cell replication data. Then, there is no need for an adjustable parameter to be estimated by fitting to the tumor data. However, the plausible asymptotes obtained in this manner spanned a large range. In one case below, the asymptote suggested by the fit to the empirical cell replication data was judged to be abnormally high. In this case, the α_N versus flux curve was followed until the biological maximum of $\alpha_{\max}$ [as given in Conolly et al. (2003)] was reached.

In three of the six regression models below, the data were restricted to the earliest exposure time (13 weeks) in Monticello et al. (1996) for which the cell proliferation rate (α_N) could be calculated. The interest in using only the 13-week exposure time also arises from observations (Monticello et al., 1991; Monticello et al., 1996) that at later times there were more frequent and severe histologic changes, which may have altered formaldehyde uptake and cell proliferation response. Consequently, given that the data in Monticello et al. (1991) for times earlier than 13 weeks could not be used as explained in the Section in Appendix D.2.2 on “uncertainty due to combining pulse and continuous labeled data,” the 13-week responses might better represent proliferation rates for use in a two-stage model of the cancer process than the rest of the Monticello et al. (1996) data.

Second, the LI data showed considerable variation among nasal sites, which may be related to the variation in tumor response among sites. Because the cell replication dose-response curves used in the cancer model represent all of the sites, it was attempted to include this variation by weighting the regression by the relative cell populations at risk at each of the sites. This was carried out for some of the models as stated below.

Finally, in one of the regression models, derived from fitting to all of the Monticello et al. (1996) ULLI data, time-dependence of α_N was considered by using weeks of exposure as a covariate. In this model, time was a regression (continuous) predictor, not a class variable, and its coefficient represents the change in $\log_{10} \alpha_N$ per week of exposure.

The following regression models for α_N versus flux, denoted in the equations below as N1–N6 and shown in Figures D-28 to D-33, as well as the hockey-stick and J-shaped curves used by Conolly et al. (2003), shown in Figures D-23, were next used as inputs to the clonal growth model for cancer:

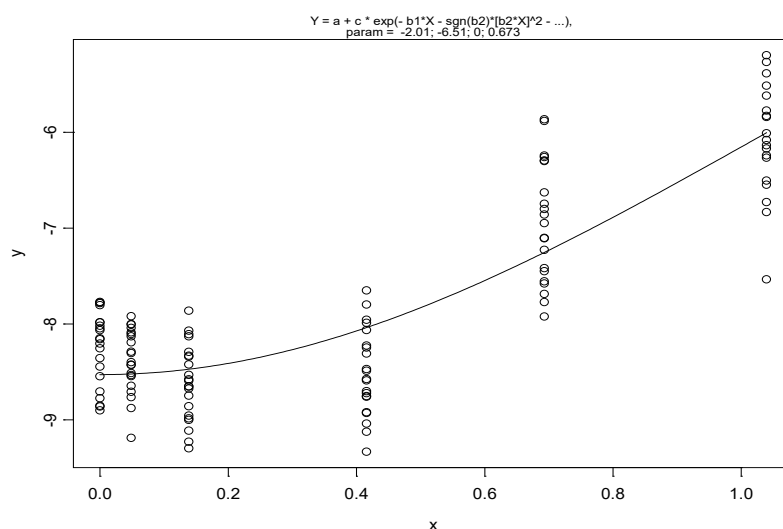


Figure D-28. Various dose-response models of normal cell replication rate; N1.

Supplemental Information—Formaldehyde (Inhalation)

Note: See text for definitions of N1–N6. N1: Quadratic; monotone increasing in flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

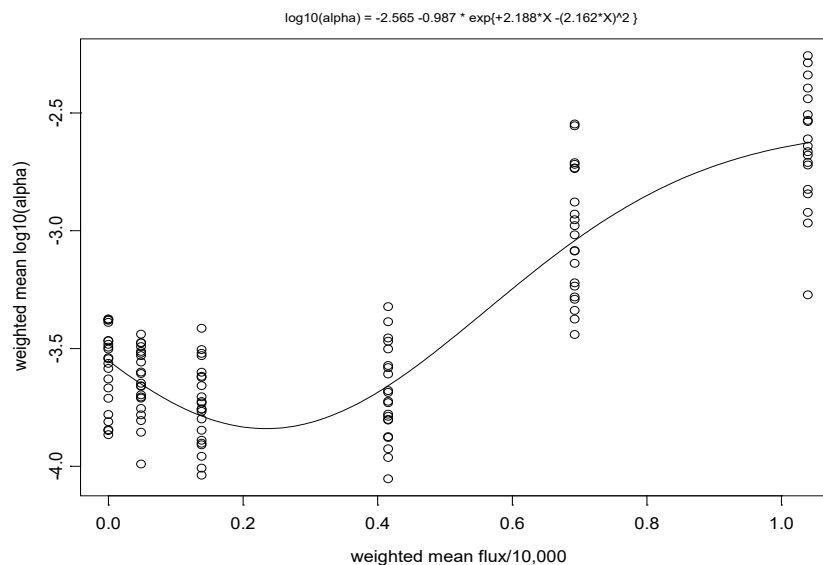


Figure D-29. Various dose-response models of normal cell replication rate; N2.

Note: See text for definitions of N1–N6. N2: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

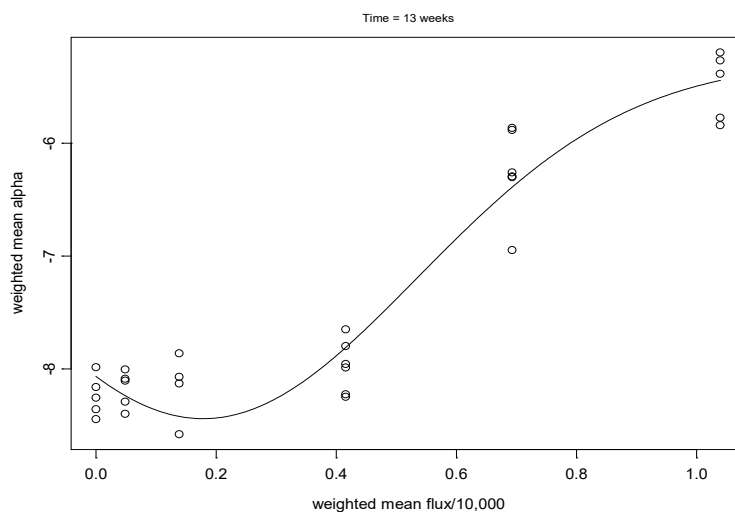


Figure D-30. Various dose-response models of normal cell replication rate; N3.

Supplemental Information—Formaldehyde (Inhalation)

Note: See text for definitions of N1–N6. N3: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to the 13-week Monticello et al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

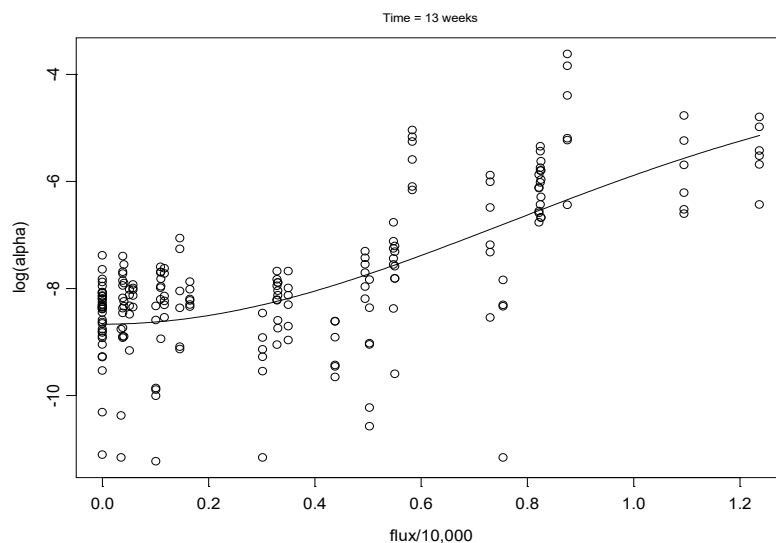


Figure D-31. Various dose-response models of normal cell replication rate; N4.

Note: See text for definitions of N1–N6. N4: Quadratic; monotone increasing in flux, derived from unweighted fit to 13-week Monticello et al. (1996) ULLI data.

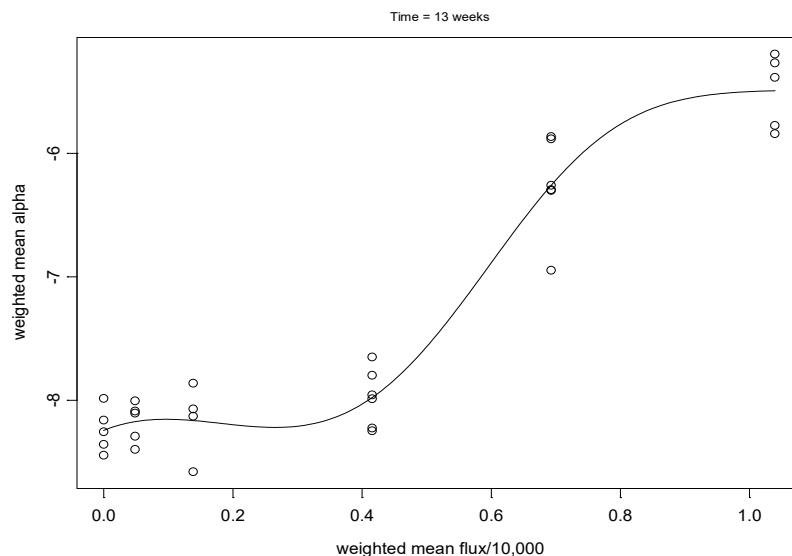


Figure D-32. Various dose-response models of normal cell replication rate; N5.

Note: See text for definitions of N1–N6. N5: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to 13-week Monticello et

Supplemental Information—Formaldehyde (Inhalation)

al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

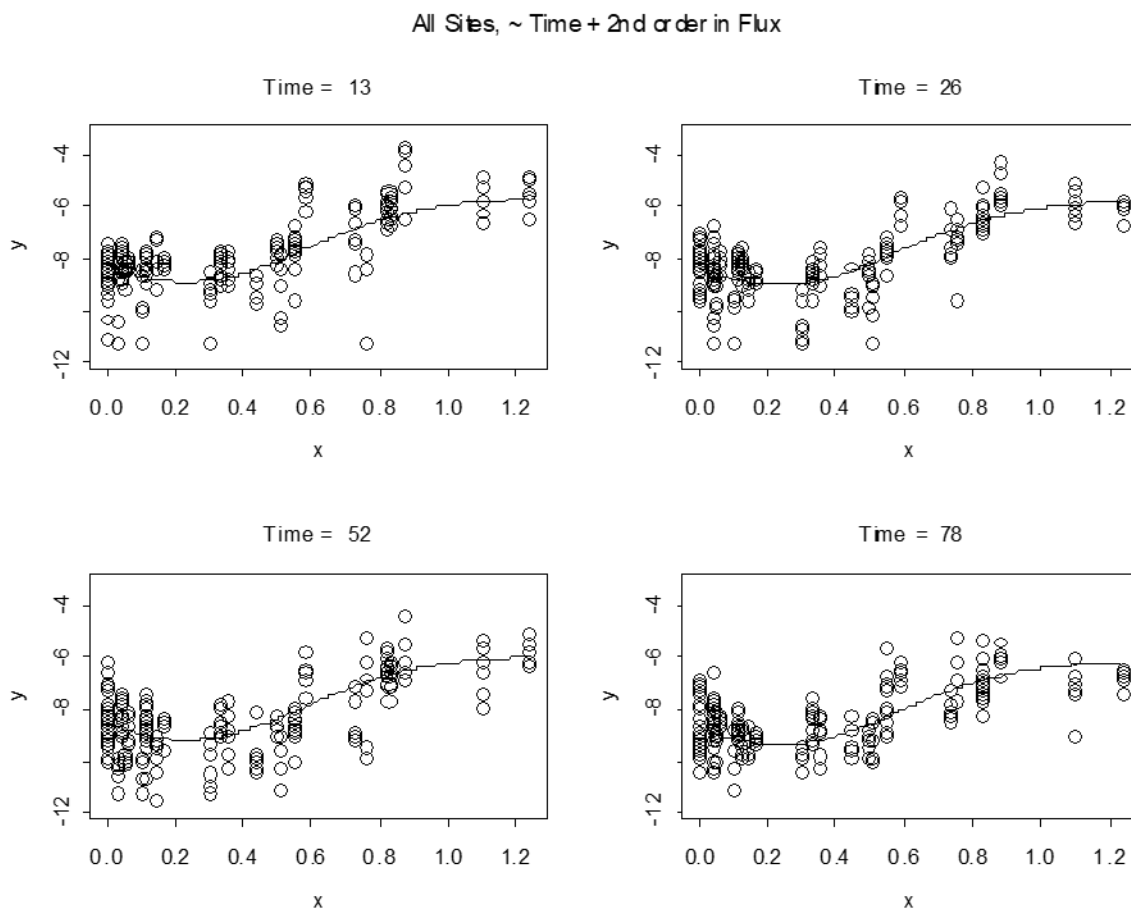


Figure D-33. Various dose-response models of normal cell replication rate; N6.

Note: See text for definitions of N1–N6. N6: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to all Monticello et al. (1996) ULLI data, using weeks of exposure as a covariate. In this model, time was a regression (continuous) predictor, not a class variable, and its coefficient represents the decrease in $\log_{10} \alpha_N$ per week of exposure time.

N1: Quadratic; monotone increasing in flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

$$\alpha_N = \text{Exp}\{-2.015 - 6.513 \times \text{Exp}[-(6.735 \times 10^{-4} \times \text{flux})^2]\} \quad (\text{D-24})$$

N2: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to all of the Monticello et al. (1996) ULLI data.

$$\alpha_N = \text{Exp}\{-5.906 - 2.272 \times \text{Exp}[2.188 \times 10^{-4} \times \text{flux} - (2.162 \times 10^{-4} \times \text{flux})^2]\} \quad (\text{D-25})$$

N3: Linear-quadratic; decreasing in flux for small values of flux, derived from fit to the 13-week Monticello et al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

$$\alpha_N = \text{Exp}\{-5.274 - 2.792 \times \text{Exp}[1.407 \times 10^{-4} \times \text{flux} - (1.986 \times 10^{-4} \times \text{flux})^2]\} \quad (\text{D-26})$$

N4: Quadratic; monotone increasing in flux, derived from unweighted fit to 13-week Monticello et al. (1996) ULLI data.

$$\alpha_N = \text{Exp}\{-3.858 - 4.809 \times \text{Exp}[-(9.293 \times 10^{-5} \times \text{flux})^2]\} \quad (\text{D-27})$$

N5: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to 13-week Monticello et al. (1996) ULLI data, using average flux over all sites for a given ppm exposure and weighting regression by estimates of the numbers of cells at each of five sites.

$$\alpha_N = \text{Exp}\{-5.488 - 2.755 \times \text{Exp}[-7.808 \times 10^{-5} \times \text{flux} + (2.349 \times 10^{-4} \times \text{flux})^2 - (2.166 \times 10^{-4} \times \text{flux})^3]\} \quad (\text{D-28})$$

N6: Linear-quadratic-cubic; initially increasing slightly with increasing flux, then decreasing slightly, and finally increasing, derived from fit to all Monticello et al. (1996) ULLI data, using weeks of exposure as a covariate. In this model, time was a regression (continuous) predictor, not a class variable, and its coefficient represents the decrease in $\log_{10} \alpha_N$ per week of exposure time.

$$\alpha_N = \text{Exp}\{7.785 \times 10^{-3} \times (\text{weeks}) - 5.722 - 2.501 \times \text{Exp}[1.103 \times 10^{-4} \times \text{flux} - (7.223 \times 10^{-5} \times \text{flux})^2 - (1.575 \times 10^{-4} \times \text{flux})^3]\} \quad (\text{D-29})$$

Uncertainty in model specification of kinetics of initiated cells

Biological implications of assumptions in Conolly et al. (2003)

The results of a two-stage MVK model are extremely sensitive to the values for initiated cell division (α_i) and death (β_i) rates, particularly in the case of a sharply rising dose-response curve as observed of formaldehyde. The pool of cells used for obtaining the available LI data ([Monticello et al., 1991](#); [Monticello et al., 1996](#)) consists of largely normal cells with perhaps increasing numbers of initiated cells at higher exposure concentrations. As such there is no way of inferring the division rates of initiated cells in the nasal epithelium, either spontaneous (baseline) or induced by exposure to formaldehyde, from the available empirical data. Conolly et al. ([2003](#)) considered $\alpha_i(\text{flux})$ as a function of $\alpha_N(\text{flux})$ as given by equation D-20. As shown in Figure D-23, α_i is estimated in Conolly et al. ([2003](#)) to be very similar to α_N , and a J- or hockey-shaped dose-response curve for $\alpha_N(\text{flux})$ necessarily results in a J or hockey shape for $\alpha_i(\text{flux})$.

The J shape for the TWA $\alpha_N(\text{flux})$ in Conolly et al. ([2003](#)) could plausibly be explained, as suggested by the examples in Conolly and Lutz ([2004](#)), by a mathematical superposition of dose-response curves describing the effects of the inhibition of cell replication by the formation of DPXs ([Heck and Casanova, 1999](#)) and cytotoxicity-induced regenerative replication ([Conolly, 2002](#)). However, as explained earlier, there is considerable uncertainty and variability, both qualitative and quantitative, in the interpretation of the LI data and in the derivation of *normal* cell replication rates from the ULLI data. While the time-weighted averaged (TWA) values of ULLI indicate a J-shaped dose response for some sites, this is not consistently the case for all exposure times and sites. It is not clear why mechanisms that might explain a J-shaped or hockey-stick dose response for normal cell replication should be expected to prevail also for initiated cells.

The next critical assumption in Conolly et al. ([2003](#)) was that made for β_i (the death rate of initiated cells), namely, $\beta_i(\text{flux}) = \alpha_N(\text{flux})$ (equation D-21). No biological justification for this assumed relationship was provided by the authors.

There are no data to evaluate the strength of these assumptions, so Subramaniam et al. ([2008](#)) studied the plausibility of various inferences that arise as a result of these assumptions. These inferences are briefly listed here.

- For flux < 27,975 pmol/mm²-hour, $\alpha_i > \alpha_N$ (see Figure D-23). Qualitatively, this concept of a growth advantage is in line with data on epithelial and other tissue types with or without exposure to specific chemicals.
- For higher flux levels in Figure D-23, the model indicates $\alpha_i < \alpha_N$. There are no data to shed further light on this inference.
- At these higher flux levels, initiated cells in the model die at a faster rate than they divide, indicating the extinction of initiated cell clones in regions subject to these flux levels. There are no data indicating formaldehyde to have this effect.

In evaluating these inferences, Subramaniam et al. ([2008](#)) point to various data that indicate that initiated cells represent distinctly different cell populations from that of normal cells with regard to proliferation response ([Schulte-Hermann et al., 1997](#); [Dragan et al., 1995](#); [Coste et al.,](#)

[1996](#); [Ceder et al., 2007b](#); [Bull, 2000](#)), have excess capacity to clear formaldehyde and, in general, are considerably more resistant to cytotoxicity, and may already have altered cell cycle control. The resistance to toxicity is manifested variably as decreased ability of the toxicant to induce cell death or to inhibit cell proliferation compared to corresponding effects in normal cells. Therefore, the influence of formaldehyde on apoptosis likely differs between normal and initiated cells.

As concluded in Subramaniam et al. ([2008](#)), taken together, there is much data to suggest that inferring $\alpha_I < \alpha_N$ at cytotoxic formaldehyde flux levels is problematic and that death rates of initiated cells are likely to be very different from those of normal cells.

In the absence of data to indicate that equations D-20 and D-21 are biologically reasonable approaches to link the kinetics of initiated cells with those of normal cells, alternate model structures other than those represented by these relationships considered by Conolly et al. ([2003](#)) were explored, given that the two-stage model is extremely sensitive to α_I and β_I . Only alternate model structures that provided a good fit to the time-to-tumor data were considered.

Plausible alternative assumptions for α_I and β_I

Therefore, in the additional sensitivity analysis presented here:

- a) initiated cell kinetics are considered to be independent of normal cells, and
- b) initiated cell replication dose response cannot take a J shape; this is motivated by the consideration that lower-than-baseline turnover rate represents an increased amount of DNA repair taking place, which may not be consistent with impaired DNA repair in initiated cells.

Thus, two alternatives were considered to equations D-20 for $\alpha_I(\text{flux})$:

$$\text{I1:} \quad \alpha_I = \gamma_1 \times [1 + \exp(\gamma_2/\gamma_3)] / \{1 + \exp[-(\text{flux} - \gamma_2)/\gamma_3]\} \quad (\text{D-30})$$

$$\text{I2:} \quad \alpha_I = \max[\alpha_I(\text{I1}), \alpha_{N\text{Basal}}] \quad (\text{D-31})$$

Here γ_1 , γ_2 , and γ_3 are parameters estimated by fitting the cancer model to the rat bioassay data. In equation D-30, α_I increases monotonically with flux from a background level of γ_1 asymptotically up to a maximum value of $\gamma_1 \times [1 + \exp(\gamma_2/\gamma_3)]$. The choice of this functional form in was considered in order to be parsimonious while at the same time allowing for a flexible shape to the dose-response curve. The sigmoidal curve allows for the possibility of a slow rise in the curve at low dose and an asymptote.

Equation D-31 is a modification of equation D-30 that restricts the rate of division of initiated cells to be at least as large as the spontaneous division rate of unexposed normal cells. There is evidence to suggest (e.g., in the case of liver foci) that initiated cells have a growth

advantage over normal cells, with or without exposure to specific chemicals ([Schulte-Hermann et al., 1999](#); [Grasl-Kraupp et al., 2000](#); [Dragan et al., 1995](#); [Coste et al., 1996](#); [Ceder et al., 2007a](#)).

In addition, in most runs, an upper bound (α_{high}) is selected for both α_N and α_I . This value is assumed to represent the largest biologically plausible rate of cell division. Following Conolly et al. (2003), in most cases α_{high} is set equal to 0.045 hours⁻¹. If a value of α_I or α_N computed using one of the above formulas exceeded α_{high} , the value of α_{high} was used in the computation rather than the value obtained by using the formula.

As noted above, Conolly et al. (2003) set the rate of death for intermediate cells, β_I , equal to the division rate of normal cells, $\beta_I = \alpha_N$. On the other hand, apoptotic rates and cell proliferation rates are thought to be coupled ([Schulte-Hermann et al., 1999](#); [Moolgavkar, 1994](#)), so that death rates of initiated cells would rise concomitantly with an increase in their division rates ([Schulte-Hermann et al., 1999](#); [Grasl-Kraupp et al., 2000](#)). Therefore, as an alternative to the Conolly et al. (2003) formulation, it is assumed that the death rate of intermediate cells is proportional to the division rate of intermediate cells.

$$\beta_I = \kappa_\beta \times \alpha_I \tag{D-32}$$

where the constant of proportionality, κ_β , is an additional parameter to be estimated by optimization against the tumor incidence data. Such an assumption has also been made by other authors ([Moolgavkar et al., 1993](#); [Luebeck et al., 1995](#); [Luebeck et al., 2000](#)).

Results of sensitivity analyses on α_N , α_I , and β_I

The number of models that might be constructed if all the possibilities listed above for α_N , α_I , and β_I are to be tried in a systematic manner clearly become exponential and daunting. (Optimally, it would have been desirable to elucidate the role of a specific modification while keeping others unchanged to determine risk.) Therefore, in order to carry out a viable sensitivity analysis while at the same time examining the plausible range of risks resulting from variations in parameters and model structures, various uncertainties were combined in any given simulation. By using the constraints described above (equations D-24 through D-32) for α_I , β_I , and α_N , 19 models were obtained that provided similarly good fits to the time-to-tumor data (which in some cases contained only five dose groups).

However, for many of these models, the optimal α_I (flux) displayed a threshold in flux even when the model used for α_N (flux) was a monotonic increasing curve without a threshold (i.e., model N4 for α_N in Figure D-31). Indeed, if a thresholded dose-response curve was plausible for α_I based on arguments of cytotoxicity, then a threshold is all the more plausible for α_N , and such models are removed from consideration.

Secondly, the basal value of α_I was required to be at least as large as the basal value of α_N . Another constraint was placed on the baseline initiated cell replication rate. In the absence of formaldehyde exposure, α_I was not allowed to be greater than two or four times α_N , even if such models described the tumor data, including the control data, very well. There are some data that suggest that baseline initiated cells have a small growth advantage over normal cells, so a huge advantage was thought to be biologically less plausible.

Finally, because most of the SCCs in the rat bioassays occurred in rats exposed to the highest formaldehyde concentration (15 ppm), the data from this exposure level have a big impact on the estimated model parameters. In most runs that incorporated the 15 ppm data, the model appeared, based on inspection of the KM plots, to fit the 15 ppm data quite well but to fit the lower exposure data less well. Because of the high level of necrosis occurring at 15 ppm, it is possible that the data at this exposure may not be particularly relevant to modeling the sharp upward rise in the dose response at 6 ppm. Furthermore, the principal interest is in the predictions of the model at lower levels to which human populations may be exposed. Consequently, in order to improve the fit of the model at lower exposures, some of the alternative models were constructed with the 15 ppm data omitted.

Sensitivity of risk estimates for the F344 rat

Figures D-34 and D-35 contain plots of the MLE of additional risk computed for the F344 rat at formaldehyde exposures of 0.001, 0.01, 0.1, and 1 ppm for eight models. Two log-log plots are provided. For those models for which the estimates of additional risk are all positive, the additional risks are plotted (Figure D-34), and, for those for which estimates of additional risk are negative, the negatives of additional risks are plotted (see Figures D-35). Only five dose groups were considered (i.e., 15 ppm data omitted) for models 8, 5, 15, and 16. Figures D-36 and D-37 show the dose-response curves for α_N and α_I for these eight cases (corresponding to those in Figures D-34 and D-35 respectively). The specification and estimated values of the parameters for these models are provided in Tables D-37 and D-38. The primary results are as follows:

- 1) Among the models considered, negative values for additional risk can arise only in models in which the dose response for normal cells is J shaped. Thus, all of the models with negative dose responses for risk have J-shaped dose responses for normal cells. However, the converse is not necessarily true as may be noted from model 8. This model has both a positive dose response for risk and a J-shaped dose response for normal cells. In this case, the strong positive increase in response of initiated cells at low dose was sufficient to counteract the negative response of normal cells.
- 2) For doses below which no tumors were observed, the risk estimates predicted by the different models span a very large range. This result points to large uncertainties in model specification (how to relate the kinetics of normal and initiated cells) as well as in parameter values. As mentioned above, the analysis does not attempt to separate the influence of the different sources of uncertainty, so this range also incorporates the uncertainty arising from the use of different control data and that due to $\alpha_{\max}$.

Supplemental Information—Formaldehyde (Inhalation)

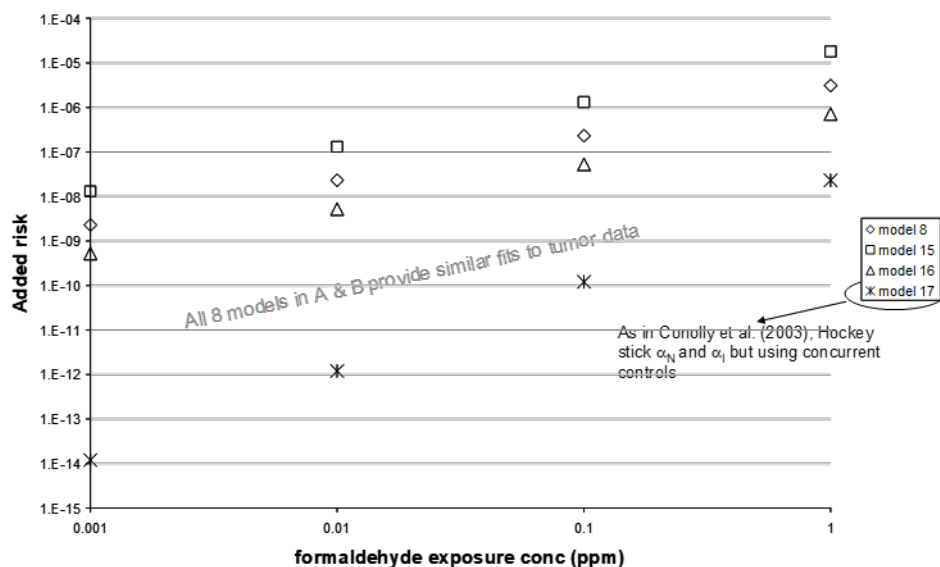


Figure D-34. BBDR models for the rat—models with positive added risk.

Note: All four models provide “similar” fits to tumor data (see text).

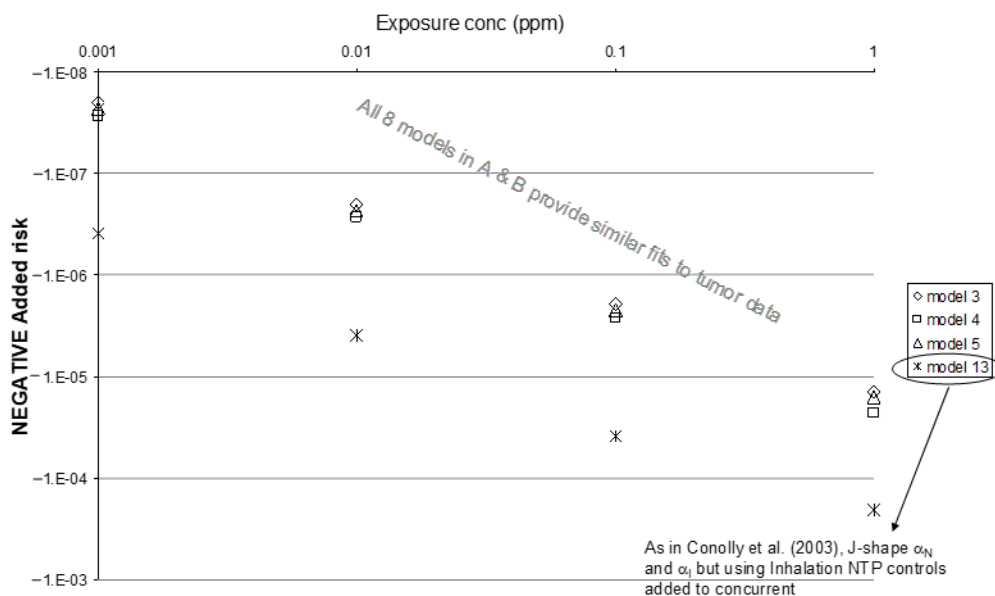


Figure D-35. BBDR rat models resulting in negative added risk.

Note: All four models provide “similar” fits to tumor data (see text).

Supplemental Information—Formaldehyde (Inhalation)

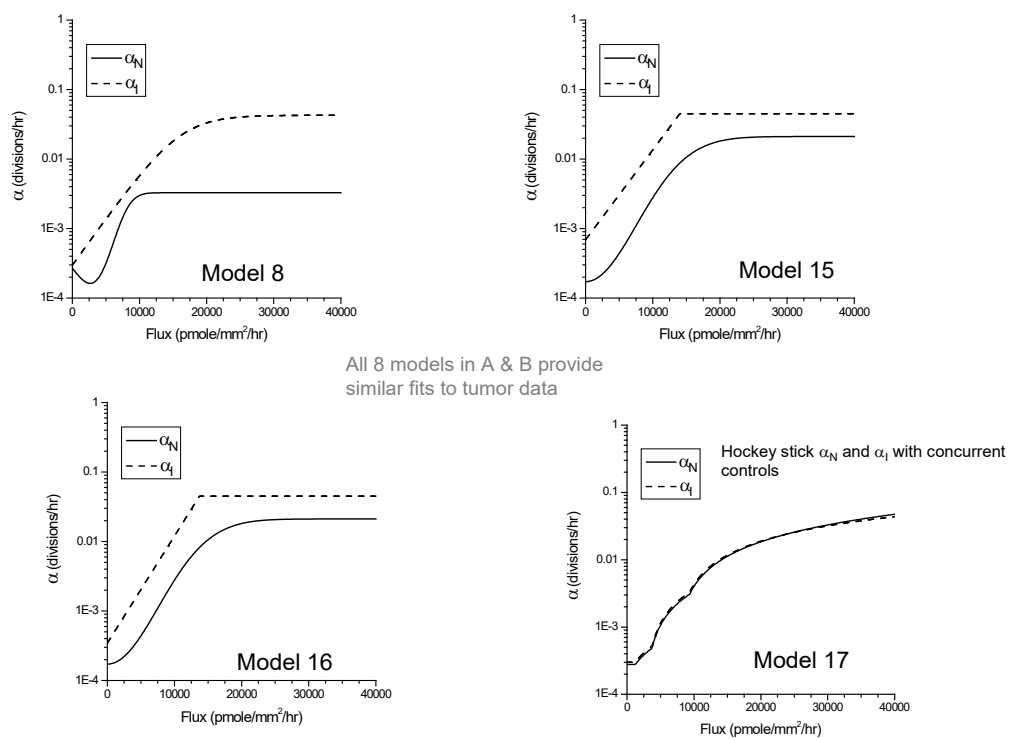


Figure D-36. Models resulting in positive added rat risk: Dose response for normal and initiated cell replication.

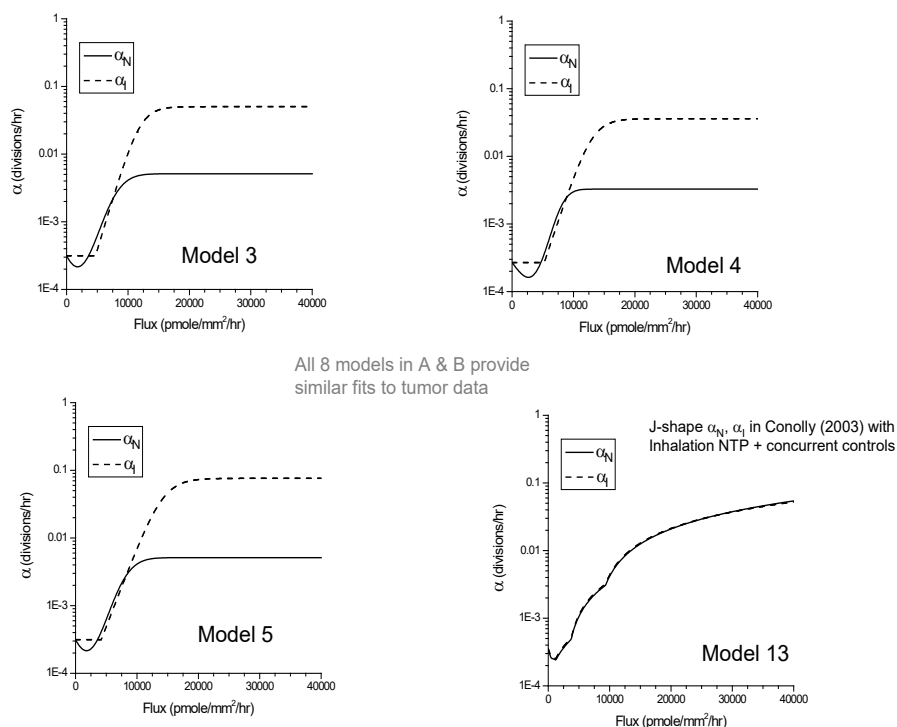


Figure D-37. Models resulting in negative added rat risk: Dose response for normal and initiated cell replication.

Table D-37. Parameter specifications and estimates for clonal growth models of nasal SCC in the F344 rat using alternative characterization of cell replication and death rates

Parameters	Model 3	Model 4	Model 5	Model 8	Model 15	Model 16
Historical controls added to concurrent	Inhalation NTP	Inhalation NTP	Inhalation NTP	Inhalation NTP	Inhalation NTP	Inhalation NTP
Number of dose groups	6	6	5	5	5	5
DPX concentration	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)	Subramaniam et al. (2007)
α_N definition	N3	N6	N3	N6	N4	N4
α_I definition	I2	I2	I2	I1	I1	I1
α_{high}	--	0.045	--	0.045	0.045	0.045
β_I definition	$\beta_I = K_\delta \times \alpha_I$	$\beta_I = K_\delta \times \alpha_I$	$\beta_I = K_\delta \times \alpha_I$	$\beta_I = K_\delta \times \alpha_I$	$\beta_I = K_\delta \times \alpha_I$	$\beta_I = K_\delta \times \alpha_I$
					$\gamma_1 \leq 4 \alpha_{NBasal}$	$\gamma_1 \leq 2 \alpha_{NBasal}$
Log-likelihood	-1,495.34	-1,495.61	-184.02	-184.22	-182.75	-186.37
μ_{NBasal}	7.518×10^{-7}	1.664×10^{-6}	8.684×10^{-7}	9.230×10^{-7}	1.037×10^{-6}	1.662×10^{-7}

Supplemental Information—Formaldehyde (Inhalation)

Parameters	Model 3	Model 4	Model 5	Model 8	Model 15	Model 16
KMU	3.884×10^{-7}	3.471×10^{-7}	0.0	0.0 (0.0, 2.093×10^{-6})	4.582E-6 (1.8×10^{-6} , 1.86×10^{-5})	0.0
KMX (KMU/μ_{NBasal})	0.5166	0.2086	0.0	0.0 (0.0, 4.696)	4.420 (1.53, 17.67)	0.0
D_0^S	214.3	199.7	261.8	254.2	423.2	245.1
D_{0F}^S	75.26	79.81	119.7	101.1	100.8	98.83
γ_1	1.164×10^{-5}	1.006×10^{-5}	3.168×10^{-5}	2.967×10^{-4}	6.888×10^{-4}	3.441×10^{-4}
γ_2	1427	1,591	1,825	3,223	4,652	2,818
γ_3	11,944	13,017	14,207	15,989	54,334	37,896
K_θ	0.9893	0.9848	0.9804	0.9504	1.006	0.9660

^SSee Subramaniam et al. (2007) for an explanation of the time delay constants D_0 and D_{0F} .

Table D-38. Parameter specifications and estimates for clonal growth models of nasal SCC in the F344 rat using cell replication and death rates as characterized in Conolly et al. (2003)

Parameters	Model 13	Model 17
Historical controls added to concurrent	All NTP	NO historical controls
Number of dose groups	6	6
DPX concentration	Conolly et al. (2000)	Subramaniam et al. (2007)
α_N definition	J shape [TWA, Conolly et al. (2003)]	Hockey [TWA, Conolly et al. (2003)]
α_I definition	eq. B-13	eq. B-13
α_{high}	--	--
β_I definition	$\beta_I = \alpha_N$	$\beta_I = \alpha_N$
Log-likelihood	-1,692.68	-1,474.29
μ_{NBasal}	1.731×10^{-6}	0.0
KMU	0.0	1.203×10^{-6} (1.0×10^{-6} , 1.427×10^{-6})
KMX ($KMU:\mu_{NBasal}$)	0.0	Infinite (0.4097, infinite)
D_0^S	239.5	243.13
D_{0F}^S	66.31	68.83
<i>multib</i>	1.047	$1.078 \times 10^{+0}$
<i>multic</i>	1.510	3.347
α_{max}	5.153×10^{-2}	0.045

^SSee Subramaniam et al. (2007) for an explanation of the time delay constants D_0 and D_{0F} .

- 1) At the 10 ppb (0.01 ppm) concentration, MLE risks range from -4.0×10^{-6} to $+1.3 \times 10^{-7}$. At this dose, models that gave only positive risks resulted in a five orders of magnitude risk range from 1.2×10^{-12} to 1.3×10^{-7} , while narrowing to a four orders of magnitude risk range from 1.2×10^{-10} to 1.3×10^{-6} at the 0.1 ppm level. This narrowing continues as exposure concentration increases, and the curves coalesce to substantially similar values at 6 ppm and above (not shown). For all these 8 models, the rat added risk at 6.0 ppm ranged from 1.8×10^{-2} to 2.1×10^{-2} .
- 2) There does not seem to be any systematic effect on additional risk that depends on whether the 15 ppm data are included in the analysis.
- 3) For all of the models except Models 13 and 17 in Figures D-36 and D-37, the additional risk varies substantially linearly with exposure at low exposures between 0.001 and 1.0 ppm (departing only to a small extent from linearity between 0.1 and 1.0 ppm). Models 13 and 17 show a quadratic dependence; these models employ the TWA J-shaped and hockey stick dose-response curves for α_N used in Conolly et al. (2003) and the same equations used by those authors to relate α_I and β_I to α_N (equations D-20 and D-21). However, the control data in Model 17 was different from those used by Conolly et al.; while all NTP controls were added to the concurrent controls in Model 13, only concurrent controls were used in Model 17.

The various model choices presented in Tables D-37 and D-38 all provided equally good fits to the time-to-tumor data although within the context of a significant qualification. It was not possible to simply use the maximized log-likelihood values as a means of comparing the goodness of fit to the tumor incidence data across all these model choices. This is because many of the model choices differed in the number of doses or in the number of control animals that were used, so the fits were compared across such models only visually.

Wherever results from the BBDR modeling are discussed, values of added risk, as opposed to extra risk, are reported. This is purely for convenience in interpretation. Because of the low background incidence, these values are only negligibly different from the corresponding extra risk estimate. The final risk (or unit risk) estimates provided in this document are based on extra risk estimates.

Confidence bounds: model uncertainty versus statistical uncertainty

For Models 15 and 17 in Figure D-36, 90% CIs for additional risk were calculated by using the profile-likelihood method. Table D-39 compares the lower and upper confidence bounds for these models for 0.001 ppm, 0.1 ppm (doses well below the range where tumors were observed), and 6 ppm (the lowest dose where tumors were observed) with the MLE risk estimates at these doses. In both cases, these intervals were quite narrow compared with the differences in risk predicted by the different models. This suggests that model uncertainty is of more consequence in the formaldehyde animal model than is statistical uncertainty. We also estimated confidence bounds using the bootstrap method for select models and determined that these estimates were in agreement with the bounds calculated using the profile-likelihood method. These results are not

presented here. We return to the calculation of confidence limits when determining points of departure (PODs).

Table D-39. Comparison of statistical confidence bounds on added risk for two models

Dose (ppm)	Model	Lower bound	MLE	Upper bound
0.001	Model 15	4.4×10^{-9}	1.3×10^{-8}	1.6×10^{-8}
	Model 17	1.2×10^{-14}	1.2×10^{-14}	1.3×10^{-14}
0.1	Model 15	4.5×10^{-7}	1.3×10^{-6}	1.7×10^{-6}
	Model 17	1.2×10^{-10}	1.2×10^{-10}	1.3×10^{-10}
6	Model 15	1.8×10^{-2}	2.1×10^{-2}	2.3×10^{-2}
	Model 17	1.3×10^{-2}	1.8×10^{-2}	3.0×10^{-2}

In conclusion, it is demonstrated that the different formaldehyde clonal growth models can fit the data about equally well and still produce considerable variation in additional risk and biological inferences at low exposures.

Statistical Methods Used in Evaluation

Parameters of the alternate models shown here were estimated by maximizing the likelihood function defined by the data ([Cox and Hinkley, 1974](#)). Such estimates are referred to as maximum likelihood estimates (MLEs). Statistical confidence bounds were computed by using the profile-likelihood method ([Crump, 2002](#); [Cox and Hinkley, 1974](#); [Cox and Oakes, 1984](#)). In this approach, an asymptotic $100(1 - \alpha)\%$ upper (lower) statistical confidence bound for a parameter, β , in the animal cancer model is calculated as the largest (smallest) value of β that satisfies

$$2[L_{\max} - L^*(\beta)] = \chi_{1-2\alpha}^2 \quad (\text{D-33})$$

where L indicates the likelihood of the rat bioassay data, $L_{\max}$ is its maximum value, $L^*(\beta)$ is, for a fixed value of β , the maximum value of the log-likelihood with respect to all of the remaining parameters, and $\chi_{1-2\alpha}^2$ is the $100(1-2\alpha)\%$ percentage point of the chi-square distribution with one degree of freedom. The required bound for a parameter, β , was determined via a numerical search for a value of β that satisfies this equation.

The additional risk is defined as the probability of an animal dying from an SCC by the age of 790 days, in the absence of other competing risks of death, while exposed throughout life to a prescribed constant air concentration of formaldehyde, minus the corresponding probability in an animal not exposed to formaldehyde. The MLE of additional risk is the additional risk computed using MLEs of the model parameters.

The method described above for computing profile-likelihood confidence bounds cannot be used with additional risk because additional risk is not a parameter in the cancer model. Instead, an asymptotic $100(1 - \alpha)\%$ upper (lower) statistical confidence bound for additional risk was

computed by finding the parameter values that presented the largest (smallest) value of additional risk, subject to the inequality

$$2[L_{\max} - L] \leq x_{1-2\alpha} \quad (\text{D-34})$$

being satisfied, with the resulting value of additional risk being the required bound. This procedure was implemented through use of penalty functions ([Smith and Coit, 1995](#)). For example, the profile upper bound on additional risk was computed by maximizing the “penalized added risk,” defined as (*additional risk – penalty*), where

$$\text{penalty} = W \times \{[(L_{\max} - L) - x_{1-2\alpha}/2]^+\}^2 \quad (\text{D-35})$$

and $[]^+$ equals the quantity in the brackets whenever it is positive and zero otherwise. The multiplicative weight, W , was selected by trial and error so that the final solution satisfied the following equation sufficiently well.

$$2(L_{\max} - L) = x_{1-2\alpha} \quad (\text{D-36})$$

The computer code was written in Microsoft Excel 2002 SP3 Visual Basic. Either the regular Excel Solver or the Frontline Systems Premium Solver was used to make the required function optimizations. Computation of confidence bounds was highly computationally intensive, and, consequently, confidence bounds were computed only for selected parameters in selected runs. For select cases, the bootstrap method was also used to calculate confidence bounds in order to confirm their accuracy. Values so calculated were found to be in agreement with those calculated by using the likelihood method.

Sensitivity Analysis of Conolly et al. ([2004](#)) Human Extrapolation Model

Uncertainties in the Human Extrapolation Model

Subsequent to the BBDR model for modeling rat cancer, Conolly et al. ([2004](#)) developed a corresponding model for humans for the purpose of extrapolating the risk estimated by the rat model to humans. Also, rather than considering only nasal tumors, it is used to predict the risk of all human respiratory tumors. The human model for formaldehyde carcinogenicity ([Conolly et al., 2004](#)) is conceptually very similar to the rat model but is not based on any data on human exposure to formaldehyde. Unlike the sensitivity analysis of the rat modeling where a number of issues were examined, a much more restricted analysis will be presented here for the sake of brevity. A more extensive analysis was carried out initially that carried forward several of the rat models in Tables D-37 & D-38 to the human, and the lessons learned from those exercises are in agreement with the more restricted presentation that follows. Table D-40 lists the major uncertainties and assumptions in the human extrapolation model in Conolly et al. ([2004](#)).

Table D-40. Summary of evaluation of major assumptions and results in [\(Conolly et al., 2004\)](#)

Assumptions^a	Rationale in (Conolly et al., 2004) or (CIIT, 1999)	EPA evaluation	Further elaboration
Cell division rates derived from rat labeling data were assumed applicable to human (except for assuming different fraction of cells with replicative potential).	There are no equivalent LI data for human or guidance for extrapolating cell division rate across species.	Enzymatic metabolism plays a role in mitosis. Therefore, we expect interspecies difference in cell division rate. Basal cell division rates in humans are expected to be much more variable than in laboratory animals.	Subramaniam et al. (2008)
Parameters for enzymatic metabolism of formaldehyde in human PBPK model for DPX concentrations: K_m varies by order of magnitude between rat and monkey but is same for monkey and human. $V_{max}:K_m$ is similar for rat and monkey but 6-fold lower for human.	See “PBPK model for Human DPX...”	See “PBPK model for Human DPX...”	“PBPK model for Human DPX...”; Conolly et al. (2000); Subramaniam et al. (2008); Klein et al. (2011)
Anatomically realistic representation of nasal passages.	Reduces uncertainty (over default calculation carried out by averaging dose over entire nasal surface).	Computer representation pertains to that of one individual (white male adult). There is considerable interindividual variability in nasal anatomy. Susceptible individuals are even more variable.	(Kimbell et al., 2001b; Kimbell and Subramaniam, 2001) ; (Subramaniam et al., 1998; Subramaniam et al., 2008)
KMU: μ_{Nbasal} is species invariant (used to estimate human).	Human cells are more difficult to transform than rodent, both spontaneously and by exposure to formaldehyde.	μ_{Nbasal} is 0 when concurrent controls or inhalation NTP controls in time frame of concurrent bioassays are used. Leads to infinitely large KMU for human.	Subramaniam et al. (2007); Crump et al. (2009); (Crump et al., 2008) .

Supplemental Information—Formaldehyde (Inhalation)

Assumptions ^a	Rationale in (Conolly et al., 2004) or (CIIT, 1999)	EPA evaluation	Further elaboration
Conservative assumptions were made. Results are conservative in the face of model uncertainties.	1) Hockey-stick dose response for α_N was included even though TWA indicated J shape. 2) Overall respiratory tract cancer incidence data for human baseline rates were used. 3) Risk was evaluated at statistical upper bound of the proportionality parameter relating DPXs to the probability of mutation.	Results in Conolly et al. (2004) are not conservative in the face of model uncertainties: (a) human risk estimates are very sensitive to use of historical controls in the analysis of the animal bioassay, (b) human risk estimates are unboundedly large when concurrent controls are used in rat model, and (c) minor perturbations in model assumptions regarding division and death rates of initiated cells lead to upper bound risks that were more than 1,000-fold greater than the highest estimates in Conolly et al. (2004).	Conolly et al. (2004); Subramaniam et al. (2007); Crump et al. (2009); (Crump et al., 2008).

^aAssumptions in this table are in addition to those listed for the BBDR model for the F344 rat.

Uncertainties in the PBPK Model for Human DPX Concentrations

Conolly et al. ([2000](#)) constructed a PBPK model for the rhesus monkey along similar lines as for the F344 rat, and used the rat and rhesus monkey parameter estimates to develop a model for human DPX concentrations. In the rhesus monkey model, they maintained the same values of k_b , k_{loss} , and k_f as in the rat model but optimized the values of V_{max} and K_m against the rhesus monkey data from Casanova et al. ([1994](#)). The resulting human PBPK model used formaldehyde flux estimates predicted by an anatomically realistic CFD modeling of the nasal passages; except for the anatomic reconstruction, there were no other human data used to inform the PBPK model.

For the human, the model used the value of K_m estimated by the rhesus monkey model and the epithelial thickness averaged over three regions of the rhesus monkey nose. The maximum rate of metabolism, V_{max} , which was estimated independently for the rat and rhesus monkey by fitting to the DPX data available for these species, was then extrapolated to the human by assuming a power law scaling with body weight (BW) (i.e., $V_{max} = a \times BW^b$), and the coefficient “a” and exponent “b” were derived from the independently estimated values of $(V_{max})_{RAT}$ and $(V_{max})_{MONKEY}$. Table D-41 gives the values of V_{max} and K_m in the Conolly et al. ([2000](#)) extrapolation.

Table D-41. Extrapolation of parameters for enzymatic metabolism to the human in Conolly et al. (2000)

Parameter	F344 rat	Rhesus monkey	Human
V _{max} (pmol/min-mm ³)	1,008.0	91.0	15.7
K _m (pmol/mm ³)	70.8	6.69	6.69

Source: Conolly et al. (2000).

In general, laws for allometric scaling across species, such as how enzymatic metabolic rates vary across organisms, are derived as empirical regression relationships based on data from multiple species and usually multiple sources of data points. For example, West and Brown (2005) demonstrate that metabolic rates scale with mass^{3/4} using data from organisms ranging over 27 orders of magnitude in mass (intracellular up to the largest organisms). In Conolly et al. (2000), the power-law relationship is derived using two data points (F344 rat and rhesus monkey for a single chemical) with log BW as *x*-axis and V_{max} on *y*-axis. Because such a regression does not have the power to delineate the curvature in the scaling function, the empirical strength of the allometric relationship derived in Conolly et al. (2000) is extremely weak for use in extrapolating from the rat to the human on the basis of body-weight. Furthermore, as noted earlier, V_{max} is highly correlated to K_m, the value of K_m appears to vary substantially between the rat and monkey, and as indicated by the large standard error using multiple methods in Klein et al. (2011), its estimation is fairly uncertain. These observations make the scaling relationship in Conolly et al. (2000) more problematic.

The following observations point to the uncertainty in the values of the parameters V_{max} and K_m in the Conolly et al. (2000) models for predicting DPXs. First, K_m varies by an order of magnitude across the rat and monkey models and considered invariant between the monkey and human models (Conolly et al., 2000). Second, the values in Conolly et al. (2000) for V_{max}/K_m, the low-dose limit of the rate of enzymatic metabolism, is roughly similar between the rat and monkey but lower by a factor of six in the human.

Another factor that can substantially influence the above extrapolation of DPXs in the human is that Conolly et al. (2000) assumed the tissue to be a well-mixed compartment with regard to formaldehyde interaction with DNA and used the amount of formaldehyde bound to DNA per unit volume of tissue as the DPX dose metric. Considering formaldehyde's highly reactive nature, the concentrations of formaldehyde and DPX are likely to have a sharp gradient with distance into the nasal mucosa (Georgieva et al., 2003). Cohen Hubal et al. (1997) concluded that the well-mixed assumption is inappropriate at exposure concentrations less than 4 ppm. Furthermore, given the interspecies differences in tissue thickness, there is uncertainty as to whether DPX per unit volume or DPX per unit area of nasal lining is the more appropriate dose metric to be used in the extrapolation. In particular, it may be assumed that the cells at risk for tumor formation are only those in the epithelium and that measured DPX data (in monkeys and rats) are an average over the entire tissue thickness. Because the epithelial DPXs in monkeys (and presumably humans) would

then be more greatly “diluted” by lower levels of DPX formation that occur deeper into the tissue than in rats, it could be predicted that the ratio of epithelial to measured DPXs in monkeys and humans would be much higher than the ratio in rats.

On the whole, these observations suggest that human extrapolations of DPX concentrations using the human PBPK model in Conolly et al. (2000) may be highly uncertain.

Sensitivity Analysis of Clonal Growth Model for Human Extrapolation

EPA (Crump et al. (2008)) carried out a limited sensitivity analysis of the Conolly et al. (2004) human model. This analysis was limited to evaluating the effect on the human model of the following. These evaluations have been the subject of some debate in the literature and at various conferences (Crump et al., 2009; Conolly et al., 2009).

- 1) The use of the alternative sets of control data for the rat bioassay data that were considered in the sensitivity analysis of the rat.
- 2) Minor perturbations in model assumptions regarding the effect of formaldehyde on the division and death rates of initiated cells (α_i , β_i).

One (of the two) adjustable parameter in the expression for the human α_i in Conolly et al. (2004) was determined from the model fit to the rat tumor incidence data while the second parameter was determined from background rates of cancer incidence in the human. Therefore, variations considered in α_i were constrained to only those that (a) did not meaningfully degrade the fit of the model to the rat tumor incidence data, as shown in Figure D-41 (i.e. these fits were indistinguishable from those shown in Figure 5-5 of the Toxicological Review), and (b) were in concordance with background rates in the human.

Crump et al. (2008). also evaluated these variations with respect to their biological plausibility. The sensitivity analysis on assumed initiated cell kinetics was thought to be particularly important because there were no data to even crudely inform the kinetics of initiated cells for use in the models, even in rats, and the two-stage clonal expansion model is very sensitive to initiated cell kinetics (Gaylor and Zheng, 1996; Crump, 1994a, b).

Effect of background rates of nasal tumors in rats on human risk estimates

Crump et al. (2008) quantitatively evaluated the impact of different control groups on estimates of additional human risk as follows:

- 1) Concurrent controls plus all NTP controls; the same as used by Conolly et al. (2004);
- 2) Concurrent controls plus controls from NTP inhalation studies;
- 3) Only concurrent controls;
- 4) Each set of control data was applied with both the J shape and hockey-stick models in Conolly et al. (2004) for $\alpha_N(\text{flux})$ and $\alpha_i(\text{flux})$ for a total of six analyses.

- 5) Uncertainties associated with α_N or α_I are not addressed. Parameters α_{max} , multfc, and KMU were estimated in exactly the same manner as in Conolly et al. (2004).

Crump et al. (2008) present the following dose-response predictions of additional risk in humans from constant lifetime exposure to various levels of formaldehyde arising from exercising the above six cases. Their plots are reproduced in Figure D-38, where the corresponding curves based on Conolly et al. (2004) are also shown for comparison.

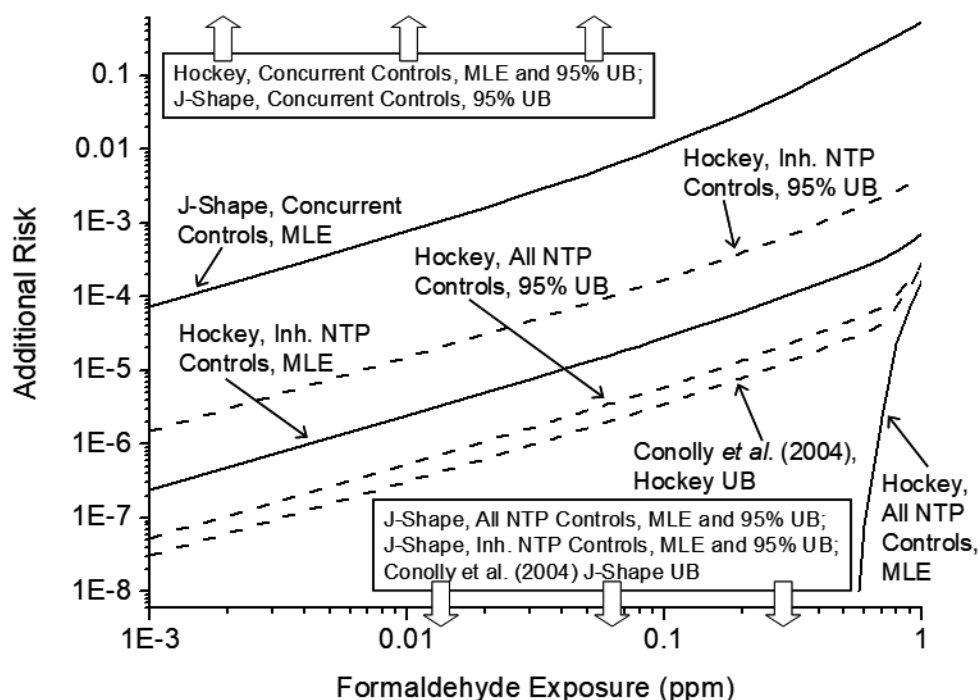


Figure D-38. Effect of choice of NTP bioassays for historical controls on human risk.

Note: Estimates of additional human risk of respiratory cancer by age 80 from lifetime exposure to formaldehyde are obtained by using different control groups of rats.

Source: Crump et al. (2008).

The lowest dotted curve in Figure D-38 represents the highest estimates of human risk developed by Conolly et al. (2004). This resulted from use of the hockey-stick model for cell division rates in conjunction with the statistical upper bound for the parameter KMU. As indicated by the downward block arrows in the figure, their corresponding estimates based on the J-shaped model were all negative for exposures below 1 ppm.

Consider next the solid curves in the figure, which show predicted MLE added risks that were positive and less than 0.5. Crump et al. (2008) next examined the added risk obtained when the MLE estimate of $(KMU;\mu_{basal})$ in these cases is replaced by the 95% upper bound of this parameter ratio. The upper bound risk estimates in Conolly et al. (2004) were calculated in a

similar manner (but using all NTP historical controls). Except for minor differences, risk estimates corresponding to such an upper bound and using all NTP controls were very similar in the two efforts ([Crump et al., 2008](#); [Conolly et al., 2004](#)).

Figure D-38 shows that the choice of controls to include in the rat model can make an enormous difference in estimates of additional human risk. For the J-shaped model for cell replication rate both estimates based on the MLE and those based on the 95% upper bound on $KMU:\mu_{basal}$ are negative for formaldehyde exposures below 1 ppm. However, when only concurrent controls are used in the model in [Crump et al. \(2008\)](#), the MLE from the J-shaped model is positive and is more than three orders of magnitude higher than the highest estimates obtained by [Conolly et al. \(2004\)](#). Using only concurrent controls, estimates based on the 95% upper bound on $KMU:\mu_{basal}$ are unboundedly large (block arrows at the top of the figure). For the hockey-stick shaped model for cell replication rate, when all NTP controls are used, the estimates based on the MLEs are zero for exposures less than about 0.5 ppm. If only inhalation controls are added, the MLEs are about seven times larger than the [Conolly et al. \(2004\)](#) upper bound estimates, and the estimates based on the 95% upper bound on $KMU:\mu_{basal}$ are about 50 times larger than the [Conolly et al. \(2004\)](#) estimates. If only concurrent controls are used, both the MLE estimates and those based on the 95% upper bound on $KMU:\mu_{basal}$ are unboundedly large.

Alternative assumptions regarding the rate of replication of initiated cells

For the human model, [Conolly et al. \(2004\)](#) made the same assumptions for relating $\alpha_I(\text{flux})$ and $\beta_I(\text{flux})$ to $\alpha_N(\text{flux})$ as in their rat model ([Conolly et al., 2003](#)). That is, these quantities were related by using equations D-20 and D-21. By extending the shape of these curves to humans, the authors' model brings the cytotoxic action of formaldehyde to bear strongly on the parameterization of the human model as well.

In the sensitivity analyses that follows, calculations similar to that presented in Table 5-40 of the Toxicological Review are continued over a large range of exposure concentrations. In these analyses, [Crump et al. \(2008\)](#) made minor modifications to the assumed division rates of initiated cells in [Conolly et al. \(2004\)](#), while all other aspects of the model and input data were kept unchanged. Two alternatives were considered for each of the hockey-stick and J-shaped models. Figure D-39 shows the hockey-stick model for initiated cells in rats. In the first modification to the hockey-stick model (hockey-stick Mod 1), rather than having a threshold at a flux of 1,240 pmol/m²-hour, the division rate increases linearly with increasing flux until the graph intersects the original curve at 4,500 pmol/m²-hour, where it then assumes the same value as in the original curve for larger values of flux. The second modification (hockey-stick Mod 2) is similar, except the modified curve intersects the original curve at a flux of 3,000 pmol/m²-hour.

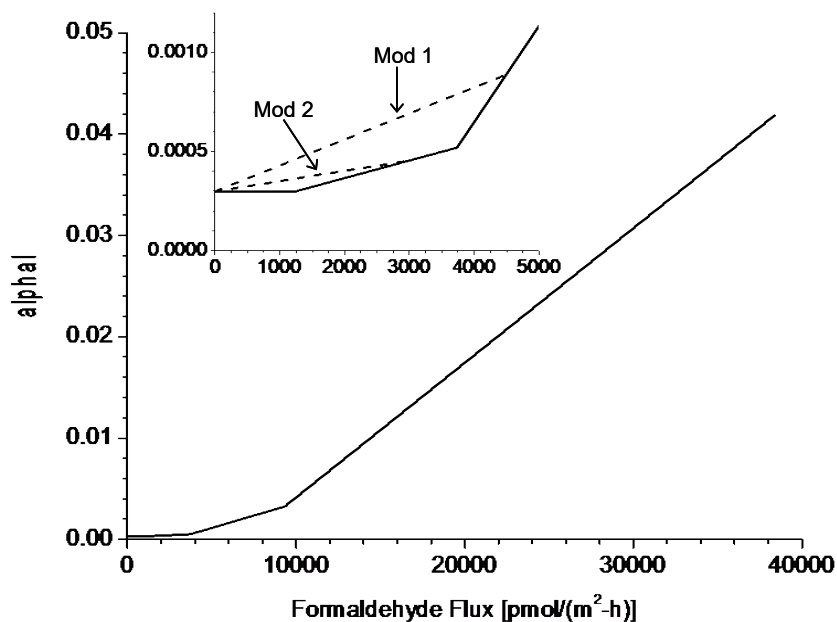


Figure D-39. Variations to the hockey-stick model for division rates of initiated cells in rats.

Source: [Crump et al. \(2008\)](#).

Figure D-40 shows the rat J-shaped model for initiated cells. In the first modification to this dose response (J-shaped Mod 1), rather than having a J shape, the division rate of initiated cells remains constant at the basal value until the original curve rises above the basal value and has the same value as the original curve for larger values of flux. In the second modification (J-shaped Mod 2), the J shape is retained but somewhat mitigated. In this modification, the division rate initially decreases in a linear manner similar to that of the original model but with a less negative slope until it intersects the original curve at a flux of 1,240 $\mu\text{mol}/\text{m}^2\text{-hour}$, where it then follows the original curve for higher values of flux.

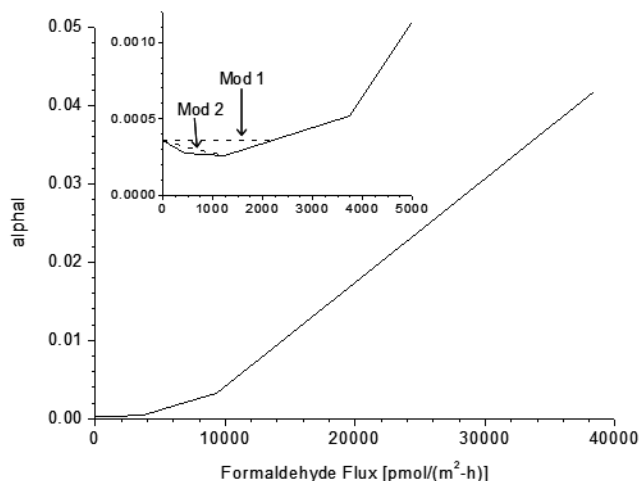


Figure D-40. Variations to the J-shaped model for division rates of initiated cells in rats.

Source: [Crump et al. \(2008\)](#).

Because the first constraint on the variation in α_1 was in concordance with the rat time-to-tumor incidence data, Crump et al. (2008) applied each of the modified models in Figures D-39 and D-40 to the version of the formaldehyde models in Subramaniam et al. (2007) that employed all NTP controls and the hockey-stick curve for α_N . These authors restricted their analysis to this case because their stated purpose was only a sensitivity analysis as opposed to developing alternate credible risk estimates. Figure D-41 reproduces [from Crump et al. (2008)] curves of the cumulative probability of a rat dying from a nasal SCC by a given age for bioassay exposure groups of 6, 10, and 15 ppm. For comparison purposes, the corresponding KM (nonparametric) estimates of the probability of death from a nasal tumor are also shown. Three sets of probabilities are graphed: the original unmodified one and the ones obtained by using hockey-stick Mod 1 and Mod 2. Crump et al. (2008) state that the changes in the tumor probability resulting from these modifications are so slight that the three models cannot be readily distinguished in this graph.³⁶ Thus, the modifications considered to the models for the division rates of initiated cells caused an inconsequential change in the fit of the model-predicted tumor incidence to the animal tumor data.

³⁶The largest change in the tumor probability resulting from this modification for any dose group and any age up through 900 days was found to be less than 0.002, a change so small that it would be impossible to detect, even in the largest bioassays ever conducted. The changes in tumor probability resulting from the other modifications described earlier were found to be even smaller. These comparisons were made in Crump et al. (2008) without reoptimizing the likelihood. The authors note that reoptimization of the model subsequent to the variations would have made the fit of modified models even better.

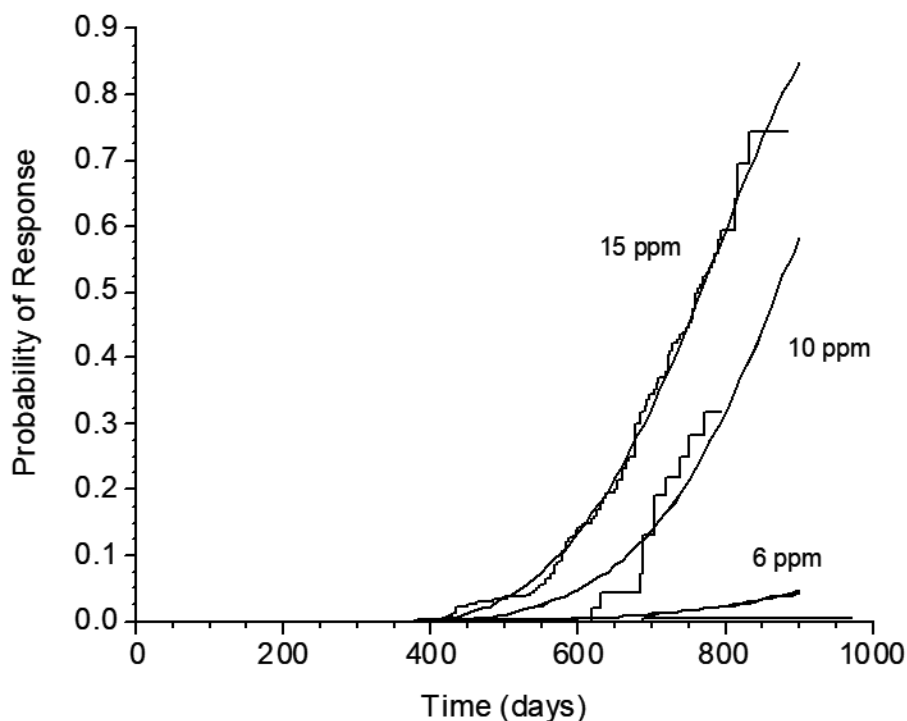


Figure D-41. Very similar model estimates of probability of fatal tumor in rats for three models in Figure D-39.

Note: The differences are visually indistinguishable. Models were derived from the implementation of Conolly et al. (2003) with the hockey-stick curves for $\alpha_I(\text{flux})$ and $\alpha_N(\text{flux})$ and variants derived from modifications (Mod 1 and Mod 2, Figure D-39) to $\alpha_I(\text{flux})$. Model probabilities are compared to Kaplan-Meier estimates. The three sets of model estimates are so similar that they cannot be distinguished on this graph.

Source: Crump et al. (2008).

The above modifications did not affect the basal rate of cell division in the model and likewise had no effect on the fit to the human background data (Crump et al., 2008).

Crump et al. (2008) noted that, although the threshold model for initiated cells in Conolly et al. (2003) was replaced with a model that had a small positive slope at the origin, the resulting curves, hockey-stick Mod 1 and hockey-stick Mod 2, could have been shifted slightly to the right along the flux axis in order to introduce a threshold for α_I without materially affecting the risk estimates resulting from these modified curves. Thus, “the assumption of a linear no-threshold response is not an essential feature of the modifications to the hockey-stick model; clearly threshold models exist that would produce essentially the same effect” (Crump et al., 2008).

Biological plausibility of alternate assumptions

Crump et al. (2008) provide many arguments to support the very small variations made to the α_I in Conolly et al. (2003) for their sensitivity analyses. These variations are found to be:

- consistent with the tumor-incidence data (Figure D-41);

- small compared with the variability and uncertainty in the cell replication rates characterized from the available empirical data (at the formaldehyde flux where α_1 was varied);
- supported (qualitatively) by limited data, suggesting increased cell proliferation at doses below cytotoxic;
- perturbations to be expected on any dose response derived from laboratory animal data because of human population variability in cell replication (the Conolly et al. (2004) modeling assumes that the formaldehyde flux levels at which cell replication, normal and initiated, exceeds baseline rates remain essentially unchanged when extrapolated to the human.)

The analyses of the cell replication data show that the data are not consistently (over each site and time) indicative of a hockey-stick or J shape as the best representation of the data; in some cases, the data appear to be more representative of a monotonic increasing dose response without a threshold. This uncertainty is particularly prominent when examining the cell replication data at the 13-week exposure time and the pooled data from the PLM nasal site from Monticello et al. (1996) (Appendix D.2.2 “Characterization of uncertainty-variability in cell replication rates”). The earliest exposure time in this experiment was at 13 weeks; it is possible that early times are of more relevance to the carcinogenesis as well as for considering typical (frequent short duration) human exposures. Meng et al. (2010) measured cell replication in the anterior lateral meatus of the F344 rat using continuous labeling on rats exposed to all the concentration levels in the Monticello et al. (1996) experiment. Labeling index (i.e., LI, as opposed to ULLI in the Monticello experiment) was measured as the percentage of BrdU-labeled cells among the total number of cells counted at the nasal site. Their data are reproduced below in Figure D-42, where the asterisk denotes the observation of a statistically significant difference from the control group (Dunnett’s test, $p < 0.01$). EPA determined that a linear regression provided good fits to all of the data ($R^2 = 0.97$) as well as to the subset of the data obtained by deleting the higher dose data at 10 and 15 ppm exposures ($R^2 = 0.84$). Thus, these data appear to be consistent with a monotonically increasing trend in the dose-response for cell replication.

For initiated cells, there are no data on which to evaluate the modifications made in Figures D-39 and D-40 to the assumption in equation 5-4. However, some perspective can be gained by comparing them to the variability in the division rates obtained from the data on normal cells used to construct the formaldehyde model. As shown in Figure D-25 and discussed further in Subramaniam et al. (2008), these data show roughly an order of magnitude variation in the cell replication rate at a given flux. As part of a statistical evaluation of these data, a standard deviation of 0.32 was calculated for the log-transforms of individual measurements of division rates of normal cells (Crump et al., 2008). By comparison, the maximum change in the log-transform division rate of initiated cells resulting from hockey-stick Mod 2 was only 0.20, and the average change would be considerably smaller. Thus, although there are no data for initiated cells, it can be

said that the modifications introduced in Crump et al. (2008) for initiated cells are extremely small in comparison to the dispersion in the data for normal cells.

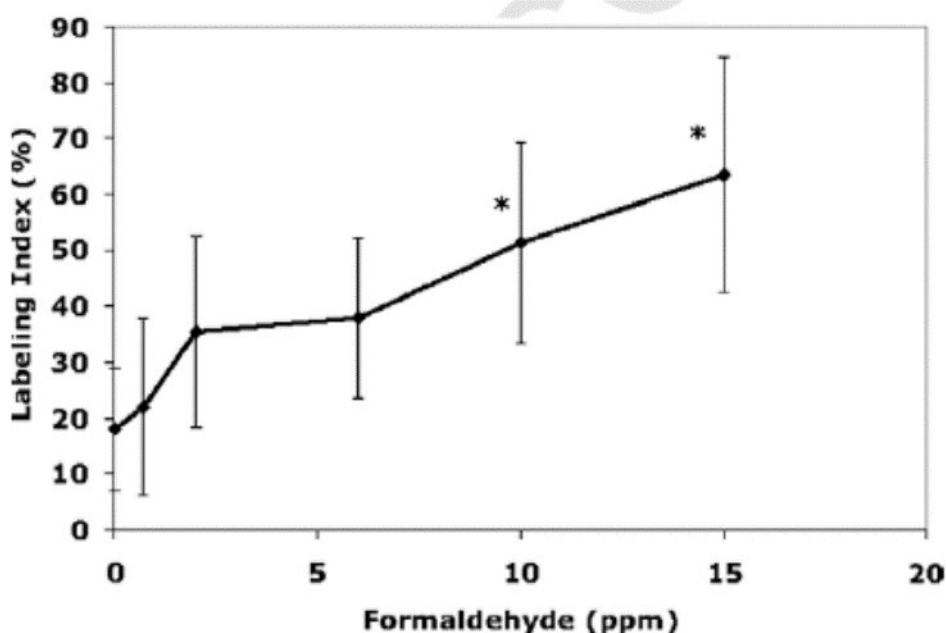


Figure D-42. Cell proliferation data from Meng et al. (2010).

The y-axis shows the percentage of BrdU-labeled among the total number of cells counted in the ALM section of the rat nose.

Reproduced with permission from (Meng et al., 2010).

Effect of alternate assumptions for initiated cell kinetics on human risk estimates

Figure D-43 contains graphs of the additional human risks estimated [in (Crump et al., 2008)] by applying these modified models or α_i and using all NTP controls, compared with those obtained by using the original (Conolly et al., 2004) model. Each of the four modified models presents a very different picture from that of (Conolly et al., 2004). At low exposures, these risks are three to four orders of magnitude larger than the largest estimates obtained by (Conolly et al., 2004).

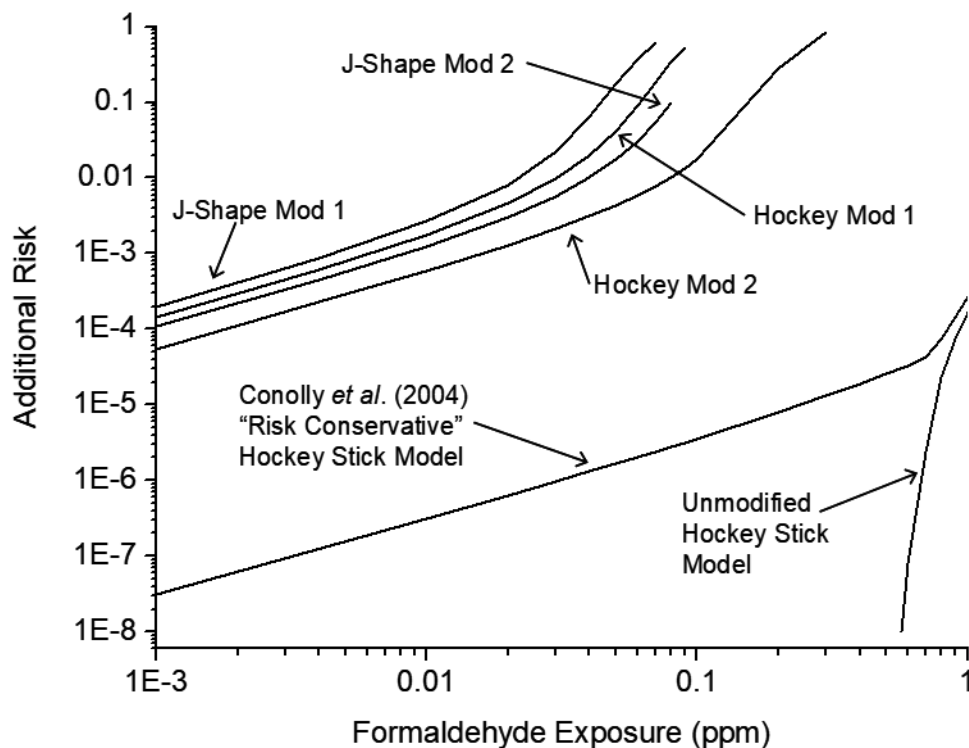


Figure D-43. Graphs of the additional human risks estimated by applying these modified models for α_i , using all NTP controls, compared to those obtained using the original (Conolly et al., 2004) model.

Source: [Crump et al. \(2008\)](#).

These results have been criticized by ([Conolly et al., 2009](#)) as being unrealistically large and above the realm of any epidemiologic estimate for formaldehyde SCC. Thus, they argue that the parameter adjustments made in ([Crump et al., 2008](#)). are inappropriate. [Crump et al. \(2009\)](#) rebutted these points by arguing that the purpose of their work was not to provide a more reliable or plausible model but to carry out a sensitivity analysis. They argued that the changes made to the model (in their analyses) were reasonable because they did not violate any biological constraints or the available data. Further, they pointed out that “by appropriately mitigating the small modifications [they] made to the division rates of initiated cells, the model [would] provide any desired risk ranging from that estimated by the original model up to risks 1,000-fold larger than the conservative estimate in Conolly et al. (2004).”

[Crump et al. \(2008\)](#) also evaluated the assumption in equation D-21 of the CIIT modeling pertaining to initiated cell death rates (β_i) by making small changes to β_i . They report that they obtained similarly large values for estimates of additional human risk at low exposures. Obtaining reliable data on cell death rates in the nasal epithelium appears to be an unusually difficult proposition ([Monticello and Morgan, 1997](#); [Hester et al., 2003](#)), and, even if data are obtained, they are likely to be extremely variable.

D.2.3. Supporting Information for Lymphohematopoietic Cancer Estimates

This section presents the attempts to derive a unit risk estimate for myeloid leukemia based on the results of the NCI cohort study ([Beane Freeman et al., 2009](#)) and discusses the uncertainties associated with the available estimates.

Beane Freeman et al. (2009) used log-linear Poisson regression models stratified by calendar year, age, sex, and race and adjusted for pay category (salary/wage/unknown) to estimate RRs for various categorical exposure groups (see Table D-42). The NCI investigators used the low-exposure category as the reference category to “minimize the impact of any unmeasured confounding variables since nonexposed workers may differ from exposed workers with respect to socioeconomic characteristics” ([Hauptmann et al., 2004b](#)). A 2-year lag interval was used to determine exposures to account for a latency period for LHP cancers.

The log-linear trend tests conducted by [Beane Freeman et al. \(2009\)](#) used exposure as a continuous variable (except for peak exposure, for which categorical ranks were used) (general model form: $RR = e^{\beta X}$, where β represents the regression coefficient and X is exposure). As shown by [Callas et al. \(1998\)](#), the Poisson regression model converges to the Cox proportional hazards model as the age strata are made infinitely small, and when age is well characterized and adjusted for, as it was in the ([Beane Freeman et al., 2009](#)) Poisson regression model, these two models yield essentially the same RR point estimates and CIs.

Dr. Beane Freeman provided EPA with the regression coefficient estimates from the log-linear trend test models for cumulative exposure for several LHP cancer subtype groupings. These estimates are presented in Table D-43. As with the NPC calculations, the nonexposed person-years were included in the primary unit risk estimate derivations and other quantitative approaches to be more inclusive of all the exposure-response data. Results for the exposed person-years only are presented for some of the unit risk estimates for comparison.

Table D-42. Relative risk estimates for mortality from multiple myeloma (ICD-8 code 203), leukemia (ICD-8 codes 204–207), myeloid leukemia (ICD-8 code 205), and other/unspecified leukemia (ICD-8 code 207) by level of formaldehyde exposure for different exposure metrics

Cancer type	Relative risk (number of deaths)				p-Trend	
					All person-years ^a	Exposed only ^b
Peak exposure (ppm)						
	0	>0 to <2.0 ^c	2.0 to <4.0	≥4.0		
Multiple myeloma	2.74 (11)	1.0 (14)	1.65 (13)	2.04 (21)	>0.50	0.08
Leukemia	0.59 (7)	1.0 (41)	0.98 (27)	1.42 (48)	0.02	0.12
Myeloid leukemia	0.82 (4)	1.0 (14)	1.30 (11)	1.78 (19)	0.07	0.13

Supplemental Information—Formaldehyde (Inhalation)

Cancer type	Relative risk (number of deaths)				p-Trend	
					All person-years ^a	Exposed only ^b
Other/unspecified leukemia	0.61 (2)	1.0 (13)	0.86 (8)	1.15 (13)	0.50	>0.50
Average Intensity (ppm)						
	0	>0 to <0.5 ^c	0.5 to <1.0	≥1.0		
Multiple myeloma	2.18 (11)	1.0 (25)	1.40 (11)	1.49 (12)	>0.50	>0.50
Leukemia	0.54 (7)	1.0 (67)	1.13 (25)	1.10 (24)	0.50	>0.50
Myeloid leukemia	0.70 (4)	1.0 (24)	1.21 (9)	1.61 (11)	0.40	0.43
Other/unspecified leukemia	0.58 (2)	1.0 (21)	0.98 (7)	0.84 (6)	>0.50	>0.50
Cumulative Exposure (ppm × years)						
	0	>0 to <1.5 ^c	1.5 to <5.5	≥5.5		
Multiple myeloma	1.79 (11)	1.0 (28)	0.46 (5)	1.28 (15)	>0.50	>0.50
Leukemia	0.53 (7)	1.0 (63)	0.96 (24)	1.11 (29)	0.08	0.12
Myeloid leukemia	0.61 (4)	1.0 (26)	0.82 (8)	1.02 (10)	0.44	>0.50
Other/unspecified leukemia	0.77 (2)	1.0 (15)	1.65 (10)	1.44 (9)	0.13	0.15

^aLikelihood ratio test (1 degree of freedom) of zero slope for formaldehyde exposure (continuous variable, except for peak exposure metric) among all (nonexposed and exposed) person-years.

^bLikelihood ratio test (1 degree of freedom) of zero slope for formaldehyde exposure (continuous variable, except for peak exposure metric) among exposed person-years only.

^cReference category for all categories with the same exposure metric.

Source: [Beane Freeman et al. \(2009\)](#).

Table D-43. Regression coefficients for leukemia, myeloid leukemia, and myeloid plus other/unspecified leukemias mortality from NCI trend test models of cumulative exposure^a

Cancer type	Person-years	β (per ppm × years)	Standard error (per ppm × years)
Leukemia	All	0.01246	0.006421
	Exposed only	0.01131	0.00661
Myeloid leukemia	All	0.009908	0.01191
	Exposed only	0.008182	0.01249
Myeloid leukemia plus other/unspecified leukemia ^b	All	0.01408	0.007706
	Exposed only	0.01315	0.007914

^aModels were stratified by calendar year, age, sex, and race and adjusted for pay category; exposures included a 2-year lag interval.

^b*p*-trend values for the myeloid and other/unspecified leukemia categories combined are 0.10 for all person-years and 0.13 for exposed person-years only.

Source: Personal communication from Laura Beane Freeman to Jennifer Jinot ([Jinot and Beane-Freeman, 2014](#))

Approaches Used for Quantitative Risk Assessment of Myeloid Leukemia

As discussed above, cumulative exposure, which incorporates both exposure intensity and duration, is the preferred exposure metric for the evaluation of long-term exposure to chemicals and effects on cancer, and it is the exposure metric of choice for the estimation of cancer risks in this assessment. EPA explored several approaches for deriving a unit risk estimate for myeloid leukemia based on cumulative exposure.

EPA considered a standard approach for deriving the unit risk estimate using the regression coefficient for myeloid leukemia and cumulative exposure; however, the *p*-value (0.44) for that regression coefficient was far from 0.05, indicating a poor model fit. The poor model fit could be due, at least in part, to inadequate statistical power, likely exacerbated by the underreporting of myeloid leukemia deaths suggested by the analyses by ([Percy et al., 1981](#); [Percy et al., 1990](#)). Table D-43 shows that the regression coefficient for all person-years for myeloid leukemia is only slightly lower than that for all leukemia, which had a lower *p*-value of 0.08 and which should include all the myeloid leukemia deaths, both specified and unspecified. The “other/unspecified” leukemias comprise a sizable portion of all leukemia deaths (almost 30%) in the cohort and presumably include a good proportion of unclassified myeloid leukemias. The results of two NCI studies done at different times to evaluate the accuracy of death certificates by comparing the underlying cause of death on death certificates to original hospital diagnoses suggest that a third to a half of leukemias not otherwise specified on death certificates were diagnosed as myeloid leukemias in the hospital ([Percy et al., 1981](#); [Percy et al., 1990](#)).³⁷ Thus, two additional approaches for deriving a unit risk estimate for myeloid leukemia, which attempted to address the underreporting of myeloid leukemias, were considered.

One approach involved using the all leukemia grouping.³⁸ Use of the all leukemia background rates in the life-table calculations (described in more detail below) might inflate the unit risk estimate by increasing the background risk relative to which the formaldehyde-related risks are calculated. However, the inclusion of any leukemia subtypes not related to formaldehyde

³⁷In the ([Percy et al., 1981](#); [Percy et al., 1990](#)) studies, only about 10% of leukemia deaths were classified as “other or unspecified” based on hospital diagnoses [versus 29% from death certificates in the ([Beane Freeman et al., 2009](#)) study], and 51% ([Percy et al., 1981](#)) and 53% ([Percy et al., 1990](#)) of leukemia deaths were myeloid leukemias based on hospital diagnoses [versus 39% from death certificates in the ([Beane Freeman et al., 2009](#)) study], suggesting that about a third or more of the “other or unspecified” leukemia deaths in the [Beane Freeman et al. \(2009\)](#) study were probably myeloid leukemias. [Percy et al. \(1990\)](#) reported in their study that “Of the nearly 600 deaths from leukemia NOS [other or unspecified] nearly 50% were originally diagnosed as myeloid... Obviously myeloid leukemia is grossly underreported on death certificates.”

³⁸The all leukemia category includes all 123 leukemias observed in the cohort. Of these, 48 (39.0%) were myeloid, 37 (30.1%) were lymphoid, and 36 (29.3%) were other/unspecified; the remaining 2 (1.6%) were monocytic leukemias (ICD-8 code 206).

exposure should theoretically dampen the exposure-response relationship (lowering the regression coefficient) relative to that for all the myeloid leukemias alone; thus, this should mitigate at least some of the effect of using the “all leukemia” background rates.

The approach considered by EPA to be the best currently available involved using a combined grouping of the myeloid leukemia and other/unspecified leukemias subcategories. The myeloid and other/unspecified leukemias grouping had a stronger association with cumulative exposure (p -trend = 0.10 for all person-years) in the Beane Freeman et al. (2009) study than did myeloid leukemia alone and it captures the unclassified myeloid leukemias with the least inclusion of nonmyeloid leukemias. There is likely more uncertainty associated with the background rates for the other/unspecified leukemias than for the specified myeloid and lymphocytic leukemia subtypes (discussed further below); however, the benefits of focusing on the myeloid plus other/unspecified leukemias rather than the broader “all leukemias” grouping in attempting to be more inclusive of all the myeloid leukemias were deemed to outweigh any additional uncertainty associated with the background rates.

Although the unit risk estimate based on the approach of using myeloid plus other/unspecified leukemias inevitably includes some nonmyeloid leukemias, it is considered by EPA to be the best of the currently available approaches for deriving a unit risk estimate for myeloid leukemia specifically.³⁹ Results for all the approaches will be presented for comparison, and it will be apparent that the different approaches yield similar unit risk estimates. Because the purpose in presenting the results from the various approaches is to compare relative quantitative differences across the different approaches, not all the sensitivity analyses that would be presented in a final assessment were performed for each approach (e.g., performing comparison analyses based on exposed person-years only).

Prediction of Lifetime Extra Risk of Myeloid Leukemia Mortality and Incidence

Lifetime extra risk estimates for myeloid leukemia mortality were calculated from the regression results using the different approaches discussed above and the same general methodology described for the NPC mortality estimates. U.S. age-specific 2006 all-cause mortality rates (NCHS, 2009) were used in the life-table programs. For the cause-specific background mortality rates, NCHS age-specific 2006–2010 mortality rates for all race and sex groups combined were used for all leukemia (http://seer.cancer.gov/csr/1975_2010/results_merged/sect_13_leukemia.pdf) and (NCHS, 2006)

³⁹Although the inclusion of cancer subtypes not necessarily causally associated with the chemical exposure in the grouping of cancers represented in the regression coefficient and the corresponding background rates for the life-table analysis is overt here, it is not uncommon that, due to data limitations, unit risk estimates based on human data reflect cancer groupings broader than what might be strictly causally associated with the chemical exposure (e.g., all leukemias, or all lung cancers). As noted in the text, any inclusion of unassociated cancer subtypes in the derivation of the regression coefficient should theoretically attenuate the coefficient in a manner that would offset the use of the unassociated subtypes in the background rates in the life-table analysis.

age-specific mortality rates were used for myeloid leukemia (ICD-10 C92) and for other/unspecified leukemias (C94-C95) ([NCHS, 2006](#)). In addition, a 2-year lag period was used, as selected by ([Beane Freeman et al., 2009](#)).

The resulting point estimates and one-sided 95% UCLs for the extra risk of myeloid plus other/unspecified leukemias are shown in Table D-44. The model predicts extra risk estimates that are fairly linear for exposures below about 0.01–0.1 ppm (0.012–0.123 mg/m³) but not for exposures above 0.1 ppm (0.123 mg/m³).

Table D-44. Extra risk estimates for myeloid plus other/unspecified leukemia mortality from various levels of continuous lifetime exposure to formaldehyde

Exposure concentration (ppm ^a)	Extra risk	95% UCL on extra risk
0.0001	1.32×10^{-6}	2.51×10^{-6}
0.001	1.32×10^{-5}	2.51×10^{-5}
0.01	1.34×10^{-4}	2.58×10^{-4}
0.1	1.59×10^{-3}	3.38×10^{-3}
1	8.40×10^{-2}	6.26×10^{-1}
10	9.81×10^{-1}	9.90×10^{-1}

^aValues used in the derivation of the unit risk estimate are presented in ppm throughout this section. To convert from ppm to mg/m³, 1ppm = 1.23 mg/m³.

Although the background mortality rates of leukemia are higher (lifetime risk of 0.0062 according to the life-table analysis) than those of NPC, the 1% extra risk level typically used as the basis for the POD for epidemiological data still corresponds to an RR estimate (2.5) that would be above the highest categorical result reported, even after adjusting the RR estimates upward relative to the 0-exposure group (because our primary analyses include the nonexposed workers). A 0.5% extra risk level yields an RR estimate of 1.8, which better corresponds to the RRs in the range of the data. Thus, the LEC value corresponding to 0.5% extra risk (LEC₀₀₅) was selected for the POD for all leukemia and for myeloid leukemia and myeloid plus other/unspecified leukemias, which have lower background rates than all leukemia (lifetime risks of 0.0031 and 0.0046, respectively).

There are insufficient data to establish the MOA(s) for formaldehyde-induced myeloid leukemia; thus, linear low-dose extrapolation was performed, in accordance with EPA's *Guidelines for Carcinogen Risk Assessment* ([U.S. EPA, 2005](#)). The EC₀₀₅, LEC₀₀₅, and IUR estimates for myeloid plus other/unspecified leukemia mortality are presented in Table D-45.

Table D-45. EC₀₀₅, LEC₀₀₅, and inhalation unit risk estimates for myeloid plus other/unspecified leukemia mortality from formaldehyde exposure based on log-linear trend analyses of cumulative exposure data from the Beane Freeman et al. (2009) study

Person-years	EC ₀₀₅ (ppm)	LEC ₀₀₅ (ppm)	Unit risk ^a (per ppm)	Unit risk (per mg/m ³)
All	0.253	0.133	3.8×10^{-2}	3.1×10^{-2}
Exposed only	0.269	0.135	3.7×10^{-2}	3.0×10^{-2}

^aUnit risk = 0.005/LEC₀₀₅.

All leukemia and myeloid leukemia have substantial survival rates⁴⁰; thus, it is preferable to derive incidence estimates. Unit risk estimates for leukemia incidences were calculated as described above for the NPC incidence estimates. Age-specific background incidence rates for 2006–2010 for leukemia and its major subtypes (myeloid and lymphocytic leukemia) from Surveillance, Epidemiology, and End Results (SEER) 18, a registry covering about 28% of the U.S. population, were obtained from the SEER website (http://seer.cancer.gov/csr/1975_2010/results_merged/sect_13_leukemia.pdf). Age-specific background incidence rates for other/unspecified leukemias were estimated by subtracting the myeloid and lymphocytic leukemia rates from the rates for all leukemia; these estimated rates would also include monocytic leukemia, but the contribution of monocytic leukemia is negligible.

The incidence-based calculation relies on the assumptions that incidence and mortality for these leukemia subtype groupings have the same exposure-response relationship for formaldehyde exposure and that the incidence data are for first occurrences of the cancers or that relapses provide a negligible contribution. The first assumption is more uncertain for all leukemia, myeloid leukemia, and myeloid plus other/unspecified leukemias than it was for NPC because these are groupings of subtypes with quite different survival rates (e.g., see footnote 40). The incidence-based calculation also takes advantage of the fact that incidence rates for these cancer types are negligible compared with the all-cause mortality rates and thus no special adjustment to the population at risk to account for live individuals who have been diagnosed with these cancers is necessary.

The EC₀₀₅, LEC₀₀₅, and IUR estimates for myeloid plus other/unspecified leukemia incidence are presented in Table D-46. The incidence unit risk estimate is about 10% higher than the mortality estimate. This difference is lower than the ~24% increase that would have been seen for specified myeloid leukemias alone (i.e., ICD-8 205). This is because the difference between age-specific mortality and incidence rates for the other/unspecified leukemias is not very large, and for some age groups the mortality rates are actually larger than the incidence rates. This irregularity is

⁴⁰Survival rates were 55.0% at 5 years for all leukemia [<http://seer.cancer.gov/statfacts/html/leuks.html>], 23.4% at 5 years for acute myeloid leukemia [<http://seer.cancer.gov/statfacts/html/amyl.html>], and 59.1% at 5 years for chronic myeloid leukemia [<http://seer.cancer.gov/statfacts/html/cmyle.html>] based on 2002–2009 SEER data.

to be expected for “other/unspecified” classifications because greater attention is given to diagnosing incident leukemia cases than to accounting for causes of death, so one would anticipate less underreporting of myeloid leukemias as incident cases than as causes of death on death certificates.

Table D-46. EC₀₀₅, LEC₀₀₅, and inhalation unit risk estimates for myeloid plus other/unspecified leukemia incidence from formaldehyde exposure based on Beane Freeman et al. (2009) log-linear trend analyses for cumulative exposure

Person-years	EC ₀₀₅ (ppm)	LEC ₀₀₅ (ppm)	Unit risk ^a (per ppm)	Unit risk (per mg/m ³)
All	0.224	0.118	4.2×10^{-2}	3.4×10^{-2}
Exposed only	0.239	0.120	4.2×10^{-2}	3.4×10^{-2}

^aUnit risk = 0.005/LEC₀₀₅.

The EC₀₀₅ and LEC₀₀₅ estimates for mortality and incidence and incidence unit risk estimates for all leukemia and for myeloid leukemia using the alternate approaches discussed above are presented in Table D-47. The same underlying life-table methodology was used for each of these approaches—only the regression coefficients and background cancer rates differed. As discussed above, and consistent with the results just presented, EPA interprets the best available approach (shaded in Tables D-46 to D-48) to be the life-table analysis using the regression coefficient and background rates for myeloid plus other/unspecified leukemias because this grouping captures the unclassified myeloid leukemias with the least inclusion of nonmyeloid leukemias.

Table D-47. EC₀₀₅ and LEC₀₀₅ estimates for mortality and incidence and incidence unit risk estimates for all leukemia and for myeloid leukemia using alternate approaches (all person-years)

Approach (by cancer type used as basis for regression coefficient and cause-specific background rates)	EC ₀₀₅ (ppm) LEC ₀₀₅ (ppm)		Unit risk estimate (per ppm) ^a	Unit risk estimate (per mg/m ³)
	Incidence	Mortality	(Incidence)	(Incidence)
Myeloid leukemia	0.378 0.127	0.468 0.157	3.9×10^{-2}	3.2×10^{-2}
All leukemia	0.156 0.0846	0.229 0.124	5.9×10^{-2}	4.8×10^{-2}
Myeloid + Other/Unspecified^b	0.224 0.118	0.253 0.133	4.2×10^{-2}	3.4×10^{-2}

Note: Shaded estimate is considered by EPA to be the best available estimate.

^aUnit risk estimate = 0.005/(LEC₀₀₅ for incidence).

^bIncidence background rates also include monocytic leukemia, but that contribution is negligible.

Summary of the Best Available Unit Risk Estimate for Myeloid Leukemia

The best available unit risk estimate for myeloid leukemia is the estimate of 4.2×10^{-2} per

ppm (3.4×10^{-2} per mg/m³) derived using incidence rates (and regression coefficient) for myeloid plus other/unspecified leukemias, for all (exposed and nonexposed) person-years.⁴¹ The results from the exposed person-years only are essentially indistinguishable (see Table D-46). The unit risk estimates from the other approaches considered are fairly close, with the unit risk estimate based on the myeloid leukemia category being virtually identical to the estimate based on myeloid plus other/unspecified leukemias and the estimate based on all leukemia being somewhat greater (see Table D-47).

Table D-48 summarizes some of the key information comparing the different approaches considered for the derivation of the unit risk estimate for myeloid leukemia.

Table D-48. Exposure-response modeling (all person-years) and (incidence) unit risk estimate derivation results for different leukemia groupings

Cancer grouping	Number of deaths in NCI cohort	Regression coefficient (per ppm × year)	SE (per ppm × year)	p-Value	Unit risk estimate (per ppm)	Unit risk estimate (per mg/m³)
Myeloid leukemia	48	0.009908	0.01191	0.44	3.9×10^{-2}	3.2×10^{-2}
All leukemia	123	0.01246	0.006421	0.08	5.9×10^{-2}	4.8×10^{-2}
Myeloid + Other/Unspecified leukemias	84^a	0.01408	0.007706	0.10	4.2×10^{-2}	3.4×10^{-2}

Note: Shaded estimate is considered by EPA to be the best currently available, although notably uncertain.

^aThis is the sum of the leukemias classified as myeloid and those classified as “other/unspecified.” At least 70–80% of this number is expected to be myeloid leukemias, assuming that a third to a half of leukemias not otherwise specified on death certificates are myeloid leukemias, as discussed above.

In summary, as discussed above, EPA explored several approaches for deriving a unit risk estimate for myeloid leukemia based on cumulative exposure. The first approach involved using the grouping of leukemias classified as myeloid leukemia on the death certificate. The regression coefficient for this grouping had a *p*-value (0.44) indicative of a poor model fit. It was reasoned that the poor model fit could be due, at least in part, to the underreporting of myeloid leukemia deaths

⁴¹Comparable to calculations done for NPC (see Section 5.2.1 of the Toxicological Review), a rough calculation was done to ensure that the unit risk estimate derived for myeloid leukemia incidence is not implausible in comparison to actual case numbers. For example, assuming an average constant lifetime formaldehyde exposure level of 20 ppb for the U.S. population, the inhalation unit risk estimate for myeloid (and other/unspecified) leukemia equates to a lifetime extra risk estimate of 8.4×10^{-4} . Assuming an average lifetime of 75 years (this is not EPA's default average lifetime of 70 years, but rather a value more representative of actual demographic data) and a U.S. population of 300,000,000, this lifetime extra risk estimate suggests a crude upper-bound estimate of 3,400 incident cases of myeloid leukemia attributable to formaldehyde exposure per year. This upper-bound estimate is well below the estimated 17,100 total incident myeloid leukemia (not including other/unspecified leukemias) cases per year calculated from the SEER myeloid leukemia incidence rate of 5.7/100,000 (age-adjusted incidence rate for AML and CML combined from 2008–2012 SEER-18 data; www.seer.cancer.gov).

discussed above. It can be seen in Table D-48 that the regression coefficient for myeloid leukemia is only slightly lower than that for all leukemia, which had a lower p -value of 0.08 and should include all the myeloid leukemia deaths, both specified and unspecified. Thus, a second approach involved using the all leukemia grouping, which includes other subtypes likely not associated with formaldehyde exposure. The approach considered by EPA to be the best available involved using the combined grouping of the myeloid leukemia and other/unspecified leukemias subcategories. The myeloid and other/unspecified leukemias grouping had a stronger association with cumulative exposure ($p = 0.10$) in the Beane Freeman et al. (2009) study than did myeloid leukemia alone and it captures the unclassified myeloid leukemias with the least inclusion of nonmyeloid leukemias. The benefits of focusing on the myeloid plus other/unspecified leukemias rather than the broader “all leukemia” grouping in attempting to be more inclusive of all the myeloid leukemias were deemed to outweigh any additional uncertainty associated with the background rates for the other/unspecified leukemias (discussed further below). It is reassuring that the unit risk estimates from the three different approaches are quite similar, with the estimate based on myeloid plus other/unspecified leukemias being essentially identical to the estimate based on the myeloid leukemia category and both those estimates being about two-thirds of the estimate for all leukemia.

Uncertainties in the Best Available Unit risk Estimate for Myeloid Leukemia

Important uncertainties associated with the best available estimate for myeloid leukemia are discussed below and summarized in Section 5.2.2 of the Toxicological Review.

- Despite the quality of the literature base for the formaldehyde assessment and the confidence in the qualitative hazard information for myeloid leukemia, the only study suitable for dose-response quantification for myeloid leukemia may be viewed as insufficient for developing a quantitative estimate of risk with an acceptable level of confidence.
 - The Beane Freeman study (2009) failed to observe an association between cumulative formaldehyde exposure and myeloid leukemia ($p = 0.44$), despite a reasonable number of cases ($n = 48$) and adequate follow-up. The peak exposure metric was marginally associated ($p = 0.07$). This result raises questions about the relative importance of the intensity of exposure and duration in the association of myeloid leukemia mortality. On the other hand, myeloid leukemia mortality increased with TSFE, cumulative exposure, and exposure duration in two other occupational cohorts (garment workers and embalmers).
 - The available animal studies do not provide evidence supporting an association between formaldehyde inhalation and myeloid leukemia. Thus, there are no animal data that can be used to support the POD estimate that can be derived from the only suitable human study.
- Analyses from NCI comparing causes of death recorded on death certificates with original diagnoses in hospital records suggest a misclassification of myeloid leukemia cases ($N = 48$), with a significant proportion reported as “other/unspecified” ($N = 36$).

Supplemental Information—Formaldehyde (Inhalation)

- In the ([Percy et al., 1981](#); [Percy et al., 1990](#)) studies, only about 10% of leukemia deaths were classified as “other or unspecified” based on hospital diagnoses [versus 29% from death certificates in the Beane Freeman et al. ([2009](#)) study], and 51% ([Percy et al., 1981](#)) and 53% ([Percy et al., 1990](#)) of leukemia deaths were myeloid leukemias based on hospital diagnoses [versus 39% from death certificates in the Beane Freeman et al. ([2009](#)) study], suggesting that about a third or more of the “other or unspecified” leukemia deaths in the Beane Freeman et al. ([2009](#)) study were probably myeloid leukemias. Percy et al. ([1990](#)) reported in their study that “Of the nearly 600 deaths from leukemia NOS (other or unspecified) nearly 50% were originally diagnosed as myeloid... Obviously myeloid leukemia is grossly underreported on death certificates.”
- Because it is likely that a proportion of myeloid leukemia cases were reported as “other/unspecified,” a more complete estimate of the association of cumulative formaldehyde exposure with myeloid leukemia might be obtained using the regression results for a combination of myeloid leukemia and other/unspecified leukemia.
- Although a unit risk estimate that combines myeloid leukemia and other/unspecified leukemia overtly includes cancer subtypes not necessarily causally related with the chemical exposure, it is sometimes the case that, due to data limitations, unit risk estimates are based on less directly causal groupings (e.g., all leukemias, or all lung cancers). The inclusion of unassociated cancer subtypes in the derivation of the regression coefficient should theoretically attenuate the association.
- A comparison of the unit risk estimates for all leukemia, myeloid leukemia plus other unspecified leukemia, and myeloid leukemia (ICD-8/9: 205) indicates that all of the estimates are within a factor of 1.5. Unit risk estimates were 3.9×10^{-2} , 4.2×10^{-2} , and 5.9×10^{-2} for all leukemia, myeloid leukemia plus other unspecified leukemia, and myeloid leukemia (ICD-8/9: 205), respectively.
- The approach for combining myeloid leukemia and other/unspecified leukemia to estimate risk, while arguably consistent with the identified misclassification of myeloid leukemia on death certificates ([Percy et al., 1981](#); [Percy et al., 1990](#)), is uncommon but retains significant quantitative uncertainties, including some inconsistencies in statistical results.
 - The combination of myeloid leukemia and other/unspecified leukemia in the regression model yields a p -value of 0.1. While the number of cases is increased by $n = 36$, cancers in this category, with the exception of the myeloid leukemia cases, were not identified to be causally related with formaldehyde exposure during the hazard evaluation. The inclusion of cancers not causally related with formaldehyde exposure would be expected to attenuate the association, but in contrast to this expectation, there was a stronger association for the regression model of other/unspecified leukemia alone ($p = 0.13$) compared to the model of myeloid leukemia alone ($p = 0.44$). There is not a clear explanation for why the association would be stronger for the more heterogeneous leukemia category.
 - There is likely more uncertainty associated with the background cancer rates in the U.S. population for the other/unspecified leukemias than for the specified myeloid

and lymphocytic leukemia subtypes. The survival rates of the other/unspecified cancers had to be estimated by subtracting myeloid and lymphocytic leukemia rates from the rates for all leukemia.

- Given the completely unknown MOA for myeloid leukemia, it is possible, and perhaps likely, that there are dose and duration effects for the development of myeloid leukemia following formaldehyde inhalation that are not fully understood.
 - Acknowledging the complexity of the different dose metrics available in the observational studies, as well as the lack of an association between cumulative exposure and myeloid leukemia in the Beane Freeman study ([2009](#)), it is possible that the specific, individual exposure metrics in this study failed to fully capture the patterns of exposure with which the development of myeloid leukemia is causally related. Importantly, this concern is independent of the identified hazard for myeloid leukemia, as myeloid leukemia mortality was increased in association with the peak exposure metric in this study (industrial workers) and others, as well as with duration-dependent metrics including TSFE, cumulative exposure, and exposure duration in two other occupational cohorts (garment workers and embalmers).
 - As information supporting a nonlinear extrapolation from the identified POD is not available for myeloid leukemia, the current approach uses a default linear extrapolation. It is possible that additional study on the development of this cancer after formaldehyde exposure could provide support for the linear extrapolation or, alternatively, support a nonlinear approach.

D.2.4. Estimates Using DNA Adduct Data in Animals and Background Incidence

[Lu et al. \(2010a\)](#) developed a highly sensitive MS method using [$^{13}\text{CD}_2$]-formaldehyde that reportedly distinguishes whether formaldehyde-induced hydroxymethyl-DNA monoadducts, in particular, the N^2 -hydroxymethyl-dG (N^2 -hmdG) adduct, originate from endogenous or exogenous sources of formaldehyde in rats and monkeys. In experiments using this technique, ([Yu et al. 2015b](#); [Moeller et al. 2011](#); [Lu et al. 2010a](#); [Lu et al. 2011](#)) quantified these mono adducts formed from both sources in various tissues of rats and monkeys: nasal cavity, bone marrow, mononuclear white blood cells, spleen, thymus, tracheal bronchial lymph nodes, mediastinal lymph nodes, trachea, lung, kidney, liver, and brain. ([Swenberg et al. 2011](#)), ([Starr and Swenberg. 2013](#)) and ([Starr and Swenberg. 2016](#)) used these adduct measurements and data on the background incidences of nasopharyngeal cancer, Hodgkin lymphoma, and leukemia in the U.S. population to develop cancer risk estimates by attributing the background incidences to endogenous formaldehyde, using the measured endogenous N^2 -hmdG adducts formed by formaldehyde in specific tissues as a biomarker of exposure. Their method, described by the authors as a “bottom-up approach” for risk estimation used the following steps:

1. DNA mono-adducts were used in the risk model as a marker of exposure (i.e., repairable) as opposed to a marker of effect (i.e., heritable mutations). While both

Supplemental Information—Formaldehyde (Inhalation)

adducts were reportedly formed by endogenous formaldehyde, only *N*2-hmdG adducts were detectable from exogenous formaldehyde.

2. Adducts formed endogenously were distinguished from those formed due to exogenous sources using $^{13}\text{CD}_2$ -formaldehyde coupled with MS methods.
3. Endogenously and exogenously formed mono-adducts were measured in various tissues: nasal cavity, bone marrow, spleen, thymus, and mononuclear white blood cells (rats); nasal cavity, bone marrow (monkeys).
4. Adducts were measured in rats after one 6-hour exposure to 0.7, 2.0, 5.8, 9.1, and 15.2 ppm formaldehyde and five 6-hour exposures to 10 ppm, and in monkeys (cynomolgus macaques) after two 6-hour exposures to 2 and 6 ppm. There were no measurements carried out in unexposed animals. Time-course data were used to derive the half-life ($t_{1/2}$) for repair of the *N*2-hmdG adduct in rats.
5. No exogenous adducts were detected in any of the distant tissues (bone marrow, spleen, thymus, white blood cells); therefore, for these tissues the adduct levels were estimated by considering the limit of detection (LOD) of the method as an upper-bound estimate. This LOD was converted to the equivalent level of *N*2-hmdG adducts per 10^7 dG.
6. The risk model assumes a linear relation between cancer incidence and *N*2-hmdG adduct levels (used as an intracellular marker of exposure) over the concentration range of endogenous adducts. The same linear model is then assumed for exogenous adducts in order to carry out an upward extrapolation to low exposures (that are not high enough to cause cytotoxicity). Unit risks for nasopharyngeal cancer (NPC), Hodgkin lymphoma (HL) and leukemia were calculated as follows:
 - a. Determine lower confidence limits on the endogenous *N*2-hmdG adduct levels measured in Step 3.
 - b. Assume the endogenous adduct level measured in rats to be the same in humans.
 - c. Convert exogenous *N*2-hmdG adduct levels from 6-hour exposure values to adduct levels to be expected under steady-state continuous exposure using the estimated $t_{1/2}$.
 - d. Assume adduct levels are a linear function of exposure (adduct) concentration, passing through the origin. Calculate the adduct per ppm ratio. Then, from (c) above, calculate the continuous adduct level corresponding to 1 ppm.
 - e. Convert the continuous adduct level corresponding to 1 ppm exposure from rat to human by assuming that adduct levels scale in proportion to formaldehyde flux to the nasal tissue in each species. For the monkey, assume that humans receive the same levels of formaldehyde flux.
 - f. Consider endogenous and exogenous *N*2-hmdG adducts formed by formaldehyde to be biochemically indistinguishable (both were similarly related to low-dose formaldehyde carcinogenicity).
 - g. Use the U.S. population background lifetime incidence probabilities of NPC (7.25×10^{-4}), HL (2.3×10^{-3}), and leukemia (1.3×10^{-2}). ([Swenberg et al., 2011](#)) consider values provided in EPA's 2010 draft Toxicological Review (for NPC) and the SEER Cancer Statistics Review (for HL and leukemia). Attribute these lifetime risks to the endogenous formaldehyde levels indicated by the adduct levels in step a (i.e., to the

- lower confidence limit on endogenous formaldehyde N2-hmdG adducts in the nose, bone marrow, or mononuclear white blood cells). Thus, calculate unit risk estimates for these specific cancers, expressed in units of risk per N2-hmdG adduct per 10^7 dG.
- h. Using the unit risk estimates determined in Step g, calculate upper confidence limit on cancer risks for the continuous steady-state exogenous adduct level calculated in Step e, which corresponds to 1 ppm inhaled formaldehyde exposure concentration.

The authors state that their risk estimates are conservative upper bounds on added lifetime risk at low environmental exposures, and cite the following reasons as support:

- The background risks of specific cancers are fully attributed to the internal dose represented by the endogenous N2-hmdG adducts measured in the corresponding tissue.
- Only N2-hmdG adducts are included (the unit risk would be lower if other higher endogenous adducts are included).
- A linear risk model is assumed.
- Exogenous adduct levels are assumed to be a linear function of exposure concentration, passing through the origin. The slope of this line is based on the mean adduct concentration at 10 ppm exposure which is an overestimate at low exposures because the actual relationship of adduct levels versus ppm is highly nonlinear (upwardly concave). This leads to a more conservative estimate for the cancer risk from step h of #7 above.
- The 95% lower confidence bound on mean adduct level is used, which can be assumed to correspond to the upper confidence bound on estimated risk.
- Monkeys appear to have lower exogenous N2-hmdG adduct levels than rats; therefore, risk estimates based on scaling rat adduct levels to humans in proportion to formaldehyde flux to nasal tissue would likely err on the side of being an over-estimate for humans.

EPA ([Crump et al., 2014](#)) evaluated the assumption in ([Swenberg et al., 2011](#)) and ([Starr and Swenberg, 2013](#)) that their use of a linear risk model necessarily yields an upper bound on the low-dose risk. Since the approach in the updated ([Starr and Swenberg, 2016](#)) is the same as in ([Starr and Swenberg, 2013](#)), the evaluation in ([Crump et al., 2014](#)) elaborated further below equally holds.

By virtue of the additivity assumption (see bullet 6f above), the effective dose to the DNA is represented by the total N2-hmdG adduct (endogenous plus exogenous) level. That is, the bottom-up approach allows the traditional dose-response curve (extra risk versus externally derived dose) to be rescaled so that the dose measure associated with zero external dose is now considered a positive dose equal to the levels found in tissues not exposed to an external source, and the line of zero extra risk is at a positive risk designated as the background risk. This is shown schematically in Figure D-44. The dashed line, showing the linearly extrapolated risk to exogenous exposures, is the central estimate of the linear slope based on the background risk P_0 of developing a specific cancer (attributed to an endogenous level of C_0). The solid curve represents a plausible true dose-response for a case in which the curve shapes upward in the (unobservable) endogenous range. It is

reasonable to assume that the shape of the true dose-response curve is differentiable at the endogenous adduct level and is concave upward at dose levels used in rodent bioassays (i.e., following typically used dose-response functions used in modeling the probability of tumor incidence, the slopes get steeper as dose increases and the second derivative is positive). Then it is clear from Figure D-44 that the bottom-up approach can never overestimate the relevant low-dose slope; any straight line between two points on the concave upward curve will underestimate the slope of the curve at the higher of the two doses. A similar argument can be made for a unit risk derived using a lower bound on C_0 to calculate an upper bound on P_0/C_0 .

It is possible, nonetheless, that the extent of underestimation discussed above (that is, from a “bottom up” linear fit to a dose-response curve) can be offset by the conservatism in attributing all cancers of the specified type to the endogenous dose. However, this is difficult to assess. If one focuses only on the specified type of tumor, the assumption on its own appears to be conservative. It is not, however, easy to ascertain whether that degree of conservatism would be greater than the under-estimation. In addition, the selection of the type of cancer is informed by, and thus dependent on, higher dose data. To the extent the higher dose data did not detect other types of cancer, the attribution of all observed cases of the selected tumor may not capture all the relevant cases.

Furthermore, the slope of increased risk with increasing adduct levels need not be linear over the range of the endogenous adducts, especially near C_0 or the lower bound on C_0 . Endogenous defensive mechanisms can become less effective in dealing with endogenous adduct levels as adduct levels increase over the endogenous range in which case the slope may be concave upward. This seems a plausible scenario, as organisms would have evolved some level of defensive mechanisms to deal with endogenous levels of adducts, yet there is an energy cost associated with over-capacity; thus, these defensive capabilities are not fully effective over the entire endogenous range, and this is consistent with the observance of “background” rates of cancer. Under this plausible scenario, the actual slope of the adduct-based unit risk estimate at the lower confidence bound on the mean endogenous *N*2-hmdG adduct level may be substantially higher than that suggested by the linear relationship assumed in ([Starr and Swenberg, 2016](#)). Thus, the slope obtained in [Starr and Swenberg \(2016\)](#) does not necessarily provide an upper bound on risk.

([Starr and Swenberg, 2013](#)) note that the bottom-up approach is based on applying the concept of additivity to background disease processes ([Crump et al., 1976](#)). However, this concept of additivity to background only assumes local linearity in the proximity of zero exogenous dose to be reasonable, while the bottom-up approach assumes linearity over a large dose range; in particular, the bottom-up approach assumes a linear dose-response below zero exogenous dose, which is not required in the concept of additivity to background. As a result, it is unclear if, overall, the bottom-up approach results in a conservative bound on risk, given that extrapolation upwards in a sublinear dose-response would underestimate risk and underestimate the slope of the dose-response curve at higher doses. This is further discussed and illustrated in ([Crump et al., 2014](#)).

Furthermore, the bottom-up approach assumes direct interaction of inhaled formaldehyde with a particular target tissue; if other sites of interaction and mechanisms are involved, the measures of DNA adducts in a specific tissue could lead to underestimates of the cancer potency when utilizing the “bottom-up” approach.

An additional uncertainty arises from the observation that while endogenous *N*2-hmdG and *N*6-hmdA adducts were both measured in rat and monkey nasal tissues, inhalation of formaldehyde resulted in a concentration-related pattern for exogenous *N*2-hmdG adducts only, and no detectable exogenous *N*6-hmdA adducts. If these differences (in regards the observation of *N*6-hmdA versus *N*2-hmdG adducts) are attributable to differences in the effects of endogenous versus exogenous formaldehyde in inducing DNA adducts, it is not clear that one can assume (as in bullet 6f above) additivity of endogenous and exogenous formaldehyde.

Thus, for multiple reasons, it does not appear to be possible to characterize the results using this approach as providing a conservative upper bound on cancer risk. Notwithstanding this limitation, the bottom-up approach in ([Swenberg et al., 2011](#)) and ([Starr and Swenberg, 2016](#)) is particularly attractive when other phenomena such as significant cytotoxicity and subsequent impact on DNA repair prior to mutations are occurring at higher doses. Because the approach does not use the higher-dose data (other than to identify the type of tumors of concern for analysis), it provides a unique perspective on risk estimates derived from these data.

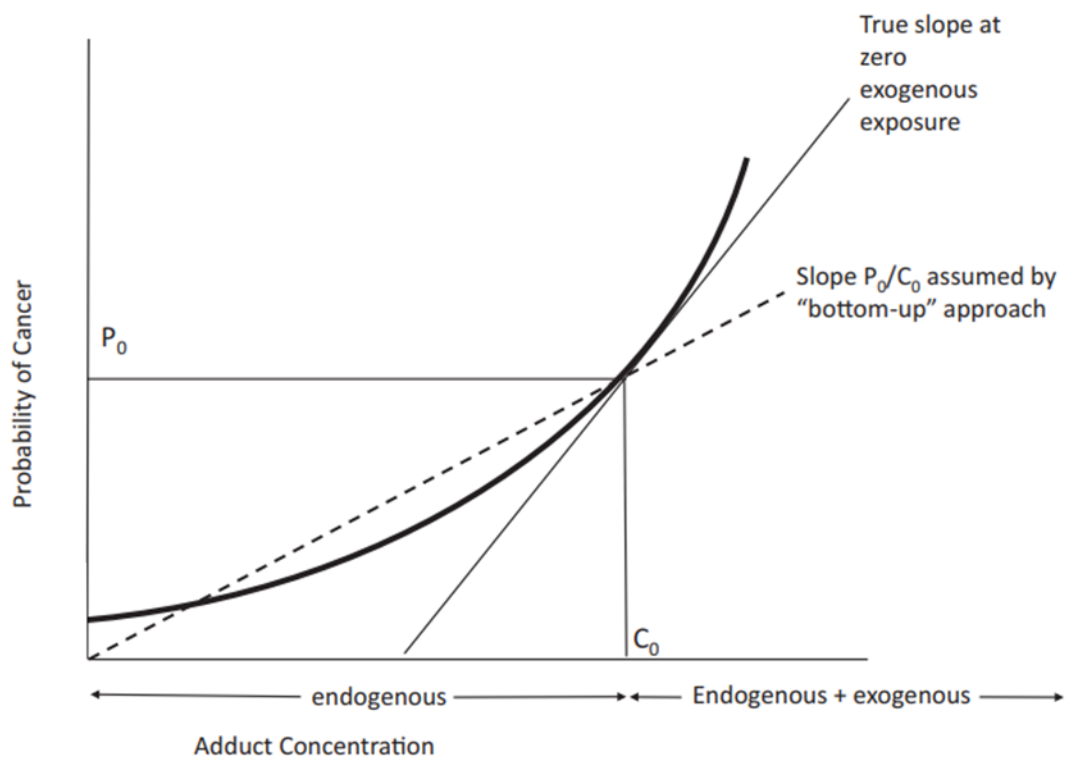


Figure D-44. Schematic of the bottom-up approach.

Source: Adapted from ([Crump et al., 2014](#)).

APPENDIX E. QUALITY ASSURANCE FOR THE IRIS TOXICOLOGICAL REVIEW OF FORMALDEHYDE

This assessment is prepared under the auspices of the U.S. Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) Program. The IRIS Program is housed within the Office of Research and Development (ORD) in the Center for Public Health and Environmental Assessment (CPHEA). EPA has an agency-wide quality assurance (QA) policy that is outlined in the *EPA Quality Manual for Environmental Programs* (see [CIO 2105-P-01.4](#)) and follows the specifications outlined in EPA Order [CIO 2105.4](#).

As required by CIO 2105.1, ORD maintains a Quality Management Program, which is documented in an internal Quality Management Plan (QMP). The latest version was developed in 2013 using [Guidance for Developing Quality Systems for Environmental Programs \(QA/G-1\)](#). An NCEA/CPHEA-specific QMP was also developed in 2013 as an appendix to the ORD QMP. Quality assurance for products developed within CPHEA is managed under the ORD QMP and applicable appendices.

The IRIS Toxicological Review of Formaldehyde is designated as Highly Influential Scientific Information (HISA)/Influential Scientific Information (ISI) and is classified as QA Category A. Category A designations require reporting of all critical QA activities, including audits. The development of IRIS assessments is done through a seven-step process. Documentation of this process is available on the IRIS website: <https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#process>.

Specific management of quality assurance within the IRIS Program is documented in a Programmatic Quality Assurance Project Plan (PQAPP). A PQAPP is developed using the EPA [Guidance for Quality Assurance Project Plans \(QA/G-5\)](#), and the latest approved version is dated April 2021. All IRIS assessments follow the IRIS PQAPP, and all assessment leads and team members are required to receive QA training on the IRIS PQAPP. During assessment development, additional QAPPs may be applied for quality assurance management. They include:

Title	Document number	Date
Program Quality Assurance Project Plan (PQAPP) for the Integrated Risk Information System (IRIS) Program	L-CPAD-0030729-QP-1-7	June 2024
An Umbrella Quality Assurance Project Plan (QAPP) for Dosimetry and Mechanism-Based Models (PBBK)	L-CPAD-0032188-QP-1-3	May 2023

Supplemental Information—Formaldehyde (Inhalation)

Title	Document number	Date
Quality Assurance Project Plan (QAPP) for Enhancements to Benchmark Dose Software (BMDS)	L-HEEAD-0032189-QP-1-3	June 2023

During assessment development, this project undergoes one quality audit during assessment development including:

Date	Type of audit	Major findings	Actions taken
July 2021	Technical system audit	None	None
August 2022	Technical system audit	None	None
June 2024	Technical system audit	None	None

During Step 3 and Step 6 of the IRIS process, the IRIS toxicological review was subjected to external reviews by other federal agency partners, including the Executive Offices of the White House. Comments during these IRIS process steps are available in the docket EPA-HQ-ORD-2010-0396 on <http://www.regulations.gov>.

During Step 4 of assessment development, the IRIS Toxicological Review of Formaldehyde (Inhalation) was released for public comment from April 14, 2022 to June 13, 2022. Following this comment period, the toxicological review underwent independent external peer review by the National Academies of Sciences, Engineering, and Medicine (NASEM) until August 9, 2023. The peer review report is available on the [NASEM website](#). Public are available in the docket [EPA-HQ-ORD-2010-0396](#).

Prior to release (Step 7 of the IRIS process), the final toxicological review was submitted for management and QA clearance. During this step the CPHEA QA Director and QA Managers review the project QA documentation and ensure that EPA QA requirements are met.

APPENDIX F. RESPONSE TO EXTERNAL COMMENTS

The IRIS Toxicological Review of Formaldehyde(Inhalation) has undergone a formal external peer review by the National Academy of Sciences, Engineering, and Medicine (NASEM) in accordance with U.S. Environmental Protection Agency (EPA) guidance on peer review ([U.S. EPA, 2015](#)). Multiple public, external peer-review meetings were held on 10/12–10/13/2022, 12/22/2022, and 1/30–1/31/2023, each of which included an opportunity for public comment. The public was also able to submit written comments to NASEM throughout the peer review. The NASEM peer review panel was tasked with providing consensus comments on the assessment approaches, key conclusions, and areas of scientific controversy or uncertainty. Supplementing the general statement of task NASEM provided to the panel (see below), EPA provided the panel with a series of charge questions (posted to the IRIS website, https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=248150, and the public docket, <https://www.regulations.gov/docket/EPA-HQ-ORD-2010-0396>) to help guide the panel's review. Prior to the external peer review, the assessment was released for public comment (04/14/2022), Step 4a of the IRIS process. Public comments were submitted to the U.S. EPA docket by 06/13/2022.

NASEM Statement of Task (Box S-1 of the NASEM report): *An ad hoc committee under the auspices of the National Academies of Sciences, Engineering, and Medicine will conduct a scientific review of EPA's draft document referred to as the Integrated Risk Information System (IRIS) Toxicological Review of Formaldehyde, plus appendices. The committee will assess whether EPA's draft document adequately and transparently evaluated the scientific literature, used appropriate methods to synthesize the current state-of-the science, and presented conclusions regarding the hazard identification analysis and dose-response analysis of formaldehyde that are supported by the scientific evidence. The committee will not conduct its own hazard assessment of formaldehyde, nor will the committee address the broader aspects of the IRIS Program.*

The comments made by the external peer reviewers and public commenters, as well as EPA's responses to these comments, are grouped according to the chapters and topic areas identified in the NASEM peer review report and bulleted below. The relevant EPA charge questions provided to the panel are also reiterated for each topic area. The consensus "findings" and tiered recommendations (see tiering description below) are directly quoted from the body of the NASEM consensus study report (see <https://www.nationalacademies.org/our-work/review-of-epas-2022-draft-formaldehyde-assessment>); topics without findings or recommendations are not included in this Appendix. In some cases, content from the NASEM study report's Appendices is included to note more specific or supplemental advice. Along with the relevant EPA charge questions, a

summary of the number of findings and recommendations from the panel on each topic area is provided at the start of each section. The reader is encouraged to consult the report in its entirety.

Public comments submitted to the EPA docket are included within each relevant topic area (including an additional “other public comments” section for general comments and comments on topics not highlighted in the NASEM report). Where public comments were made on topics raised by the external peer reviewers, they are noted along with the external peer review comments. When comments from the public and peer review panel on a topic differed, the recommendations of the panel are prioritized. As needed, additional public comments not fitting within the topics outlined by the peer reviewers are included in a separate section at the end of each chapter-based section. As the public comments received in the docket were voluminous, EPA previously developed an organized summary of the comments received and provided this summary to the panel at the beginning of the peer review and posted it in the docket. This summary compilation was used to populate the public comments in this Appendix. The summary includes indexing to the specific documents and page numbers for the quoted comments below. Please note that this summary did not include public comments provided directly to NASEM after announcing the external peer review in 2022 or comments submitted to the EPA docket regarding the length of the public comment period or a requested correction to an affiliation. In this summary, some comments were truncated for brevity⁴²; the reader is encouraged to consult the EPA docket for the full text of the public comments received: <https://www.regulations.gov/docket/EPA-HQ-ORD-2010-0396>).

External Peer Reviewers were asked to prioritize their comments to indicate their relative importance as follows (these instructions are duplicated from the charge questions and NASEM report):

- Tier 1: Necessary Revisions – Please use this category for any revisions you believe are necessary to adequately support and substantiate the assessment conclusions in the formaldehyde (inhalation) assessment. For Tier 1 recommendations, please clearly describe and justify each necessary revision.
- Tier 2: Suggested Revisions – Please use this category for any revisions you encourage EPA to implement to strengthen the analyses or scientific basis for the assessment conclusions, or to improve the clarity of the presentation in the formaldehyde (inhalation) assessment.

⁴² Comments that have been truncated or which have additional details or supporting information in the docket are noted as such in the sections below [Disclaimer: while EPA made every effort to compile accurately and comprehensively the submitted public comments in these courtesy tables, it is possible some information could be missing or inaccurate. Footnote callouts were deleted, and a few minor grammatical corrections were made to some comments for clarity. The EPA docket is the primary source that should be consulted for comments on this assessment.]

- Tier 3: Future Considerations – Please use this category for any advice you have for scientific exploration that might inform future work. While these recommendations are generally outside the immediate scope or needs of the formaldehyde (inhalation) assessment, they could inform future assessments or research efforts.

Tier 1 Recommendations and Tier 2 Suggestions were considered taking into account the extent to which those suggestions would impact the conclusions or quantitative analyses in the assessment and the level of effort to implement. For this assessment, all Tier 1 and Tier 2 suggestions were addressed or implemented. Tier 3 Future Considerations are generally outside of the immediate scope or need of the Toxicological Review but may inform future analyses; two of the four Tier 3 considerations received from peer reviewers on this assessment were addressed or implemented (see below). Most of the Findings and general comments received did not require an EPA response.

External peer reviewer and public comments regarding requests for additions of clarifying text or editorial or grammatical corrections have been made throughout the assessment as appropriate; these comments and responses have not been tracked in this Appendix.

F.1. NASEM REPORT CHAPTER 2: “METHODS AND ORGANIZATION”; CHARGE QUESTION 1: ASSESSMENT DEVELOPMENT METHODS AND ORGANIZATION

F.1.1. Charge Question(s) and Peer Review Comment Summary

Charge Question 1: Assessment Development Methods and Organization.

The Toxicological Review describes and applies a systematic review process for identifying, screening, and evaluating pertinent studies, and then for prioritizing the evidence to inform hazard and dose-response decisions. This process is described in the Toxicological Review’s Preface on Assessment Methods and Organization, with documentation primarily in Appendix A.5 [Appendix B in the revised assessment]. Please answer parts (a) and (b).

- a. Please comment on whether the methods for assessment development (Preface on Assessment Methods and Organization) and the organization of the assessment are clear and transparent.
- b. Please comment on whether there is sufficient documentation on methods and criteria for the following:
 - Identification of epidemiologic, experimental, and mechanistic studies (please identify any additional peer-reviewed studies that would significantly alter key assessment conclusions and thus should be incorporated prior to finalization)⁴³.

⁴³ If newly identified studies that meet the assessment PECO criteria are found by, or brought to the attention of, the panel during the peer review, EPA asks the panel to judge whether the studies would significantly alter

- Critical evaluation of individual studies or sets of studies.
- Assessment of the weight of evidence (i.e., evidence integration).
- Selection of studies and data sets for deriving toxicity values.

Peer Review Comment Summary

- 1 Tier 1 Recommended Revision
- 7 Tier 2 Suggested Revisions (2 have multiple parts)
- 0 Tier 3 Future Considerations
- 14 Consensus Panel Findings (numbered below) and 1 Conclusion

F.1.2. Response to Prior National Research Council (NRC) Reports

Peer Review Findings and Comments

- **Finding (1):** The 2022 Draft Assessment responds to the broad intent of the 2011 NRC report.
- **Finding (2):** The 2022 Draft Assessment is responsive to the broad intent of the 2011 NRC review of EPA's 2010 Draft Assessment and the 2014 NRC review of the IRIS process.

Related Public Comments

- **Public Comment (University of California San Francisco):** "The extent to which EPA has addressed the 2011 NAS recommendations is unclear. We are generally concerned with whether the 2011 NAS recommendations for the formaldehyde assessment were fully taken into consideration in this assessment. Given the critical feedback from the NAS, we were surprised to see that the NAS report is rarely mentioned in the draft Formaldehyde Toxicological Review. It would be appropriate to more thoroughly outline how changes to the assessment were incorporated to address the concerns raised by this committee of experts."
- **Public Comment (Troy Corporation):** "Based on the 2010 National Academy of Sciences (NAS) IRIS formaldehyde review and their 2011 recommendations to the EPA it [is] difficult to discern if these were followed in the preparation of the current assessment. The 2010 NAS document clearly indicated EPA did not identify modes of action and failed to utilize a "weight of evidence" approach towards endpoints such as nasal pharyngeal cancer (NPC), sino-nasal cancer, lymphohematopoietic cancer (LHP), nervous system effects and systemic toxicity. Unfortunately, the 2022 IRIS assessment also did not follow these recommendations and many of the conclusions for these endpoints are the same as in 2010."

key conclusions (e.g., the draft RfC or IUR) and, if so, how. For any studies judged to significantly alter key conclusions, please recommend where and how such studies should be incorporated in the final assessment.

EPA Response (to both public comments above): The responses and actions made in response to the NAS (2011) comments were provided in Appendix D of the external review draft. Please see the consensus peer review panel findings on this topic from the external peer review panel above, which finds the 2022 draft to be broadly responsive to prior NAS recommendations.

F.1.3. Alignment of the Assessment Methods with the State of Practice

Approach to Documentation of Methods

Peer Review Findings and Comments

- **Finding (3):** EPA did not develop a set of specific protocols for the 2022 Draft Assessment in a fashion that would be consistent with the general state of practice that evolved during the prolonged period when the assessment was being developed. Instead, EPA described the assessment methods across the three documents that together make up the 2022 Draft Assessment: The Main Assessment (789 pages), accompanying Appendices (1059 pages), and an Assessment Overview (192 pages).
- **NASEM Panel Conclusion:** Pre-published protocols are essential for future IRIS assessments to ensure transparency for systematic reviews in risk assessment.

EPA Response to Finding 3 and the Conclusion: Although no action is necessary for the IRIS formaldehyde assessment, EPA agrees with the Conclusion regarding future IRIS assessments. As noted in the consensus peer review report, “the procedures used by the IRIS Program were evolving over the period during which the 2022 Draft Assessment was being developed. The IRIS Handbook, which would provide detailed and codified guidance for developing IRIS assessments and make the IRIS process transparent to stakeholders...was released in draft in 2020 and finalized in 2022...Further, work on the 2022 Draft Assessment was suspended in 2018 after the draft’s completion in 2017...The use of prereview—i.e., publicly released—protocols to document the methods to be used in an IRIS systematic review began in 2018 (EPA, 2018).” Since 2018, the IRIS Program has published more than a dozen assessment protocols.

- **Finding (4):** The committee’s review of the 2022 Draft Assessment documents the challenges faced by users of the assessment in navigating the voluminous documentation and understanding the methods used and evidence assessed. EPA needs to revise the Draft Assessment to ensure that the methods used for each outcome can easily be found by providing a linked roadmap and merging the descriptions of the methods used for each outcome in a single location.

- **Tier 1 Recommended Revision (Recommendation 2.1):** EPA should revise its assessment to ensure that users can find and follow the methods used in each step of the assessment for each health outcome. EPA should eliminate redundancies by providing a single presentation of the methods used in the hazard identification and dose-response processes. A central roadmap and cross-references are also needed to facilitate access to related sections across the different elements of the assessment (e.g., appendixes, main document) for the different outcomes analyzed.

EPA Response to Tier 1 Recommended Revision 2.1: The revised assessment incorporates extensive changes and additions in response to this recommendation. Some of the most notable revisions are bulleted below.

- A single assessment methods section (Section 2 in the revised Toxicological Review) akin to a Protocol, stating all the methods used in the assessment, has been added to the beginning of the Toxicological Review. The methods describe both the general methods applied based on study type (e.g., epidemiology studies) as well as by topic area (e.g., sensory irritation).
- The Appendices have been revised to have a complementary presentation to the Assessment Methods section. The revised Appendices are now the location for the transparent documentation of the application of those methods (e.g., literature search and study evaluation in Appendix B of the revised assessment) to the identified studies on each health effect- and mechanism-specific topic area.
- A document map has been added to the assessment methods, and cross-referencing to topic-specific methods has been added throughout the Toxicological Review.
- The Assessment Overview has not been retained (please note that the intent of this document was to briefly summarize the findings from the Toxicological Review, not serve as a separate piece of analysis).
- For the literature searches, the methods and documentation have been revised to improve clarity and transparency as follows:
 - The PECO criteria for each search were clarified and now have a consistent presentation in the assessment methods, with explanations provided for many of the specific inclusion/exclusion criteria. The language used to describe specific inclusion or exclusion criteria has been standardized across health effects-study type.
 - A new, streamlined HERO page (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/4051) for documentation of screening decisions was developed, providing documentation more consistent with current practice within the IRIS Program.

Supplemental Information—Formaldehyde (Inhalation)

- The documentation of the search results for a given health effect- or mechanism-specific search (including electronic searches and the systematic evidence map-based results) have been consolidated by topic area in both Appendix B and in the HERO page.
- For the study evaluations, the following changes were made:
 - The Study Evaluation section begins with an overview (Section 2.3.1 in the revised assessment) describing the commonalities in the approaches used across study types for the overall study confidence classification (*high, medium, low, not informative*), and the domain-specific classifications (Good, Adequate, Deficient).
 - The Study Evaluation overview section also includes a discussion of the evaluation criteria and classification scheme used for three major study types. A summary of the approach use for mechanistic studies is also included in this overview section. The criteria covered for each study type include:
 - For observational epidemiology studies: Population selection, Information bias (relating to outcome classification and exposure measurement in residential and school settings and in occupational settings), potential for confounding, analytic approach, and other sensitivity considerations;
 - For experimental studies in animals: Exposure quality, test subjects, study design, endpoint evaluation, and data considerations and statistics; and
 - For controlled exposure studies in humans: Exposure assessment, outcome classification, consideration of potential observer and subject bias, consideration of confounding, and results presentation.
 - This overview section is followed by domain-specific sections describing the application of the evaluation criteria described in the overview. The criteria are presented in a tabular format and now more transparently describe how ‘good’, ‘adequate’ and ‘deficient’ ratings are drawn for each domain based on general and topic area-specific considerations.
 - To enhance consistency in documentation of study evaluations, the documentation tables (now in Appendix B.3) were revisited to ensure the criteria were consistently applied, both within a topic area and across studies of similar type (e.g., in vivo experimental animal studies).
 - The graphics (“caterpillars”) previously included to summarize the judgments for epidemiology studies were removed and replaced with simplified text and documentation (e.g., shading of domains resulting in lower confidence ratings) that more directly map to the evaluated domains.

Supplemental Information—Formaldehyde (Inhalation)

- Evidence synthesis approaches have been revised as follows:
 - Synthesis judgments are now included for each evidence stream, separate from evidence integration judgments.
 - Each synthesis section includes a brief recap of the methods used, as well as increased cross-referencing, and a listing of studies that met PECO criteria but were classified as *not informative* and thus not synthesized.
 - Each synthesis section is summarized in bullets documenting the considerations (i.e., modified Bradford-Hill considerations) and formaldehyde-specific data that were most influential to the evidence synthesis judgment.
 - Evidence synthesis summary judgments are drawn and presented during evidence synthesis rather than only as a component of evidence integration.
 - Several synthesis sections not impacting the primary assessment conclusions (e.g., acute and short-term exposure impacts on pulmonary function; attempts to estimate a unit risk for myeloid leukemia) are now briefly summarized in the Toxicological Review, with the more detailed analyses moved to the Appendices.
- Evidence integration approaches and documentation have been revised as follows:
 - The evidence profile tables have been revised and now separately present the evidence-based factors that increased or decreased certainty within and across the evidence streams.
 - Terms including ‘other inferences’ and ‘relevant exposure circumstances’ (now “sufficient exposure conditions”) are more clearly defined and contextualized.
- The dose-response methods and documentation have also been significantly revised, including:
 - A table of more detailed methods for selecting studies for dose-response analysis has been added to the assessment methods (Section 2.7 in the revised Toxicological Review).
 - Individual tables documenting the rationale for advancing certain formaldehyde-specific studies for dose-response analysis (based on the methods laid out in Section 2.7) have been added for each health effect with a strong enough hazard judgment to support dose-response analysis in Section 5.
 - Modeling for many of the studies advanced for dose-response analysis has been re-performed or confirmed using updated methods (e.g., using the current version of BMDS).
- **Tier 2 Suggested Revision (Recommendation 2.2):** In updating the assessment in line with the Tier 1 Recommendation 2.1, EPA should further clarify the evidence review and conclusions for each health outcome by giving attention to the following:

Supplemental Information—Formaldehyde (Inhalation)

- [2.2 a] Using a common outline to structure the sections for each health outcome in order to provide a coherent organization that has a logical flow, by adding an overview paragraph to guide readers at the start of sections for each of the various health domains, and including hyperlinks to facilitate cross-walking among sections within the document;
- [2.2 b] Moving lengthy, not directly used information to an appendix;
- [2.2 c] Including a succinct executive summary in the Main Assessment; and
- [2.2 d] Performing careful review and technical editing of the documents for consistency across the multiple parts of the Draft Assessment, including across the Assessment Overview and Appendices. (The Assessment Overview could be entirely removed if the above recommendations were carried out.)

EPA Response to Tier 2 Suggested Revision 2.2: EPA has revised the assessment as follows:

- [2.2a] Summaries following a consistent format provide an overview for each section and include callouts to the relevant methods. The cancer sections are structured identically, based on considerations outlined in the EPA cancer guidelines ([U.S. EPA, 2005](#)). In addition, for both noncancer and cancer health effects, summary sections recapping the evidence synthesis judgments were added after each synthesis piece, based on each Hill consideration impactful to the judgments.
 - [2.2b] Several synthesis sections not impacting the primary assessment conclusions (e.g., acute, and short-term exposure impacts on pulmonary function; attempts to estimate a unit risk for myeloid leukemia) were moved to the Appendices and are now only briefly summarized in the Toxicological Review.
 - [2.2c] The Executive Summary at the beginning of the Toxicological Review provides a succinct summary of the key assessment conclusions and their basis.
 - [2.2d] The documents have been edited for clarity and consistency. The Assessment Overview, which was not intended as a separate document of EPA analyses but rather as a summary of the main messages from the analyses presented in the Toxicological Review, has not been retained as part of the revised assessment.
-
- **Un-Tiered, related peer review comments (Appendix E):** Several clarifications and corrections on this topic were noted in Appendix E, including:
 - [a] “In some cases, Tables and Figures included in the Appendices are not cited in other documents (e.g., Figures A-24 to A-26).”
 - [b] “As an example of inconsistencies that EPA needs to address, Figure A-36 shows 20 human studies and 35 animal studies for inclusion. Table A-93, which summarizes the animal data for developmental and reproductive toxicity and animal studies, contains

29 rows, with one row containing two studies (Vosoughi et al., 2012, 2013), resulting in 30 total animal studies (not 35 as stated in Figure A-36)."

- [c] "EPA needs to document whether the evidence integration summary tables in the Main Assessment and Assessment Overview are identical."
- [d] "Figure 2-3 needs to be modified to more accurately show representative ranges of outdoor and indoor formaldehyde concentrations."

EPA Response to Un-Tiered Comment: The following changes were incorporated.

- [a] EPA has reviewed the Tables and Figures to ensure they are called out in the text.
- [b] EPA has reviewed and clarified a number of inconsistencies, particularly related to counts of identified literature.
- [c] The Assessment Overview has not been retained in the revised assessment.
- [d] The figure referenced (in Section 5.1.5 of the revised Toxicological Review) has been revised and no longer presents estimates of ranges of outdoor and indoor formaldehyde concentrations. A summary of estimates of the median levels of formaldehyde in homes and outdoor air has been added to the text for context.

Related Public Comments

- **Public Comment (University of California San Francisco):** "'EPA did not clearly develop a pre-published protocol. EPA, in its Charge Questions to the Peer Review Committee, stated that 'The IRIS Program decided to conduct a reassessment of formaldehyde inhalation from scratch on the basis of that review, using transparent and predefined systematic review methods.' We are deeply concerned that we were unable to find an IRIS Assessment Plan or Protocol released in advance of the assessment for peer review comment. Although there were myriad delays of this document, it is troubling that such a protocol may not have been released, which violates the IRIS process for conducting assessments and the stated intent to used 'transparent and predefined systematic review methods.' We have commented extensively on the importance of pre-defined protocols, which are foundational to such assessments and play an important role in reducing bias and ensuring transparency.'"
- **Public Comment (American Chemistry Council):** "In developing the 2022 Draft Assessment, EPA failed to fully implement this first step. EPA never released an IRIS assessment plan, which would have included scoping and problem formulation materials. Rather, on its website it states that this step is "not applicable" without explaining why this step is not applicable. By not following the IRIS process, EPA denied the public multiple opportunities to provide valuable input into key formative elements of the 2022 Draft Assessment. Consequently, the 2022 Draft Assessment was not based on a fully developed record of available information."

Supplemental Information—Formaldehyde (Inhalation)

“The 2022 Draft Assessment does not discuss scoping or problem formulation activities for the “newly developed” assessment...Consistent with the recommendations from the NASEM (2011) review of the 2010 Draft Assessment, EPA should have engaged in an explicit scoping and problem formulation process to specifically address best available evidence-based health outcomes in the context of mode of action.”

“...The 2022 draft Formaldehyde Review contains no discussion of problem formulation activities or an Assessment Plan. There is very little discussion of how EPA arrived at the Populations, Exposures, Comparators, and Outcomes (PECO) statements, and no discussion of EPA's hypotheses regarding critical endpoints for formaldehyde assessment. To meet the NASEM recommendations, EPA should have organized the draft Formaldehyde Review around plausible MOAs and/or hypotheses regarding the most critical formaldehyde effects. At a minimum, EPA should revise the draft to include discussion regarding problem formulation and scoping activities that occurred in 2012 and again in 2021, particularly considering the many changes to the IRIS Program in the 10 years between initiation of the assessment and its release.”

“For example, EPA has not planned, issued, nor taken comment on a systematic review protocol for formaldehyde, unlike the other sixteen chemicals to be assessed according to the Agency's February 2022 IRIS Program Outlook. However, the Feb. 8 EPA letter claims that “[t]he systematic review methods used in the current draft IRIS formaldehyde assessment formed the basis for the methods presented in the IRIS Handbook, which was favorably reviewed by the NASEM in November 2021.” While subsequent process-oriented reviews may offer context to the panel reviewing the 2022 IRIS assessment, they do not resolve NASEM critiques of the previous IRIS assessment of formaldehyde.” *[Please see docket for details and supporting information.]*

EPA Response (to both public comments above): NAS (2011) understood implementing SR methods would take years and made it clear that EPA should not wait until SR is fully implemented to produce IRIS assessments. With respect to formaldehyde specifically, “The committee recognizes that revision of the approach will involve an extensive effort by EPA staff and others, and it is not recommending that EPA delay the revision of the formaldehyde assessment to implement a new approach.” SR enhancements such as releasing IRIS Assessment Plans (IAPs) and protocols, are a relatively recent activities (begun in 2017 and 2018, respectively). The current formaldehyde draft was in progress well before this timeframe. While EPA did not publicly release a protocol prior to initiating the current draft, the methods previously described in the Preface (now in a standalone “Assessment Methods” section) and used to develop the draft are consistent with the current SR methods outlined in the IRIS Handbook, which has been peer-reviewed by NASEM. Further, the key science issues and chemical-specific considerations that IRIS assessment protocols describe were detailed in NAS's (2011) report, which directly informed the current draft. EPA Note: Some

recommendations attributed to NAS (2011) in the public comments above (e.g., on a process for scoping and problem formulation) could not be confirmed after consulting the 2011 report.

Evidence Identification (Steps 1 and 2)

Peer Review Findings and Comments

- **Finding (5):** EPA’s literature search strategies for the 2022 Draft Assessment are adequate and consistent with the state of practice at the time. EPA appears to have sufficiently harmonized the methods for the pre- and post-2016 literature searches that were conducted using two different methods and were consistent with the state of practice at the time. Although the search strategies are adequately documented, the origins of the various PECO statements are less clear. In particular, across noncancer outcomes, the rationale for study exclusion on the basis of the populations, exposures, and outcomes is not well documented. For the literature searches, EPA used four databases, curated reference lists in published reviews, and “other national or international health assessments of formaldehyde,” consistent with prior NASEM guidance (See Appendix C [case study: Hanrahan et al., 1984, human sensory irritation]). As an example, see the literature search documentation for sensory irritation (pp. A-231–232).
- **Tier 2 Suggested Revision (Recommendation 2.3):** EPA should expand the text explaining the choices of the elements of the PECO statements.

EPA Response to Tier 2 Suggested Revision 2.3: As noted previously, the PECO criteria for each search were clarified and now have a consistent presentation in the assessment methods (Section 2.2 of the revised Toxicological Review), with explanations provided for many of the specific inclusion/exclusion criteria. The language used to describe specific inclusion or exclusion criteria has been standardized across health effects by study type (e.g., epidemiology studies).

Related Public Comments

- **Public Comment (American Forest and Paper Association):** “We wanted to address the Agency to the fact that two of our Formaldehyde (FA) manuscripts were not cited in the Review (Golden 2011, Golden and Holm 2017). It is important to note that these manuscripts report on critical aspects in the FA literature that have not been addressed in the Review. Including this information would supply useful perspective on the complex literature regarding the relationship between FA and asthma and sensory irritation and lead the Agency to different conclusions. EPA also does not include or discuss other publications that deserve more careful consideration. Our examination of the Review shows the following:
 - EPA relies on two papers to derive a Point of Departure (POD) for asthma that show no positive association with the disease
 - EPA failed to review and incorporate critical and relevant literature

Supplemental Information—Formaldehyde (Inhalation)

- EPA failed to discuss studies accurately and transparently
- These failures have led to the lack of a proper Weight of Evidence integration using the Best Available Science
- EPA is overly conservative in its use of uncertainty factors leading to ‘acceptable concentrations’ that are far below other recent regulatory evaluations of FA that use a practical Weight of Evidence approach
- This conservatism has led to EPA-derived ‘acceptable’ concentrations that exist in virtually no locations on earth.”

EPA Response: Both manuscripts noted by the commenter are reviews and tagged to the project for this assessment as supplemental materials. Reviews and other documents without primary data are typically tagged as such [see IRIS Handbook; ([U.S. EPA, 2022](#))]. Separately, regarding the first bullet in the comment, a NOAEL is an example of a POD that can be based on a study that does not show an effect for a hazard identified based on the available evidence base as a whole. The other bulleted comments are not specific to the content of the assessment and thus no response is provided. However, comments elsewhere in this Appendix that contain content specific to this assessment may include EPA responses relevant to the intent of this public comment.

- **Public Comment (ToxStrategies):** “U.S. EPA’s literature search and/or review of their literature search results incorrectly filtered out relevant MOA evaluations that the agency should have considered.”

EPA Response: As previously described, reviews, opinions, and other articles without primary data were tracked as supplemental materials in HERO. While such materials can help to identify relevant evidence and hypotheses, the IRIS Program develops its own MOA evaluations, consistent with EPA guidelines.

- **Public Comment (Anonymous):** “Attached are several recent articles and reviews which may be relevant to EPA and NASEM reviewers, including several peer-reviewed pieces published since 2000 which do not appear in the main text or appendices of the draft assessment. [Hartwig et al. 2020; Chang et al. 2021; Protano et al. 2021; Agathokleous and Calabrese 2021]”
[Please see docket for details and supporting information.]

EPA Response: None of the articles above contain primary data. As previously described, studies with no primary data are considered as supplemental materials and tracked in HERO. The articles above are tagged as such in the formaldehyde HERO page.

- **Public Comment (Natural Resources Defense Council):** "It is unclear what EPA used for its study inclusion and exclusion criteria. For example, NIEHS comments expressed concern that EPA's literature search was inadequately broad, and unjustifiably limited. NIEHS noted that, "the limited set of search terms is unlikely to capture all animal studies of hypersensitivity" and may exclude other important studies. Moreover, the public is unable to fully access the Systemic Evidence Maps, which are crucial to convey critical information such as how the study inclusion and exclusion criteria were applied to particular studies, and how that may limit the data set used for the evaluation. NIEHS identified this concern as "Tier 1 – Necessary Revision", which NRDC agrees with."

EPA Response: Please note that the systematic evidence map (SEM) used to update the literature from 2016-2021 was included in Appendix F of the external review draft, not as a separately released file. In the current assessment, the SEM has been integrated into the Assessment Methods section (Section 2.2), the Appendix documentation of literature searches (Appendix B.2), and the formaldehyde HERO page (link above). Regarding the other NIEHS comment on literature search terms, some of the animal studies of hypersensitivity were identified through the immune conditions-specific search and others were identified through a separate search focusing on mechanistic information relevant to immunological and inflammatory changes. These studies were ultimately judged by EPA as informative of mechanism for the purposes of this assessment and are discussed in detail in Appendix C.7. No "missed" studies on hypersensitivity were identified by peer-reviewers or public commenters.

- **Public Comment (Troy Corporation):** "...While there is an extensive section of text denoting criterion for assessment study selection it is not clear why certain studies were chosen for a particular toxicological endpoint."
"In addition, the assessment literature review did not capture many of the new more recent scientific studies industry has prepared since the last IRIS assessment. Since 2010, there has been a continual effort by industry and academia alike to provide additional data on key formaldehyde inhalation toxicological endpoints. This new data adds to an already extensive data set on this compound. These new studies include the reanalysis of epidemiology studies, examination of the effects of endogenous versus exogenous formaldehyde and portal of entry effects, examination of formaldehyde exposure to asthma, mode of action studies and sub-chronic animal inhalation studies. In total approximately 73 additional studies have been conducted and based on the current assessment, have not been incorporated into the current document."

EPA Response: The criteria for inclusion and exclusion of studies and effects for each hazard synthesis are described in the Assessment Methods (Section 2.2 of the revised assessment).

Specifically, regarding the “73” studies noted by the commenter, these overlap with 76 studies submitted by the American Chemistry Council in a letter to EPA in June 2022. Of these 76 studies, 7 met the assessment PECO criteria. The 69 remaining studies contained no primary data, or were out of scope (e.g., no health-related information). Of the seven studies that meet the assessment PECO criteria, all seven were/are cited.

As previously described, reviews and studies with no primary data are considered as supplemental materials and tracked in HERO. Such studies are often not cited in IRIS assessments.

- **Public Comment (United States Chamber of Commerce):** “Moreover, over the last decade, more than 40 peer reviewed studies have demonstrated safe thresholds for formaldehyde, including important methodologies published in recent weeks, award-winning re-analysis of key data, and several lines of evidence demonstrating the safe use of formaldehyde for EPA regulatory purposes. For example, ‘A Review of Stable Isotope Labeling and Mass Spectrometry Methods to Distinguish Exogenous from Endogenous DNA Adducts and Improve Dose–Response Assessments’ published by the American Chemical Society in December 2021. Unfortunately, many of these high-quality studies and reviews are ignored, briefly dismissed, or misused by EPA in the draft assessment (as noted in comments filed by the Small Business Administration).”

EPA Response: EPA conducts an independent analysis of the studies meeting PECO criteria that contain primary data. Reviews and other studies with no primary data (all of the 40+ papers noted by the commenter) are considered as supplemental materials and tracked in HERO. EPA has considered in detail many of the cited supplemental studies and discussed a subset of those in the assessment, including support for the conclusion regarding lack of evidence for remote distribution of exogenous formaldehyde. The adduct data and the experiments based on the stable isotope labeling and mass spectrometry (SILMS) method alluded to above, including Lai et al., 2016; Lu et al., 2010, 2011; Moeller et al., 2011; and Yu et al., 2015, were crucial to supporting EPA’s conclusions in Section 3.1 (Toxicokinetics). EPA extensively evaluated (in Section 3.1.1 and Appendix C.1.12) the models in papers by Schroeter et al., 2014 and Campbell et al., 2020 which accounted quantitatively for the influence of endogenous formaldehyde on exogenous formaldehyde uptake. The NASEM consensus report (see section on “EPA’s use of toxicokinetic data and dosimetry models” in that report) found EPA’s evaluation and use of these models thorough and appropriate.

- **Public Comment (American Chemistry Council):** “There are numerous studies that should have been evaluated in the Draft Assessment, but were not cited or referenced. EPA has excluded or dismissed a number of key studies, reviews, responses, and presentations, with a

majority having been presented in correspondence and presentations by the ACC Formaldehyde Panel to the Agency since 2011.”

“Routinely over the last decade, the Panel has met with and provided IRIS staff information on new scientific findings and publications related to the deficiencies identified in the NASEM review of the 2010 Draft IRIS Assessment of Formaldehyde. It is therefore unclear why many of these studies have not been included in the 2022 Draft Assessment after such longstanding outreach and communication with EPA on the part of the Panel. A representative sample of the engagements and communications with the EPA have been provided as attachments to this submission.”

“The 2022 Draft Assessment does not respond to NASEM’s recommendation to clearly document its methods for study selection...As noted, systematic review methods have developed substantively since the NASEM 2011 review. The IRIS Handbook includes a highly detailed and structured process for literature search and selection. However, the process for inclusion and exclusion of studies from IRIS assessments, including formaldehyde, diverges from current best practices. For example, there are numerous places in the systematic review when EPA can choose to exclude studies without justification, including after refinement of the study plan or organization of the hazard assessment.”

“The 2022 draft Formaldehyde Review is not clear on the methods for study selection, and in some cases appears to deviate from best practices for systematic review, particularly for studies published between 2016 and 2021. Specifically, for the 2016-2021 studies, after comparing these studies to the PECO criteria, EPA assessed whether studies were “potentially impactful” - a subjective process to further narrow the body of evidence. EPA states this process relies on “expert judgment by two reviewers.” Particularly concerning is that aspects of study quality assessment are intertwined in these criteria - for example, the criteria pertaining to animal studies with multiple dose levels. While multiple doses are preferred to evaluate dose-response relationships in animal toxicity studies, some single-dose studies may still be informative for critical endpoints or MOAs and therefore should not be excluded before full evaluation. Additionally, criteria related to selecting relevant mechanistic data are vague, with no guidance other than inclusion of only those “most directly related to the mechanistic uncertainties identified in the 2017 draft.” The subjective selection of recently published studies is apparent in the failure to include numerous critical re-analyses and MOA assessments, as detailed in the comments submitted by ACC’s Formaldehyde Panel.”

“The systematic review of literature contained in the Draft Assessment was inadequate. The Draft Assessment itself verifies that the systematic review was bifurcated and rushed, and that simple literature searches were performed pre-2017 and post-2017...The above indicates that, against EPA policy, a full systematic review of the literature was not conducted before release of the Draft Assessment for peer-review. Also, inadequate, and incomplete science cannot be

justified by a desire to “expedite” an update to a 12-year-old draft document. This deficiency must be addressed before any further revisions are initiated.”

“The criteria for inclusion/exclusion of studies published after 2017 are unclear. The Draft Assessment gives the appearance of subjectively excluding evaluation of any studies that did not impact conclusions made before 2017, in confidential internal review drafts that remain unavailable to the public and were not externally peer-reviewed.” *[Please see docket for details and supporting information.]*

EPA Response: Please see EPA responses above on this topic, including EPA’s response to public comments on the systematic review methods utilized in the formaldehyde assessment versus those presented in the IRIS Handbook (Approach to Documentation of Methods, above in Section F.1.3), which are also pertinent here. As previously described, the HERO database was used to document study screening decisions. And, as above, reviews and commentaries without primary data, including re-analyses of MOA assessments, are considered as supplemental information.

Study Evaluation (Steps 3 and 4)

Peer Review Findings and Comments

- **Finding (6):** EPA provides overall and outcome-specific evaluation criteria that are generally consistent with the common domains for risk-of-bias analysis and their application in practice. However, the criteria are found in several different locations in the documents and in some cases are inconsistently presented and integrated across the documents. As a result, the committee was challenged to reconstruct the study evaluation approach and how the criteria were applied for study evaluation.
- **Finding (7):** The considerations listed for classification of study confidence and evaluation of each study by at least two independent experts are adequate. However, the committee’s case studies (see Box 2-1 and Appendix C) revealed inconsistencies in how evaluation criteria were described and applied in EPA’s evaluation of human and animal studies.
- **Tier 2 Suggested Revision (Recommendation 2.4):** EPA should thoroughly review the Draft Assessment documents to address issues of consistency and coherence so as to ensure that its methods can be applied and replicated with fidelity. The reviews for each outcome in Chapters 4 [noncancer health effects] and 5 [cancer] provide more specific guidance.
 - *Related committee comments* (from p. 33, noting that similar comments were provided elsewhere related to laying out 'criteria' for confidence selection for noncancer effects): "For cancer outcomes, the risk-of-bias domains are described generally for each outcome, but there is no clear description of how judgments were made concerning the classification of studies as having high, medium, or low confidence or not being

informative for each risk-of-bias domain." ... "Comparable tables for different outcomes may not have sufficiently parallel descriptions." AND "These tables have two columns for exposure and for study design and analysis (see, e.g., Table A-33). The descriptions in the table cells sometimes mention risk-of-bias domains described elsewhere in the document. However, the documentation is not specific to each domain as a heading in the tables (because there are only two table headings rather than six for the high, medium, low, not informative classification)."

- *Related committee comments:* "For pulmonary function, while the study evaluation criteria provided in Table A-43 appear to be appropriate, they do not overlap with the methodologic issues mentioned in the text."
- *Related committee comments:* "Appendix A document presents a table (Table A-43) of criteria used to categorize study confidence for epidemiological studies as high, medium, low, and not informative. This table considers two aspects: (1) exposure, and (2) study design and analysis. Neither the Main Assessment nor the Assessment Overview references Table A-43."

EPA Response to Tier 2 Suggested Revision 2.4: Tables have been added to the assessment methods clarifying considerations for each study evaluation domain applied in the context of each health outcome (Section 2.3 of the revised Toxicological Review). The narrative text and tabular content have been reviewed for consistency and updated. The table noted above is now referenced in the assessment.

In making these criteria more explicit and rechecking their consistent application across studies, several changes were made in confidence characterizations of individual studies or of specific analyses or outcomes within studies, although none of these changes affected the evidence synthesis or integration judgments.

Changes in Sensory Irritation study evaluations:

- [Alexandersson and Hedenstierna \(1989\)](#) was changed from *low* to *medium* because a potential selection for healthy workers and attenuation of the measure of association was the only limitation of significance identified.
- [Berglund et al. \(2012\)](#) was changed from *high* to *medium* because measures to blind participants to exposure was not described and an uncommon endpoint measurement was used.

Changes in Allergy and Asthma study evaluations:

- [Branco et al. \(2020a\)](#) was previously classified as *low* confidence; this was changed to *medium* for the dichotomized analysis but kept at *low* for the continuous (per IQR). This change was made because the concern regarding the analytic strategy

that did not take into account the shape of the dose-response was more pronounced for the continuous variable than for the dichotomized variable.

- [Huang et al. \(2017\)](#) was previously classified as *low* confidence; this was changed to *medium* for the rhinitis analysis but kept at *low* for the eczema and asthma analyses. This change was made based on the greater uncertainty regarding validation of truncated version of ISAAC questionnaire for the latter two outcomes compared to the former; the rhinitis questions were similar to those used in other studies, whereas the eczema and asthma questions were more limited.
- [Mi et al. \(2006\)](#) was changed from *medium* to *low* confidence, consistent with other studies with a limited (4 hour) exposure measurement period.
- [Raaschou-Nielsen et al. \(2010\)](#) was changed from *low* to *medium* confidence because EPA to be consistent with other studies in which limitations which were expected to lead to a reduced (attenuated) effect estimate.
- [Li et al. \(2019\)](#) and [Yu et al. \(2017\)](#) were previously classified as two separate *low* confidence studies, based primarily on reporting deficiencies in each. EPA's re-review that the 2019 is essentially a replication of the 2017 study protocol using a later birth cohort. The missing information in each paper was found in this other paper, although the study authors did not respond to several requests from EPA for confirmation regarding the exposure measurement period and exposure levels for each study cohort. Based on this information, EPA present these as a single *medium* confidence study.

Changes in Nervous Systems Effects evaluations:

- The review resulted in changing the confidence conclusions for two of the studies based on the criteria for exposure assessment ([Bellavia et al., 2021](#)) (*medium* to *low*) and other limitations including small number ALS deaths ([Pinkerton et al., 2013](#)) (*high* to *medium* confidence).
- In ensuring consistency in evaluations across studies of a particular type (e.g., in vivo experimental animal studies), three *in vivo* studies examining mechanistic changes received a revised rating (*high* to *medium* confidence) Sorg, 2001, 626595, Tsukahara, 2006, 626805, and Hayashi, 2004, 626503

Related Public Comments

- **Public Comment (American Chemistry Council):** "The Draft Assessment often relies on animal studies performed with novel and unvalidated test methods to reach conclusions. As an example, as described further in these peer-review comments, each of the animal studies relied upon for developing organ specific reference concentrations (osRfCs) in the Draft Assessment, relied on novel methods, such as measuring seminiferous tubule diameter, without providing

any hematoxylin and eosin (H&E) stained tissue section histopathological evaluation by a trained pathologist, or measuring metal concentrations in lungs or testes without even providing critical information such as body weights, food consumption, hematology, clinical signs, sufficient number of animals for statistical evaluation, etc. as is expected of any well conducted toxicology study using validated endpoints for assessing toxicity.”

“A critically deficient evaluation domain for the assessment of formaldehyde is methanol co-exposure. Any study (epidemiological or animal) with methanol co-exposure should generally be classified as uninformative rather than “low confidence” when related to systemic effects. The Draft Assessment overstates in their conclusions “...the evidence indicates that inhalation of formaldehyde likely causes increased risk of xxx” for studies that were confounded by methanol.”

“Epidemiology studies without any analytical sampling should have been excluded from the determination of hazard and/or dose-response...Below is an example of an epidemiology study, Taskinen et al. (1999), that was graded with medium confidence. This paper should not have been carried forward due to multiple deficiencies, including a lack of analytical data (Table 1-58, p. 1-415)”

“The study quality assessment framework used in the 2022 draft appears different from that described in the Handbook, allows for significant reviewer subjectivity, and ultimately is unclear on how confidence classifications were determined. The depiction of EPA's evaluation of study confidence process (see Figure II) is convoluted, and the study evaluation tables (e.g., Appendix Tables A-105 and A-106) that summarize results of individual studies are difficult to read and interpret.”

“Further, the EPA approach to study quality assessment focuses on three areas: (1) reporting quality, (2) risk of bias, and (3) study sensitivity, but primarily emphasizes risk of bias. Certain categories are more important to judging study quality than others. For example, exposure characterization is a critical evaluation domain for epidemiological studies, including the formaldehyde literature, which is plagued by studies with poor surrogates for exposure (e.g., studies relying on next of kin reporting embalming practices of deceased workers). The 2022 draft includes a lettered “grading” system for exposure assessment; this system, which is poorly described, does not appear in the Handbook or in any other agency study evaluation framework. Additionally, risk of bias is only one facet of internal validity (bias reflects a systematic error only, while internal validity is also impacted by other non-systematic errors).”

“Overall, EPA does not sufficiently address the NASEM recommendations relating to evaluating the quality and reliability of individual studies and must improve this in their general approach to study quality evaluation. EPA should revisit its framework for evaluating exposure assessment in epidemiological studies of formaldehyde exposure and revise its process for evaluating mechanistic information to ensure it is a uniform and reproducible methodology across endpoints. EPA should consider aligning with the exposure

characterization domain within the data quality evaluation framework developed for Toxic Substances Control Act (TSCA) risk evaluations.

"The 2022 Draft Assessment conclusions are speculative and lack scientific confidence because EPA applied a flawed and subjective study evaluation method, especially in the analysis and interpretation of the epidemiology data...While EPA has made progress in this area, the study evaluations presented in the 2022 Draft Assessment need improvement and reflect two distinct but related issues: 1) several important deficiencies remain in the evaluation framework provided in the IRIS Handbook and 2) the 2022 Draft Assessment fails to follow the IRIS Handbook."

"The 2022 draft incorporates a defined methodology and detailed discussions of mechanistic and MOA information for some endpoints, but not others. The Supplemental Information details a set of criteria for judging the strength of the evidence for mechanistic events associated with non-cancer respiratory effects (see Table A-64). The draft also provides evidence tables summarizing general study characteristics and findings for mechanistic studies of respiratory effects. The 'utility and notes' column provides the overall confidence rating for that study; however, details on the rating are provided only for some low confidence studies." "EPA's analysis and interpretation of epidemiology data in the 2022 Draft Assessment is flawed. Not only does the study evaluation framework deviate from that required in the IRIS Handbook, but also EPA does not clearly communicate the results of its study evaluation. EPA also fails to consistently apply its own IRIS rating system for the robustness of exposure categorization, fails to thoroughly consider exposure latency, implements a highly subjective methodology for evaluating study reporting, biases, and sensitivity, and selectively interprets studies according to particular exposure metrics. All of these oversights lead to a lack of confidence in EPA's conclusions. Specific issues are described in detail below." *[Please see docket for details and supporting information.]*

EPA Response: Confidence classifications and the supporting rationale (emphasizing limitations that reduce confidence) are provided for approximately 1,000 individual studies in the IRIS formaldehyde assessment. The overall goal of these study evaluations was to evaluate the extent to which the study results are likely to represent a reliable, sensitive, and informative presentation of a true response. The study evaluation approach and evaluation criteria are presented in Section 2.3 of the Toxicological Review (and in the Preface of the external review draft). The study evaluation judgments for each study are documented in Appendix B.3 (and in Appendix A of the external review draft), and limitations identified in Appendix B.3 that could substantively change a result presented in the study, or the interpretation of that result, are highlighted within each respective hazard synthesis (Sections 3.2 and 3.3). Consideration of factors noted by the commentor are included in the study evaluation process, including a primary focus of EPA's evaluation on exposure assessment (e.g., see Section 2.3.9 for methods for rating exposure in the cancer epidemiology

studies, which was specifically raised by the commentor). While study evaluations have been refined for epidemiological studies in the IRIS Handbook compared to methods used in the formaldehyde assessment, the core considerations are similar. This is not surprising as the methods used in the formaldehyde assessment shaped the methods presented in the IRIS Handbook.

The NASEM committee was supportive of EPA's study evaluation approach and even conducted case studies to evaluate consistency of how studies were considered. The committee did not have any Tier 1 recommendations on this topic. EPA has revised the document to address the committee's findings and Tier 2 commendations (see above). EPA's response to public comments on the systematic review methods utilized in the formaldehyde assessment versus those presented in the IRIS Handbook are discussed above (Approach to Documentation of Methods) and are also pertinent here. Please note that TSCA does not have a finalized data quality evaluation framework.

Evidence Synthesis (Steps 5 and 6)

Peer Review Findings and Comments

- **Finding (8):** In general, the strength-of-evidence categories are appropriate for the human and animal evidence streams and consistent with the state of practice.

Related Public Comments

No related public comments were submitted.

Evidence Integration (Step 7)

Peer Review Findings and Comments

- **Finding (9):** In general, the IRIS Program's approach to evidence integration is appropriate and grounded in methods used by EPA and many other entities for reaching causal conclusions. Expert judgment is involved in this phase of the assessment, and the IRIS Program appears to draw on appropriate multidisciplinary groups to evaluate the evidence.
- **Finding (10):** The committee identified aspects of the process for further consideration and reformulation. First, the IRIS Program's schema blurs the distinction between evidence synthesis and integration, combining them into a single step, as exemplified by the summary tables for each health outcome that include both "evidence judgment" and "hazard determination." (e.g., Table 1-4). A clear distinction between synthesis and assessment of the overall strength of evidence would be preferable conceptually and more consistent with the state of practice. [goes on to indicate the following: "expanded [integration] narrative descriptions would be useful for documenting the rationale for the classifications made. The tables are useful but terse, as is the accompanying text... The terms used—demonstrates, indicates, suggests, or is inadequate—are aligned with the IRIS Handbook, but are not obviously

hierarchical...[and] also are used inappropriately, as in the inherent proposition that it is the evidence itself that “demonstrates” or “indicates.” The use of these terms represents an unnecessary source of inconsistency with the state of practice.”]

- **Tier 2 Suggested Revision (Recommendation 2.5):**

- [2.5 a]: The assessment should be edited to more sharply demarcate the synthesis and the integration of evidence discussions.
- [2.5 b]: EPA should expand the narrative descriptions of the evidence integration step, or should follow published methodology while providing detailed explanation of any adaptations.

EPA Response to Tier 2 Suggested Revision 2.5:

EPA appreciates the NASEM committee findings on the evidence integration conclusions of “demonstrates” and “indicates” as not being clearly hierarchical. However, these phrases are used in the IRIS Handbook ([U.S. EPA, 2022](#)), which underwent Agency review, Interagency review, and public NASEM peer review in 2021. That said, the IRIS Program understands there may be potential confusion and defines these terms in the IRIS Handbook, along with descriptions of patterns of findings across evidence that lead to different evidence integration judgments. The definitions are also presented in the formaldehyde assessment (Section 2.6).

- 2.5a: In the revised assessment, synthesis judgments are more clearly drawn prior to and separately from the evidence integration judgments. Summary sections after each evidence synthesis section have been added to better demarcate the synthesis judgments and the supporting rationale. These sections also use bullets to describe factors (e.g., consistency) influencing synthesis judgments and include the synthesis judgment. In addition, corresponding edits were made to the description of the evidence synthesis and integration framework methods.
- 2.5b: In the revised assessment, the evidence integration step follows the methodology published in the IRIS Handbook, with revised documentation consistent with the application of that approach in the evidence profile tables that support each evidence integration narrative.

Related Public Comments

No related public comments were submitted.

Use of Mechanistic Evidence

Peer Review Findings and Comments

- **Finding (11):** Overall, the committee found that EPA has been thorough and transparent in identifying the scientific literature with regard to mechanistic evidence. However, the definition of “impactfulness” and how this concept was applied are not well described. Similarly, the term “other inferences” is used in the sections on integration of cancer evidence, but the term is not explained.
- **Tier 2 Suggested Revision (Recommendation 2.6):** To increase the transparency of the evaluation of mechanistic data, EPA should clarify key terms (e.g., “impactfulness,” “other inferences”) and their application to specific studies. “Impactfulness” can be defined (in Table F-12 and elsewhere), and “other inferences” can be explained in discussing the approach to evidence integration in the “Preface on Assessment Methods and Organization.”

EPA Response to Tier 2 Suggested Revision 2.6:

- The “other inferences” are now better defined in the assessment methods (Section 2) and the specific inferences relevant to each health effect-specific decision more clearly documented during evidence integration.
- The definition for “impactfulness” (which primarily applies to the SEM literature update) has been revised for clarity (see Section 2 of the revised Toxicological Review).

Related Public Comments

- **Public Comment (Natural Resource Defense Council):** “Regarding the assessment of the weight of evidence (i.e., evidence integration), NRDC strongly supports EPA's reasoned approach regarding mechanistic information, stating that mechanistic events and associations are preferred, but that, “the lack of mechanistic data explaining an association did not discount results from human or animal health effect studies” (p. 38). To that end, NRDC disagrees with the comment from ATSDR, arguing that the lack of mechanistic information should be treated not as a data gap, but as a “reason to down-grade the evidence findings.” NRDC supports EPA’s determination that, “Mechanistic understanding is not a prerequisite for judging the evidence, and thus absence of knowledge should not be used as a basis for decreasing strength NTP (2015); NRC (2014a). The human relevance of animal findings is assumed unless there is sufficient evidence to the contrary [see IARC (2006); U.S. EPA (2005a)].” (p. 40) This is also consistent with the IARC Preamble, updated January 2019 following a global process of public comment and scientific peer review. EPA could update the IARC reference in the text.”

EPA Response: No response needed as comment was supportive of the assessment, which is consistent with EPA guidelines.

- **Public Comment (American Chemistry Council):** "For the evaluation of MOA information, EPA notes that 'in general, studies relevant to mechanistic interpretations informing hazard identification were not individually evaluated.' However, the sections that follow indicate that some data were assessed for risk of bias and other domains, but with differing methods for each endpoint. For non-cancer respiratory mechanistic studies, EPA follows the general Handbook framework for rating these studies as low, medium, or high confidence. For mechanistic studies of non-cancer extra-respiratory effects, such as circulating blood cells, however, it appears no confidence evaluations were conducted, despite the fact that some of these studies have been used to inform carcinogenic hazard in the LHP malignancies (see page A-554)."

"Finally, for epidemiological studies of genotoxic endpoints, a third process was used, which is summarized as follows: '...an overall conclusion of "no obvious bias" was used if no concerns were identified. for studies with a potential bias identified, the potential bias or issue was summarized in the comment row. For each assay (e.g., chromosomal aberrations, CBMN, Comet assay), factors related to assay methods that could affect the endpoint values were identified using published reviews from collaborations that compared assay methods across epidemiological studies (Fenech, 2020; Moller et al., 2020; Bonassi et al., 2011; Fenech et al., 2011; Valverde and Rojas, 2009; Bonassi et al., 2005).' Interestingly, on page A-222, the mechanistic epidemiological study by Zhang et al., (2010), which has been all but refuted in a number of peer-reviewed publications (see, for example, Gentry et al., 2013; Mundt et al., 2017) is discussed only briefly by EPA; the comment column simply states, 'small sample numbers, no obvious bias.' The Gentry and Mundt papers are also cited, but the re-analyses presented in these studies are largely dismissed and not integrated with Zhang et al. (2010) or any of the other human studies evaluating markers of genotoxicity."

"The Draft Assessment relies on multiple assumptions [regarding MOA] rather than considering alternative viewpoints supported by data in the formaldehyde literature."

"Discussion of MOA is more often speculative than grounded in empirical demonstration."

"EPA misinterprets Recio et al. (1992) to support an implausible genotoxic mode-of-action for carcinogenicity."

"EPA provides no criteria for its process for considering the strength of the mechanistic studies for genotoxic findings. EPA should standardize its approach to considering mechanistic and MOA studies and apply it consistently across endpoints. Mechanistic and MOA data should not be relegated to 'supplemental information' but more fully integrated into the assessment beginning early in the IRIS process."

"The 2022 Draft Assessment does not incorporate best practice recommendations from NASEM and other agencies on the evaluation and integration of MOA information...Other systematic review frameworks recommend evaluating mechanistic information and information on potential MOAs with the same level of rigor as the other lines of evidence

(animal and human). WHO (2021) is particularly clear on this matter, stating: ‘Regarding mechanistic and pharmacokinetic data (two separate types of data), these are often described as being contextual or associated with sub-questions. However, if these data are anticipated to be critical in making determinations of hazard or dose– response, such data should be included in the review and subjected to the same appraisal and structured evaluation process applied for other data types (emphasis added).’ ...The 2022 Draft Assessment, however, does not appear to implement any of these best practices for the use of mechanistic studies and MOA information. Mechanistic studies and information supporting (or not supporting) MOA are largely relegated to “supplemental” information and considered only in the evidence integration phase in the context of their ability to inform hazard conclusions based on human and animal evidence.”

“The 2022 Draft Assessment does include more discussion of MOAs relative to the 2010 Draft Assessment. EPA acknowledges that inhaled formaldehyde is not distributed to an appreciable extent beyond the respiratory tract to distant sites. EPA, therefore, assumes inhaled formaldehyde acts via a different pathway. Although EPA looks at endpoints that could be key events in an MOA, EPA has not proposed a biologically plausible MOA...In fact, the postulated MOA is lacking any experimental data to support EPA’s conclusion that inhaled formaldehyde that EPA acknowledges is not distributed to sites beyond the portal of entry and causes cancer.” “The 2022 Draft Assessment’s treatment of MOA and mechanistic data renders it inappropriate for regulatory use...EPA’s dismissal of the importance of MOA and mechanistic information is inconsistent with the Agency’s own approach to human health hazard assessment for a given chemical under the Toxic Substances Control Act (TSCA), one of the stated objectives for the 2022 Draft Assessment. EPA’s guidelines governing application of systematic review in TSCA risk evaluations characterizes the ‘the availability of a fully elucidated mode of action (MOA) or adverse outcome pathway (AOP)’ as ‘highly preferred.’ These guidelines argue that ‘[m]echanistic evidence may provide support for biological plausibility and help explain differences in tissue sensitivity, species, gender, life-stage or other factors,’ emphasizing that ‘EPA/OPPT plans to prioritize the evaluation of mechanistic evidence instead of evaluating all of the identified evidence upfront. This approach has the advantage of conducting a focused review of those mechanistic studies that are most relevant to the hazards under evaluation.’”
[Please see docket for details and supporting information.]

EPA Response: A large part of the document (spread out over many sections of the Toxicological Review and Appendices) is devoted to bringing all the mechanistic information to bear on evaluating MOA. Section 2.3 describes the methods for evaluating the mechanistic evidence and Appendix B.3 documents the results of individual mechanistic study evaluations. Sections 2.5 and 2.6 provide the framework for EPA’s synthesis of this evidence and detail how the mechanistic evidence informs the overall evidence integration decisions, respectively.

Specifically regarding cancer MOA, Section 3.2.5 discusses the evaluation of the respiratory cancer MOA in the context of EPA's guidelines and Section 3.3.3 outlines and evaluates the mechanistic evidence relevant to LHP cancers. Relevant to these interpretations Section 2.3.6 details the methods used to evaluate individual mechanistic studies most relevant to immune-, inflammatory-, or tissue injury-related changes, while Section 2.3.10 describes the methods used to evaluate the genotoxicity studies (the results of these evaluations are documented in Appendix B.3.6 and Appendix C.3, respectively). Also note the analyses probing the temporal- and dose-dependence of the two primary MOAs contributing to nasal cancer development in Section 3.2.5 and Appendix D.2., as discussed in other EPA responses below, and the use of nonlinear approaches (Section 5.2.1) to addressing one MOA contributing to upper respiratory tract cancers.

As noted above the NASEM committee found that "... EPA has been thorough and transparent in identifying the scientific literature with regard to mechanistic evidence." The committee did not have any Tier 1 recommendations on this topic and EPA has revised the document to address the committee's Tier 2 comments. It is noted that the IRIS Program's approach, and the approach used in this assessment, for consideration of mechanistic evidence is consistent with approaches being implemented by other health agencies and EPA programs (i.e., prioritizing mechanistic evidence instead of evaluating all of the identified evidence upfront). Although some revisions have been applied to the MOA syntheses in the revised assessment, the underlying approach was not changed.

- **Public Comment (United States Chamber of Commerce):** "...the SBA cautioned EPA against 'making definitive conclusions about causes based on evidence that only demonstrates association and without identifying a mode of action' while identifying nearly a dozen key studies excluded by EPA and repeatedly warning that EPA 'does not have a mode of action or mechanistic evidence' supporting its core conclusions.¹⁰"
"Additional interagency concerns were identified during internal review, such as the Agency for Toxic Substances and Disease Registry (ATSDR) statement that the lack of mechanistic data 'needs to be a reason to down-grade the evidence findings. It is significant.'"

EPA Response: EPA guidelines [e.g., ([U.S. EPA, 2005](#))] and the IRIS handbook methods ([U.S. EPA, 2022](#)) do not support downgrading confidence ratings in the evidence conclusions when there is a lack of mechanistic understanding. Further, the evidence integration approach applied in this assessment explicitly evaluates the support for causation based on application of adapted Hill considerations for causality. EPA Note: Interagency reviewer (e.g., ATSDR; SBA) comments were on a draft that was revised prior to public release. Please consult the full interagency review comments in the EPA docket.

- **Public Comment (ToxStrategies):** “The adoption of the KCC approach by the U.S. EPA indicates that risk assessment is, depending on one’s point of view, (d)evolving into an anti-hypothesis, anti-expert, anti-MOA approach of simply demonstrating that a chemical has certain characteristics in common with other carcinogens as opposed to understanding how a chemical causes a specific tumor. It is noteworthy that lead author on the KCC approach (Smith et al. 2016; Smith et al. 2020) is also a coauthor on epidemiological studies linking inhaled formaldehyde exposure to leukemia (Zhang et al. 2010; Zhang et al. 2009). Instead of using the KCC approach, the U.S. EPA should follow its own guidance for the risk assessment of carcinogens as described in U.S. EPA (2005).”

“Page 1-302 states that formaldehyde has been shown to be genotoxic or mutagenic in a variety on in silico and in vitro test systems. To what is EPA referring regarding in silico test systems? A search of the Toxicological Review for the term “silico” revealed no additional hits. A search of the Supplemental Information for the term “silico” revealed one hit in a citation for Yoo & Ito (2018a). Searching for “Yoo” revealed the study in Table F-12” Mechanistic studies relating to respiratory tract cancers, focusing on genotoxicity additional hits” where the study was listed as “not impactful.” As was discussed in Thompson et al. (2020), BBDR in silico modeling suggests that formaldehyde-induced nasal tumors in rats could be predicted with little or no direct mutagenic contribution from DNA-protein crosslinks as the nasal tumors could be explained by mutations accumulating from increased regenerative cell proliferation (Conolly et al. 2003).”

“Pertinent to the release of the US EPA’s External Review Draft of Toxicological Review of Formaldehyde(Inhalation) I was surprised to see that a 2020 review article I coauthored on the mode of action of formaldehyde-induced nasal tumors in Critical Reviews in Toxicology¹ was not cited anywhere in the 193-page Assessment Overview, 789-page Toxicological Review, or the 1058-page Supplemental Information documents². This omission by the U.S. EPA is unfortunate as there are critical data integration topics in the review article that might have informed U.S. EPA’s assessment. Moreover, Thompson et al. (2020) is an update of the mode of action (MOA) for formaldehyde-induced nasal tumors published by McGregor et al. (2006), which the U.S. EPA did cite in their assessment. As such, the omission of Thompson et al. (2020) is disappointing.”

“Below are comments relevant to the missing Thompson et al. (2020) article (and others), additional comments relevant to the MOA evaluation for formaldehyde-induced nasal tumors, as well as separate comments related to some of the noncancer evaluation in the U.S. EPA (2022) assessment. Broadly speaking, these comments highlight the following:

- Failure to integrate relevant science into the MOA for nasal tumors in rodents
- Failure to response appropriately to previous review by the National Research Council
- Failure to adhere to U.S. EPA MOA guidance and practices

Supplemental Information—Formaldehyde (Inhalation)

- Failure to consider nonlinear approaches for protecting against upper respiratory tract cancers
- Failure to use appropriate benchmark dose modeling software and practices
- Poor organization of the various assessment documents”

[Please see docket for details and supporting information.]

EPA Response: EPA did not adopt the 10 key characteristic of carcinogens (KCCs) approach for evaluating carcinogenicity. EPA uses this as a tool to organize the available mechanistic information but relies on the EPA cancer guidelines MOA framework when conducting the analyses. The 2021 NASEM peer review report on the IRIS Handbook was supportive of using key characteristics as an organizational construct for mechanistic evidence, including for carcinogenicity. With respect to publications not cited in the assessment, please see prior EPA responses (for instance, the comment by Troy Corporation) about how reviews and studies with no primary data are considered as supplemental materials and tracked in HERO (Section 5.2.1). Please also see the specific descriptions in the Toxicological Review that describe how the relevant information was identified (Section 2) and EPA guidelines were adhered to in evaluating the respiratory cancer MOA (Section 3.2.5), as well as the consideration of nonlinear approaches to addressing one MOA contributing to upper respiratory tract cancers, including derivation of an RfC (Section 5.2.1). EPA has updated the BMDS modeling, as described in responses to NASEM and public (including from ToxStrategies) comments below, primarily in Appendix F.3.8.

Finding on Evidence Integration

Peer Review Findings and Comments

- **Finding (12):** Evidence integration is the critical last step in hazard identification, and transparency is essential, requiring a sufficiently comprehensive narrative documenting the decision-making process. In completing its determination of hazard identification in the 2022 Draft Assessment, EPA drew on long-standing approaches for inferring causation, including the Bradford Hill considerations, first enumerated in a 1965 publication (Hill, 1965). Nonetheless, the 2022 Draft Assessment deviates from those long-established approaches in several respects, including (1) blurring of the boundary between evidence synthesis and integration, and (2) the choice of terminology used to describe the strata in the four-level schema for classifying the strength of evidence. Additionally, in some instances the narratives concerning the evidence integration step are too terse to explain fully why EPA came to its confidence conclusions.

EPA Response to Finding 12: As previously described above under “Evidence Integration (Step 7)”, the assessment has been revised to clearly demarcate evidence synthesis judgments from evidence integration and to more clearly document the different factors that influenced the

evidence integration confidence conclusions for each health effect. Regarding the evidence integration framework, the committee seemingly mistakenly interpreted the 5-level evidence integration schema applied in the external review draft as 4 levels because the category of 'not likely' was only mentioned as a footnote (since it wasn't used for this evidence base). However, in the revised assessment, the 5th evidence synthesis category of "compelling evidence of no effect" and the 5th evidence integration category of "strong evidence supports no effect" are now described in the Assessment Methods (see Section 2 of the revised Toxicological Review), although these categories do not apply to any of the hazard synthesis for this assessment. Regarding the terminology used, as noted above, the IRIS Program understands there may be potential confusion and defines the terms used for evidence integration in the IRIS Handbook, along with descriptions of patterns of findings across evidence that lead to different evidence integration judgements. These definitions are also presented in the assessment (Section 2.6).

Related Public Comments

No related public comments were submitted.

Dose-Response Assessment (Step 8)

Peer Review Findings and Comments

- **Finding (13):** The committee found EPA's considerations for study inclusion to be reasonable, although the discussion of those considerations in multiple places in the documents made it more difficult to determine what the considerations were and how they were applied.
- **Finding (14):** Although EPA provides criteria for study inclusion in the dose-response assessment, it does not include any discussion of how these criteria were applied to the specific studies chosen for dose-response. Using the Hanrahan et al. (1984) study as a test case, there are inconsistencies between the characteristics of the study and EPA's criteria for studies that would be selected for dose-response analysis.

EPA Response to Findings 13 and 14: More specific criteria for study selection for dose-response analysis are now laid out in methods (Section 2.7 of the revised Toxicological Review) and documentation has been added for each health effect and study in the dose-response section (see Section 5). Specifically, in response to Finding (14), Sections 5.1 and 5.2 of the Toxicological Review now also includes tables and expanded discussions of the rationale for selecting and advancing studies for noncancer and cancer dose-response analysis and, ultimately, for selecting the RfC and IUR. This includes a specific rationale for each *medium* or *high* confidence study supporting why it was or was not advanced for POD derivation. Given the expanded study selection documentation now provided, the initial section on dose-response analysis is now presented as two separate sections: study/endpoint selection and POD derivation.

EPA has reviewed the Hanrahan et al. (1984) study and the Liu et al. (1991) study for selection for dose-response analysis and, as outlined in Section 5, decided not to advance either individually. Rather, they were advanced together as complementary studies and advanced the combined dose-response data to draw strength from each other (which helps to mitigate several limitations specific to one of the studies but not the other), in keeping with the example provided in the NASEM peer review report.

- **Tier 2 Suggested Revision (Recommendation 2.7):** EPA should consider using information from studies that are complementary to each other to derive benchmark concentrations for outcomes of interest (see also Appendix D [point of departure analysis using two studies: a case study]). For example, multiple studies can be complementary by widening the exposure scale, broadening the age groups, and including vulnerable or susceptible groups.

EPA Response to Tier 2 Suggested Revision 2.7: As noted above, EPA provides additional analyses on sensory irritation building from the recommendation above in the revised assessment (see Appendix D.1.1 of the revised assessment, which builds from methods described in Appendix D of the NASEM 2023 report) by combining the dose-response data from Hanrahan et al. (1984) and Liu et al. (1991). Using the data available and building on approaches outlined by the NASEM committee, EPA now estimates a BMCL from the combination of these studies. Although concerns about the precision of the estimate remains, which impacts confidence in the derived toxicity value (see related comments below), the derived POD is considered much more stable and appropriate as compared to the BMCL based on Hanrahan et al. (1984) alone.

- **Tier 2 Suggested Revision (Recommendation 2.8):** Given that EPA has requested additional information from some study authors, the authors of the Liu et al. (1991) study could be approached for additional information that would help EPA reconstruct an overall dose-response graph (see also Appendix D [examples of issues identified by the committee]).

EPA Response to Tier 2 Suggested Revision 2.8: EPA has not been able to locate a working email address for KS Liu and has found no recent publications. EPA contacted a study co-author, Dr. Ken Sexton, at two email addresses found on publications seeking any additional information on the dose-response modeling; however, both attempts were not delivered. Note that EPA also contacted Dr. Krzyzanowski regarding additional information on the regression modeling details from Table 5 of that study and received a response.

Although EPA was unable to acquire additional information in relation to the study by Liu et al. (1991), additional modeling efforts related to this study were successful, expanding on the committee's efforts on this topic presented in Appendix D of the NASEM report (see Appendix D.1).

Related Public Comments

- **Public Comment (American Chemistry Council):** "NASEM has provided several recommendations regarding the development of toxicity values, including that toxicity values should be more representative of the body of evidence, and should use formal methods for combining multiple studies. It is critical that EPA address NASEM recommendations regarding developing toxicity values to better reflect the state-of-the-science in this field and demonstrate how the systematic review process informs the development of the values."
"Subjective exclusion of "evidence against" (not supporting) the conclusions reached in the Draft Assessment is contradictory to EPA guidelines...The Draft Assessment, however, sets aside both animal and mechanistic information and solely calculates Reference Concentrations (RfCs) and Inhalation Unit Risks (IURs) based on individual study results. Toxicokinetic, dosimetry, animal, and mechanistic data are not integrated into the assessment of epidemiology results when calculating exposure limits/IURs, when they appear to contradict numerically identified associations from epidemiology." *[Please see docket for details and supporting information.]*

EPA Response: Note that the draft RfC in the external review draft was based on multiple studies and health effects, consistent with prior NRC (2011) recommendations. And, while a single epidemiology study was used to estimate the draft IUR due to limitations in the evidence base, toxicokinetic, dosimetric, and mechanistic modeling approaches were applied to the animal data to derive complementary unit risk estimates based on the animal cancer data. These decisions in line with prior recommendations are retained in the revised assessment. In addition, as noted above, EPA followed NASEM's recommendation to jointly model the dose-response from the two complementary studies of formaldehyde exposure in mobile homes and sensory irritation.

F.1.4. Other Public Comments on Assessment Methods and Organization

- **Public Comment (American Chemistry Council):** "Ensuring and maximizing the quality of the 2022 Draft Assessment requires the application of 'a "weight-of-evidence" approach that considers all relevant information and its quality [...]' Equally important, the substance of the information must be 'accurate, reliable and unbiased,' which entails the use of 'the best available science and supporting studies conducted in accordance with sound and objective scientific practices [...]' EPA must ensure that the information presented in the 2022 Draft Assessment 'is comprehensive, informative, and understandable.'
"Due to a deficient systematic review, information that was, or should have been, known to the Administrator was excluded from the Draft Assessment."
"The 2022 Draft Assessment does not fully incorporate NASEM (2011) systematic review recommendations or current best practices for implementing systematic review in chemical toxicity assessments."

“The 2022 Draft Assessment does not follow guidance in the IRIS Handbook. The 2022 Draft Assessment makes no specific reference to the IRIS Handbook with the exception of a single footnote. However, some of the general processes mirror, at least in part, the IRIS Handbook. In other parts, it deviates from the IRIS Handbook more substantively. There are several places in the 2022 Draft Assessment with language and tables that are borrowed from the IRIS Handbook, but, in some cases, subtle changes were made that, while easily missed, are not unsubstantial.”

“The 2022 Draft Assessment does not consider IRIS Handbook recommendations regarding MOA.”

“The frequent and inconsistent use of subjective terms, results in an overly complicated Draft Assessment that is confusing and difficult to interpret. As shown in Table 1, the term “low concentration” can range from about 0.002 to 0.4 mg/m³, “high concentration” from about 3 to >12 mg/m³, and “excessive concentrations from about 7 to ≥20 mg/m³, the overlapping and ill-defined subjective terms makes interpretation of statements such as those highlighted in Table 2 almost impossible to put into context. The level of subjectivity in the language used in the Draft Assessment must be reduced to increase transparency in study interpretations.”

“There are many instances of language supporting a subjectively predetermined outcome.”

“The Draft Assessment frequently combines “statistics not reported” with “not statistically significant” and undefined “significant” determinations. The basis for statistical and biological relevance as treatment-related is not transparent.”

[Please see docket for details and supporting information.]

EPA Response: The NASEM peer review panel supported EPA’s weight-of-evidence conclusions for all included health effects. As a reminder, the IRIS Program sought feedback from the NAS on the evolving IRIS systematic review methods in 2014 and 2018 and, in 2020–2022, the completed draft IRIS Handbook ([U.S. EPA, 2022](#)) received public comment and peer review by NASEM⁴⁴. The systematic review methods developed and applied in the external draft of the IRIS formaldehyde assessment (2021) to make it responsive to the NAS (2011) report served as the basis for many of the methods in the IRIS Handbook (and thus the methods are consistent). It is separately noted that NAS (2011) indicated EPA should not wait until systematic review is fully implemented to produce IRIS assessments. However, as previously described, several aspects of the approaches in the draft assessment have been adjusted in the revised assessment to be more consistent with current practices in systematic review. Regarding terms usage, it is acknowledged that there can be differences in descriptions such as ‘high’ and ‘low’ exposure, which often differs depending on the

⁴⁴ <https://nap.nationalacademies.org/catalog/26289/review-of-us-epas-ord-staff-handbook-for-developing-iris-assessments> (2022) <https://nap.nationalacademies.org/catalog/25086/progress-toward-transforming-the-integrated-risk-information-system-iris-program> (2018) <https://nap.nationalacademies.org/catalog/18764/review-of-epas-integrated-risk-information-system-iris-process> (2014)

context of the studies being described (e.g., sets of residential human studies on one outcome versus experimental animal studies on another); attempts were made to ensure inclusion of the specific exposure context rather than these terms alone. Regarding statistical significance, which is not the only consideration in synthesis decisions, the assessment is consistent with descriptions in the IRIS Handbook.

- **Public Comment (United States Chamber of Commerce):** "... we strongly encourage EPA to revise the 2022 draft IRIS assessment and incorporate the best available science and practices for systematic review. A formaldehyde IRIS assessment that does not consider the weight of scientific evidence could lead to unwarranted regulations that would ripple through the supply chain."

EPA Response: The assessment has been revised based on the consensus comments from the NASEM peer review panel and other specific public comments, including recommendations that improve the clarity of the methods applied to the assessment (e.g., the weight of evidence, or evidence integration, approach and documentation). However, the scientific conclusions in the external draft were supported by the NASEM panel and these conclusions, while more transparent and better supported, are fundamentally unchanged in the revised assessment.

- **Public Comment (ToxStrategies):** "Overall, the document is difficult to follow as pertinent information is scattered amongst three separate documents: an Assessment Overview, a Toxicological Review, and Supplemental Information. This reviewer found it difficult to follow the assessment whether reading printed hard copies or a computer screen. In some places there are lengthy passages without any supporting references, making it difficult for the reader to know which studies the U.S. EPA is using to support a given statement, even for readers familiar with the formaldehyde literature. As written, topics are covered in differing degrees of depth in multiple places. Perhaps an alternative approach would be to have combined the Assessment Overview and Toxicological Review into two or three separate documents with as minimal overlap as possible. Possible alternatives include portal of entry (POE) vs systemic effects or cancer vs non-cancer effects—perhaps with dose-response analysis in a separate document altogether. The current approach needs serious reconsideration."

EPA response: See the EPA Response to Tier 1 Recommended Revision 2.1.

- **Public Comment (Troy page 1):** "It was difficult to assess and understand EPA's conclusions for many of the toxicological endpoints identified in the assessment and this was compounded by the incorporation of the draft IRIS Handbook recommendations and additional explanation on the process for the determination of the acceptability of studies. EPA's approach towards

endpoint selection is inconsistent and confusing since it is not clear if they followed the recommendations in the draft IRIS handbook or chose another approach.”

EPA Response: The assessment follows the IRIS Handbook approach ([U.S. EPA, 2022](#)). The presentation of the methods have been clarified to increase transparency in response to the peer review Tier 1 Recommended Revision 2.1 (above). This includes increased clarity regarding endpoint and study selection methods in Section 2 of the revised assessment.

F.2. NASEM REPORT CHAPTER 3: TOXICOKINETICS; CHARGE QUESTION 2: TOXICOKINETICS

F.2.1. Charge Question and Peer Review Comment Summary

Charge Question 2: Toxicokinetics.

Several assumptions and interpretations were applied in the Toxicological Review that were based on current research (although the draft acknowledges that uncertainties remain). Please answer parts (a), (b), and (c) considering the extent to which the available science on the toxicokinetics of inhaled formaldehyde is clearly presented and appropriately applied in the assessment of potential respiratory and systemic (i.e., nonrespiratory) health hazards.

- a. Please comment on the Toxicological Review conclusion that inhaled formaldehyde is not likely to be distributed in appreciable amounts beyond the respiratory tract to distal tissues. This conclusion underpins the organization of the assessment and several key assumptions.
- b. Please comment on the Toxicological Review assumptions (based on [a]) that:
 - Inhaled formaldehyde is not directly interacting with tissues distal to the portal-of-entry (POE) to elicit systemic effects.
 - Formaldehyde levels in the blood or at systemic sites are not appreciably changed as a result of formaldehyde from exogenous sources (inhalation).
 - Inhaled formaldehyde does not cause appreciable changes in normal metabolic processes associated with formaldehyde in distal tissues. Therefore, studies examining potential associations between levels of formaldehyde or formaldehyde byproducts in tissues distal to the POE (e.g., formate in blood or urine; brain formaldehyde levels) and health outcomes are not considered relevant to interpreting the human health hazards of inhaled formaldehyde.
- c. Please comment on the Toxicological Review evaluation of considerations regarding endogenous formaldehyde in assessing the health risk due to inhaled formaldehyde.

Peer Review Comment Summary

- 0 Tier 1 Recommended Revisions

- 3 Tier 2 Suggested Revisions
- 0 Tier 3 Future Considerations
- 6 Consensus Panel Findings (numbered below; one Finding has multiple parts)

F.2.2. Distribution of Inhaled Formaldehyde; Metabolism, Binding, and Removal of Inhaled Formaldehyde

Peer Review Findings and Comments

- **Finding (1):** EPA concluded that inhaled formaldehyde is not distributed to an appreciable extent beyond the respiratory tract to systemic sites; thus, inhaled formaldehyde is not directly interacting with tissues distal to the portal of entry to elicit effects. EPA's conclusions regarding systemic delivery of inhaled formaldehyde are based on its expert judgment, with the support of available scientific evidence.
- **Finding (2):** Despite the lack of evidence regarding systemic delivery of formaldehyde to distant sites, the biological basis for observed systemic effects (described in Chapters 4 and 5) remains unclear. Additional research is needed to address this apparent discrepancy.

Related Public Comments

- **Public Comment (United States Chamber of Commerce):** "ATSDR also stated that EPA's assumptions regarding the toxicokinetics of formaldehyde exposure used to develop systemic reference concentrations '...doesn't make any sense' and that EPA should examine confounding toxicities 'before making any evidence conclusion for formaldehyde.'"
- **Public Comment (American Chemistry Council):** "Since the NAS (2011 review), numerous studies have conclusively demonstrated that inhaled formaldehyde is not systemically circulated through the blood or other systems. Furthermore, there is now direct molecular dosimetry evidence that, at concentrations below 15 ppm, there is no detectable systemic delivery of inhaled formaldehyde, at detection limits over a thousand times lower than endogenous levels of formaldehyde. The Draft Assessment, however, fails to take this evidence into account and incorporate the recommendations of the NAS (2011) peer-review by developing RfCs for reproductive and developmental effects (Table 2-10) that rely on the assumption of systemic delivery of inhaled formaldehyde."

"Over the last four decades, toxicokinetic and toxicodynamic studies have consistently demonstrated a lack of systemic distribution of inhaled formaldehyde concentrations up to 15-20 ppm when directly measured in tissues (Heck et al., 1985; Casanova et al., 1988). More recent publications quantified protein and DNA biomarkers of formaldehyde exposure confirming that there is no systemic distribution of formaldehyde air concentrations ≤ 15 ppm (Lu et al. 2010, 2011; Edrissi et al. 2013, 2017; Yu et al. 2015; Lai et al. 2016; Leng et al. 2019)."

“Data from multiple sources provide strong evidence that formaldehyde is not systemically distributed and, therefore, could not directly cause any systemic effect. The Draft Assessment assumes that formaldehyde acts via an unknown pathway to elicit systemic effects (e.g., female reproductive or developmental toxicity, male reproductive toxicity, nervous system toxicity, and leukemia).” *[Please see docket for details and supporting information.]*

EPA Response (to both public comments above): The assessment conclusions regarding systemic effects are based on an assumption of a lack of distribution of inhaled formaldehyde to systemic sites, a toxicokinetic conclusion supported by the peer reviewers. This includes concluding that exogenous formaldehyde is not systemically distributed to remote tissues; that inhaled formaldehyde does not directly interact with cells in the bone marrow is not likely; and that in vitro studies of direct administration of formaldehyde to cells from distal tissues are not informative to the assessment. However, a lack of understanding of how inhaled formaldehyde can cause systemic effects without systemic distribution is insufficient to outweigh the formaldehyde-specific evidence that those effects are happening. EPA Note: Interagency reviewer (e.g., ATSDR) comments were on a draft that was revised prior to public release. Please consult the full interagency review comments in the EPA docket.

F.2.3. EPA’s use of Toxicokinetic Data and Dosimetry Models

[Please note that this section includes multiple comments relevant to cancer dose-response modeling.]

Peer Review Findings and Comments

- **Finding (3):** The committee found that EPA used toxicokinetic data as follows:
 - [a] EPA used these data to support its assumption that “inhaled formaldehyde is not distributed to an appreciable extent beyond the respiratory tract to systemic sites.” Thus, EPA assumed that inhaled formaldehyde does not directly interact with tissues distal to the portal of entry to elicit effects. EPA’s conclusions regarding systemic delivery of inhaled formaldehyde are based on its expert judgment, with the support of the available scientific evidence.
 - [b] EPA concluded that studies examining potential associations between levels of formaldehyde or formaldehyde by-products (e.g., formate) measured in distal tissues and health outcomes were not relevant to inhaled formaldehyde. This conclusion is consistent with EPA’s state-of-practice methods and supported by the available scientific evidence.
 - [c] EPA concluded that formaldehyde toxicokinetics show significant route-to-route difference (e.g., inhalation versus oral). With few exceptions (e.g., genotoxicity), EPA focused solely on inhalation studies. The committee supports this decision and found it to be consistent with EPA’s state-of-practice methods.

- [d] EPA used toxicokinetic data as a primary consideration informing causality during its evidence synthesis step, as “an explanation for any observed differences in responses across route of exposure, other aspects of exposure, species, or life stages.” Toxicokinetic data were used to examine consistency across studies and to evaluate biological gradient/dose-response data. Overall, the committee found that the portions of the 2022 Draft Assessment describing the toxicokinetics of formaldehyde and associated computational models are well organized and extensive. The 2022 Draft Assessment accurately reflects current understanding of the toxicokinetics of inhaled formaldehyde. The literature review in the 2022 Draft Assessment appears to be up to date and includes relevant studies published as of the assessment’s release date. The use of these data in the synthesis step is consistent with EPA’s state-of-practice methods.
- **Finding (4):** The use of these models [dosimetry, CFD models, including use in deriving cRfC for respiratory pathology] is appropriate and consistent with EPA’s state-of-practice methods.
- **Tier 2 Suggested Revision (Recommendation 3.1):** To enhance transparency, the summary tables (e.g., Tables 24, 43, and 44) should explicitly identify the models used to derive flux values. Table 44 should clearly indicate whether the BBDR models used here are equivalent to Models 1 and 2 identified in Table 43 and the text. Table 46 should indicate which flux model was used.

EPA Response to Tier 2 Suggested Revision 3.1: The model used for flux is added in the main text and also added as a footnote where appropriate (i.e., the application of the dosimetry model is now summarized in Sections 5.1.2 [e.g., Footnote 49] and 5.2.1 [e.g., Table 5-37] of the revised Toxicological Review, with the detailed evaluation and discussion presented in Appendix C.1.12). The values in “Table 2-23” (Table 5-38 in the revised Toxicological Review) are not from EPA models (1 & 2) but as derived by Conolly et al (2004) based on their models. This is now clarified in the revised text. Table 5-38 compares risk estimates predicted by the human extrapolation models in Conolly et al (2004) with those derived by EPA from the NCI occupational epidemiology data.

- **Finding (5):** The 2022 Draft Assessment generally provides a thorough discussion of the strengths and weaknesses of the available models, including an extensive discussion of how EPA used them in the assessment.
- **Tier 2 Suggested Revision (Recommendation 3.2):** To increase transparency, EPA should provide additional clarification regarding its decision not to use the BBDR model to extrapolate rat nasal carcinogenicity to humans. Criteria used by EPA to determine whether the models

would be adequately robust for this purpose are not readily available in the 2022 Draft Assessment. Likewise, EPA should provide additional support for its decision not to use the BBDR model (Conolly et al., 2004) for this extrapolation because of its conclusion that formaldehyde-induced mutagenicity, modeled as proportional to DPC concentration, is not relevant to formaldehyde's carcinogenicity.

EPA Response to Tier 2 Suggested Revision 3.2: There are two separate recommendations in the above. First, regarding model predictions, EPA's evaluation of model robustness considered established criteria on model specification, calibration and prediction. These criteria have been added to the sub-section on "Extrapolation using a biologically based dose-response model" in section 5.2.1.

The second comment pertains to the inferences from the model results in Conolly et al (2003, 2004) [i.e. "it's conclusion" = "model's conclusion"]. EPA's decision to not use the BBDR modeling for extrapolation was on account of instabilities in model predictions, and not on account of the inference in Conolly et al. (2004) regarding formaldehyde-induced mutagenicity as not relevant to its carcinogenicity. Therefore, the underlined text in the paragraph below from the draft 2022 Toxicological Review is now deleted to improve the clarity of this paragraph. The underlined deleted text does, however, relate to a major uncertainty that is discussed in Section 5.2.1 and Appendix D.2.2. Also see the response to the public comment by TERA.

"A clonal expansion model ([Conolly et al., 2004](#)), as well as possible variations of this model, developed for extrapolation of the rat nasal cancer risk to human exposure scenarios are carefully evaluated. Predictions using these models for humans are found to be not robust at any exposure concentration. Furthermore, a key model inference in Conolly et al. (2003), (Conolly et al., 2004) that formaldehyde-induced mutagenicity, modeled as proportional to DPX concentration, is not relevant to its carcinogenicity is found to be extremely uncertain. Accordingly, the clonal expansion modeling of the rat data is employed to derive multiple PODs and corresponding HECs but it is not used for extrapolating to human exposure scenarios."

- **Finding (6):** Documentation of the dosimetry methods used is variable in the 2022 Draft Assessment, especially with regard to some summary tables (for example, Table 2-27). Discussion of the comparison of and basis for unit risk estimates for nasopharyngeal cancer in humans and nasal squamous cell carcinomas in rats mentions the use of CFD and PBPK models without identifying the specific models that were used.
- **Tier 2 Suggested Revision 3.3:** To increase transparency, EPA should address these shortcomings by updating tables and text to better document its dosimetry methods.

EPA Response to Tier 2 Suggested Revision 3.3: Citation of the relevant models have been added, as described above.

Related Public Comments

- **Public Comment (University of North Carolina at Chapel Hill):** "The IRIS Draft did not recognize the importance of development of chemical-specific biomarker of formaldehyde exposure/molecular dosimetry data in its evidence-based risk assessment...Due to the absence of data for formaldehyde-specific biomarkers, the EPA IRIS Draft could not unambiguously assess the differential contribution of formaldehyde when confounding factors, such as methanol co-exposure, when present...How could the IRIS draft conduct rigorous evidence-based risk formaldehyde assessment using only data based on non-formaldehyde specific approaches that do not involve formaldehyde-specific biomarkers and could not provide accurately measure the contribution of exogenous formaldehyde under the substantial endogenous background of formaldehyde?" "Recommendations: The EPA IRIS Draft needs to discuss the limitations of available data/methodologies, highlight the significance of SILMS-generated formaldehyde-specific biomarker data, and integrate dosimetry data provided by the rich formaldehyde-specific biomarker data into risk estimates of formaldehyde."

"The IRIS Draft misunderstood or underestimated the significance and utility of stable isotope labeling and mass spectrometry methods in understanding the potential for formaldehyde carcinogenicity and its use in characterizing dose-response in quantitative risk assessment...The ability to distinguish DNA damage caused by exogenous formaldehyde from substantial background endogenous formaldehyde-induced lesions is crucial. Accurate molecular dosimetry of both endogenous and inhaled formaldehyde is essential for science-based risk assessment of formaldehyde exposure." "Recommendation: The EPA IRIS Draft needs to discuss the utilities and advantage of SILMS and, thus, to use the rich datasets we generated over the last decade to improve evidence-based risk assessment of formaldehyde."

"The IRIS Draft did not appreciate and interpret key data on the kinetics studies of formaldehyde-induced DNA adducts... The EPA IRIS Draft noticed the results of the time-course exposure study by stating 'rat nasal N2-hmdG adduct formation was also positively associated with exposure duration, with adducts accumulating to levels ≥ 5 times higher after 28 days of exposure to 2.5 mg/m³ compared to single exposures.' However, the IRIS Draft did not provide any discussion on the repair/loss and half-life of formaldehyde-induced DNA adducts, which is quite surprising as information on the fate and repair/loss kinetics is essential to assess mutagenicity and carcinogenicity of a genotoxicant like formaldehyde." "Recommendation: The IRIS Draft needs to be revised to discuss the kinetics data on formaldehyde-induced DNA adducts, half-life and the steady state of exogenous DNA adducts to more accurately characterize mutagenicity and genotoxicity of formaldehyde in an evidence-based risk assessment." *[Please see docket for additional details and supporting information.]*

EPA Response: The adduct data and the experiments alluded to were crucial to supporting EPA's conclusions in Section 3.1 (Toxicokinetics). EPA concluded that exogenous formaldehyde is not systemically distributed to remote tissues; that it is likely that inhaled formaldehyde does not directly interact with cells in the bone marrow; and that in vitro studies of direct administration of formaldehyde to cells from distal tissues are not informative to the assessment.

With regard to dose-response modeling, the experimental data provided by the internal biomarkers measured in these studies were used by Starr and Swenberg (2016) in attempts to bound human cancer risks. These estimates were carefully evaluated by EPA, concluding that the method does not necessarily provide an upper bound on the slope of the dose-response at low exogenous exposures (see Section 5.2.5). The analyses supporting the conclusion that this "bottom up" approach does not provide a reasonable upper bound are described in Appendix D.2.4. In addition, Section 3.1 reviews and discusses the large uncertainties associated with these attempted bounding estimates (e.g., high variability in endogenous formaldehyde levels; model-based predictions that any external exposure causes some increase over background levels).

It is separately noted that the dose-response modeling conducted by EPA evaluates risk that is additive to background (which includes responses that may be attributed to the effects of endogenous exposure) due to inhaled or exogenous exposure concentrations. EPA carried out a sensitivity analysis based on the revised dosimetry in Schroeter et al., and found that the use of this model does not appreciably alter the BMD modeling results (i.e. the POD used for linear extrapolation in estimating the nasal cancer unit risk). This is explained in Section 5.2.1. Overall, as noted in the NASEM Finding (5), the assessment "...provides a thorough discussion of the strengths and weaknesses of the available models, including an extensive discussion of how EPA used them in the assessment."

- **Public Comment (Thomas B. Starr):** "...All of EPA's and Crump et al.'s (2014) claims regarding the 'bottom-up' approach are no more than speculative 'hand-waving'. In what follows, we address and rebut these claims regarding our approach with formaldehyde-specific data."
[Please see docket for details and supporting information.]
- **Public Comment (University of North Carolina at Chapel Hill):** "The EPA IRIS Draft used speculations, instead of any data, to decline the adoption of the bottom-up approach that offers the opportunity to serve as an independent 'reality check' on low-dose risk estimates. The EPA IRIS Draft concluded that 'the bottom-up approach does not necessarily provide an upper bound on the slope of the dose-response at low exogenous exposures, primarily because the ratio of background tumor incidence to internal endogenous concentration at the true target tissue may underpredict the slope of the dose-response above that endogenous concentration.' The EPA IRIS Draft further stated that 'This is further discussed in Crump et al. (2014)' yet without providing any data to support such a claim in the Draft. In fact, Crump et al

hypothesized in the Letter to the Editor that 'A sublinear dose-response relationship over the endogenous range is clearly plausible on biological grounds. For example, it is likely that baseline levels of DNA repair enzymes and other protective systems evolved to deal with endogenous DNA damage would work most effectively for lower levels of endogenous adducts.' (Crump et al. 2014) Once again, no data were provided to support such a hypothesis in either Crump et al. 2014 or the EPA IRIS Draft. Without development of data to the contrary, the approach taken by Swenberg et al. remains fully supported by existing data and is not questioned by unfounded hypotheses." "Recommendation: The EPA IRIS Draft must be revised to provide specific evidence, instead of speculations, for decision-making in terms of the utility of the bottom-up approach in low-dose risk estimates."

EPA Response (to both public comments above): NASEM's peer review did not indicate a concern with EPA's position on the "bottom-up" approach. The commenter's 2013 and 2016 papers suffer from the same fundamental flaw mentioned in Section 5.2.5, such that results from their bottom-up bounding approach cannot be considered plausible upper bounds. The multiple reasons for this conclusion are detailed in Appendix D.2.4. In making this determination, EPA is not necessarily assuming a concave upward dose-response over the endogenous range but identifying a plausible scenario under which the dose-response curve pertaining to endogenous formaldehyde could take this shape. EPA demonstrated that the bottom-up approach would not give an upper bound in this case. Appendix D.2.4 also identifies other concerns with the calculations in the "bottom up" approach. In view of NPCs being rare cancers, EPA also carried out a bounding calculation as a reality check and demonstrated that the unit risk estimate derived for NPC incidence is not implausible in comparison to actual case numbers (see Section 5.2.1).

- **Public Comment (Toxicology Excellence for Risk Assessment):** "EPA should follow its own guidelines and develop a low dose cancer extrapolation based on a dual MOA. The proposed hockey-stick approach of EPA's own award winning scientist, Rory Conolly, would likely approximate the end result, but other approaches might be considered, such as that suggested by Dourson et al. (2008) for acrylamide, or published by McGregor et al. (2006) or by Thompson et al. (2020) for formaldehyde, the latter effort which was adopted by the European Union. See in particular Table 6 and Figure 8 of Thompson et al. (2020)." *[Please see docket for details and supporting information.]*

EPA Response: EPA extensively explored the use of the dual MOA models developed by Dr. Conolly and colleagues, as well as alternative model structures based on the authors' overall approach. EPA calculations were reviewed by the NASEM committee which found them "... highly responsive to the recommendations of the 2011 NRC committee regarding the use of CFD and BBDR models and modeling of time-to-tumor and time-to-death data." In regard to the relative

contributions of the dual MOAs, the modeling effort in Conolly et al. (2003) inferred that the tumorigenicity of formaldehyde in Fisher 344 (F344) rats could be explained on the basis of cytotoxicity-driven regenerative cell proliferation alone, without any contribution from a direct formaldehyde-induced mutagenic component. However, Conolly et al. (2003, 2004) did not examine the uncertainties in this conclusion and did not recognize that alternatives indicated by these uncertainties could profoundly impact low dose human extrapolation. Subramaniam et al. (2007) evaluated this inference in Conolly et al. (2003) by examining the contribution of the mutagenic component in their model as a percentage of the predicted probability of tumor--see Table IV of Subramaniam et al. (2007). The model actually attributes formaldehyde's mutagenic potential to be responsible for 58%–74% of the added tumor probability in the range of exposures where tumors were observed (6.0 ppm – 15.0 ppm). In the low dose region, this fraction varies between 2% and 80% depending on the characterization of the dose-response curve for cell replication. Namely, the predicted probability was dependent on whether a J-shape or hockey-stick shape is assumed for the dose response for cell proliferation.

Overall, the available evidence does not support a conclusion that cytotoxicity-driven regenerative cell proliferation is the sole driver of formaldehyde-induced carcinogenicity, nor do the data support that a significant contribution of mutagenicity at lower doses is unlikely.

The extensive EPA analyses of the nasal cancer MOA evidence resulted in the selection of a linear low-dose extrapolation in calculations of nasal cancer unit risk estimates (Section 5.2).

- **Public Comment (American Chemistry Council):** “EPA’s approach to the evaluation of BBDR modeling in the 2022 Draft Assessment was misguided and inconsistent with EPA’s own cancer guidelines... Dr. Conolly concludes that EPA should consider the uncertainties of the BBDR model relative to the hidden uncertainties embedded in the empirical dose-response functions that they prefer, since lack of explicit description of mechanism does not avoid accountability for the mechanism, especially when so much relevant data are available... Results from [Starr and Swenberg’s] evaluation are important in bounding the potential risks for cancer in consideration of the endogenous production of formaldehyde.” *[Please see docket for details and supporting information.]*

EPA Response: EPA’s Cancer Guidelines prefer the use of a biologically based dose response (BBDR) model but require that such models [as well as models derived by empirical curve fits alone] need to be robust. The BBDR model referred to by this commenter was found to be numerically unstable on multiple accounts (Section 5.2.1 of the revised Toxicological Review and Appendix D.2.2); thus, it was not possible to bound human risk at any exposure concentration using this model. Extremely sensitive parameters in the BBDR modeling were also determined by fitting model predictions to the observed tumor incidence in the animal bioassay. Thus, as discussed in Crump et al. (2010), extrapolation using the BBDR model not only did not eliminate the

uncertainties encountered in empirical modeling but introduced significant new sources of uncertainty. Please see EPA response above to the comment by UNC, Chapel Hill regarding the model developed by Starr and Swenberg.

- **Public Comment (Ramboll):** “The overall formaldehyde dataset for F344 rat nasal tumors and related mechanistic information is probably the most extensive such dataset in existence for any chemical. Given that the USEPA’s own Guidelines for Carcinogen Risk Assessment (USEPA 2005) clearly state a default preference for data-driven risk assessment, its multi-decade effort to develop a cancer risk assessment for formaldehyde, and its own significant resources in computational toxicology and risk assessment, it is curious that USEPA has not developed a BBDR model for formaldehyde. USEPA IRIS has, however, devoted considerable resources (Crump et al. 2008; Subramaniam et al. 2007; Subramaniam et al. 2008) to evaluate the CIIT BBDR model (Conolly et al. 2003; Conolly et al. 2004). Those of us who have developed biologically motivated models in support of risk assessment understand that no model is perfect, and that model analysis and iterative refinement support reductions in model uncertainty. It would have been interesting and informative, and consistent with their own Cancer Guidelines, if USEPA IRIS had chosen to use their analyses to develop a refined version of the CIIT model. Instead, USEPA has rejected the CIIT BBDR model and turned to more empirical approaches. In making this choice, USEPA is effectively saying that relevant mechanistic data are confusing and increase rather than decrease uncertainty, a position that is inconsistent with the 2005 guidelines. In this regard, it is worth noting that I [Dr. Rory Conolly] was employed in USEPA/ORD from 2005 through my retirement in February 2000 but was not consulted by IRIS on their evaluation of the CIIT BBDR model.”

EPA Response: EPA devoted considerable resources to explore fully utilizing the CIIT BBDR model for extrapolation by developing better input parameters as well as alternative BBDR model structures to those in the Conolly et al. (2003, 2004) effort. EPA’s efforts drew from the same mechanistic data utilized by these authors. These are indicated in the Toxicological Review (Section 5.2.1) and further detailed in Appendix D.2.2, as described above. EPA’s improvements over the CIIT effort covered all facets of the modeling including (a) an improved likelihood function that differentiated between whether a death occurred without a tumor or with an incidental tumor or with a fatal tumor; (b) a revised PBPK model for formaldehyde DPX that improved upon the original model developed by Conolly et al. (2000); (c) improved representation of the cell labeling data that allowed characterization of the temporal and regional variation in the dose-response for cell replication rates; and (d) alternative model structures for the division and death rates for initiated cells. However, 2-stage clonal growth models for formaldehyde based upon these different modifications all suffered from similar numerical instabilities (Appendix D.2.2). EPA’s efforts are

consistent with its Cancer Guidelines that prefer the use of a biologically based dose response (BBDR) model but require that such models need to be robust.

Dr. Conolly's assertions are incorrect. EPA consulted with Dr. Conolly on multiple occasions. First, EPA organized a three-day expert consultation in 2004 that included Drs. Rory Conolly, Julia Kimbell, Fred Miller, Linda Hanna, Chris Portier, George Lucier, and Kenny Crump. A summary of this consultative meeting and a separate report by Dr. Conolly were provided in Appendix H of the 2010 peer review draft report. The recommendations of this panel provided the basis for EPA's evaluation of the CIIT modeling in the current Toxicological Review. Subsequently, Dr. Conolly was an internal reviewer on Subramaniam et al. (2007), Subramaniam et al. (2008), and Crump et al. (2008), and the authors provided EPA management a detailed disposition of Dr. Conolly's comments prior to internal clearance of the manuscripts. Furthermore Dr. Conolly commented on EPA's analyses of the BBDR models in the peer-reviewed literature. All these peer-reviewed publications, including exchanges in the form of letters to the editor, were completed prior to EPA's 2010 draft assessment.

- **Public Comment (Ramboll, continued):** "A full examination of the uncertainties associated with empirical modeling of dose-response, side-by-side with the uncertainties that USEPA has identified in the CIIT BBDR modeling, would be informative. For example, one could ask about the relative uncertainty of dosimetry based on CFD modeling in anatomically realistic models of rat and human nasal passages versus empirical modeling of dose-response that does not specifically address dosimetry at all. Ignoring dosimetry does not, and should not, remove concern for the role of dosimetry as a determinant of dose-response. Uncertainty about dosimetry exists for any model of toxicant/carcinogen dose-response. Is a dose-response analysis that explicitly describes dosimetry inherently more uncertain than an approach that wholly ignores dosimetry? This same argument, comparing data-driven and empirical descriptions, can be applied to each of the main components of the BBDR model. Empirical models effectively hide explicit uncertainties about the shapes of the dose-response curve because the empirical models do not acknowledge the mechanistic determinants of dose-response; the other hand, explicitly describes these determinants. This situation is reminiscent of the early days of PBPK modeling (1980's -1990's) where PBPK models were sometimes said to increase uncertainty in the characterization of pharmacokinetics relative to empirical PK models. The need here, both for PBPK models and for the BBDR modeling for formaldehyde, is to step back and ask what determines the behavior of interest and to then realize that only relevant data and its inclusion into the dose-response analysis can address these uncertainties. BBDR modeling is simply a tool for integrating data on the mechanistic determinants of dose-response to provide a capability for predicting the entire dose-time response surface."

EPA Response: EPA agrees that the BBDR modeling allowed for integrating a vast amount of mechanistic information used in conjunction with the tumor incidence data. EPA used the BBDR modeling of the rat data to determine the point of departure (POD) for the low dose extrapolation, and a POD was chosen at a benchmark response of 0.005 extra risk—a lower level than what would have been traditionally possible with the tumor data—because the dose-response was informed by precursor data in the form of increased cell replication as characterized by labeling index measurements. However, for reasons discussed in response to a previous public comment above by the ACC, the BBDR model did not eliminate the uncertainties encountered in empirical modeling but, in addition, introduced significant new sources of uncertainty. On other aspects of this comment, please see EPA responses above (e.g., to the public comment by the ACC).

Public Comment (Ramboll, continued):

“Issue 3- Labelling index data: ... Notwithstanding the variability in the data, a clear J-shape is apparent [figure B-18 in Appendices], with an initial decrease in labeling with increasing flux to a nadir in the labeling index slightly below a flux of 2,000 pmol/(mm²- hr). At higher flux values, which are associated with cytolethal effects of inhaled formaldehyde, the labeling index increases, reflecting regenerative cellular proliferation subsequent to cell killing. Heck and Casanova (1999) suggested that DNA-protein crosslinks (DPX) due to inhaled formaldehyde act as physical blocks to the progression of the replication complex along the DNA strand, and thereby effectively reduced the rate of cell replication...The theory proposed by Heck and Casanova (1999) is supported in more recent literature (e.g. Nakamura and Nakamura 2020)...

The J-shaped dose-response for labeling index has significant implications for formaldehyde risk assessment. In the CIIT BBDR model, formaldehyde exerts a low dose linear directly mutagenic effect as a function of the formation of DPX and, simultaneously, the J-shaped dose-response for the rate of cell division. The human version of the CIIT BBDR model (Conolly et al. 2004) predicted a J-shaped dose-response for tumor risk, with risk only increasing above background at about 2 ppm inhaled formaldehyde. This result was, of course, dramatically different from USEPA's assessments, which identify inhalation unit risks in the range of a few ppb. In this context, USEPA makes much of the variability in the labeling index data (see Time variability of labeling data, Appendices, p. B-54). The variability is used to justify alternate dose-responses that do not include the J-shape, including dose-responses that are monotonically increasing in cell division rate as a function of flux/inhaled ppm (e.g. Figures B-24 & B-25, Appendices, p. B-63). These alternative dose-responses can dramatically change the risks predicted by the BBDR model. Given the size and consistency of the Monticello et al. (1991, 1996) dataset, these alternative dose-response exercises are less than convincing... I suggest that USEPA should provide a clearly stated justification for use of cell replication dose-responses that are not J-shaped. If the size and quality of the labeling index data described by Monticello et al. (1991, 1996) is not to be trusted, then what is?

... I agree that calculation of division rates as a function of both time (age of rat) and flux is preferable. In fact, this two-dimensional approach is being used in an ongoing update of the BBDR model that is not otherwise considered here. However, USEPA is using this and similar criticisms of the CIIT BBDR modeling of the labeling index data to justify use of alternative, much more empirical dose-response models that do not capture our understanding of the biological processes involved. As noted above, the one-dimensional expression for division rate as a function of flux retains the main qualitative feature of the labeling index dataset, its statistically significant J-shape. Does USEPA really think that an empirical dose-response function that completely ignores the CIIT bioassay labeling index dataset provides greater certainty in the prediction of human risk?"

EPA response: EPA did include the J-shaped characterization of the dose-response for cell division in several models used to examine the impact of the variability and uncertainty in cell replication rates. See Figure 5-8 and Table 5-40 of the Toxicological Review as well as Figures D-29, D-30, D-32, D-33 and D-35 in Appendix D.2.2 for EPA's consideration of J-shaped characterization of dose-response for cell division rate. All the BBDR-based dose-response curves evaluated in Appendix D.2.2 provide equally good fits to the observed tumor incidence data (i.e. fidelity to the sensitive calibration of the model is retained in these analyses). EPA's effort characterized the considerable temporal and regional variability in the labeling index measurements unlike the approach in Conolly et al. (2003) where a time-weighted averaging of the labeling index measurements was carried out over regions that received vastly different levels of flux. The J- shape of the cell replication dose-response had no bearing on EPA's disposition of the human BBDR extrapolation model which was numerically unstable regardless of the shape (the assessment demonstrates this for both the J- and hockey-stick shapes) (Section 5.2.1 and Appendix D.2.2).

Public Comment (Ramboll, continued):

"Labeling index data for bioassay exposure durations from 1 day through 6 weeks (Monticello et al. 1991) were measured by injecting BrdU while subsequent time points (Monticello et al. 1996) used osmotic minipumps implanted for 3 days. Calculation of a division rate constant, which is needed as an input to the clonal growth model, from labeling index data, requires an estimate of the length of the interval over which tissue is exposed to the labeling agent. Since this estimation is problematical for injection studies, the injection data were first transformed to equivalent minipump data using a factor of 6.83, which was the ratio of minipump labeling index to injection labeling index for all the control data. This factor was calculated from a total of 40 data points, 20 from pulse labeling and 20 from pump labeling. Conolly et al. (2003) then used a formula for the conversion of labeling index data into division rate constants due to Moolgavkar and Luebeck (1992). USEPA correctly note that that pulse label data should not be used with this formula. However, they apparently fail to understand

that the pulse label data were transformed into equivalent pump data before they were used for calculation of division rate constants (see Maintext p. 2-71 and Appendices, p. B-54, Uncertainty due to combining pulse and continuous labeled data). Thus, the USEPA statements that the use of the Moolgavkar and Luebeck (1992) formula is "extremely uncertain" (Maintext, p. 2-71, line 26) and "problematic" (Appendices, p. B-54, line 17) and is itself problematical."

EPA Response: EPA disagrees and has added further explanatory text to the revised Appendix (now D.2.2). It is important to understand the biological conditions under which Moolgavkar and Luebeck (1992) is applicable. Moolgavkar and Luebeck (1992) caution against applying their formula to pulse labeled data because, as indicated in their paper, it is unclear as to how long the (pulsed) label is available in the tissue. The reason this is a critical issue is that it takes about 4–8 hours of labeling for the relation between labeling index and the rate of cell proliferation to stabilize (see for example the data in Novak and Fedoroff ([1996](#))). Therefore, one should be concerned that a 2-hour episode of pulse labeling will tend to represent the pool of cells currently in S-phase rather than inform the rate of recruitment into that pool. Consequently, it is not evident that adjustment by a multiplier can produce a reasonably correct estimate of labeling index and cell division rate for the pulse-label data of Monticello et al. (1991) that is comparable to those from the continuous-label data of Monticello et al. (1996), especially not for purposes of combining and jointly analyzing the resulting estimates of cell proliferation rates.

Public Comment (Ramboll, continued):

"Issue 6- Use of historical controls: ...When the CIIT BBDR model was developed, we worked with 13 control tumors and did not anticipate the possibility of there being no control tumors. Is it reasonable to use this issue to justify rejection of the CIIT model? USEPA describes numerous alternative versions of the CIIT model in the IRIS Main text and Appendices and in the supporting publications (Crump et al. 2008; Subramaniam et al. 2007; Subramaniam et al. 2008). Thus, the ability within USEPA to develop alternative versions of the BBDR is not a constraint. Is there a reasonable alternative for scale-up of the CIIT rat BBDR model to the human version that USEPA could have implemented?...

The ratio of human to rat basal mutation rates described above and used in Conolly et al. (2004) is a means of accounting for Peto's paradox in the scale-up of the BBDR model from the rat to human. While that approach cannot be used when there are no control tumors, alternative methods of scaling are available. For example:

- [1] Set up a two-stage clonal growth (MVK) model for the rat nose as in Conolly et al. (2003).
- [2] Without specifying exposure to formaldehyde or any other chemical, adjust pmutr to produce a small lifetime tumor incidence, say 10⁻⁶. Repeat with increasing values of pmutr until lifetime tumor incidence is 100%. This exercise will need to

draw on relevant literature for biologically reasonable values of the growth advantage for intermediate cells (cells with one mutation) relative to normal cells (e.g. Conolly and Kimbell 1994).

- [3] Set up the MVK model for the human respiratory tract as in Conolly et al. (2004).
- [4] As in 2 above, adjust pmuth to obtain end of life tumor risks from 10⁻⁶ to 1.0 (100%).
- [5] The ratio of pmuth to pmutr at given levels of lifetime risk will describe rat to human scaling of the MVK model. The values of pmuth will be several orders of magnitude smaller than the corresponding values of pmutr.
- [6] This scaling would then be used with the human BBDR model to evaluate cancer risks associated with exposure to formaldehyde. For example, the calculated scaling factor(s) would be used to adjust the value of KMUrat, which defines the relationship between DPX and pmutr, to obtain KMUhuman."

EPA Response: Two points may be noted. First, EPA did use the rat BBDR model to derive a POD for determining a unit risk based on nasal cancers in the rat. Because the model used precursor data, this POD was derived below the range of the observed tumor incidence (Table 5-37, Section 5.2.1). Second, as described in Section 5.2.1, the human BBDR extrapolation model was found to be numerically unstable on two accounts: (1) in the rat to human scaling of the parameter associated with formaldehyde's mutational action, and (2) in the extreme sensitivity of minor perturbations on the assumed kinetics of initiated cells. The approach proposed above by the commenter purports to address only the instability that arises from scaling the parameter associated with formaldehyde's mutational action. Exploration of the proposed alternative method of scaling represents future research possibilities on other chemicals. Importantly, EPA's approach to use the human cancer unit risk estimate for the IUR was supported by the external peer reviewers.

Public Comment (Ramboll, continued):

"Issue 7- Initiated cells: ... USEPA (see Maintext, p. 2-73, Kinetics of initiated cells) and Crump et al. (2008) perturb the values of these optimized parameters, particularly the I cell division rate, and show that BBDR model predictions of tumor incidence changes quite dramatically. This is, however, expected behavior when the optimized value of a sensitive parameter is arbitrarily changed.... USEPA appears to not understand that the richness of the datasets for N cells and SCC highly constrains the kinetics of I cells, and specifically the value of the I cell division rate constant. It is just plain wrong to say that there are no relevant data for I cells." *[Please see docket for details and supporting information.]*

EPA Response: In some cases where clonal growth models have been successfully applied, *quantitative* information relevant to initiated cells are available in the form of preneoplastic foci. No such data are known for the case at hand. EPA however applied the same constraints as Conolly et al. (2003), namely that initiated cells ought to have only a very small growth advantage over normal cells and the criterion that the fit to the rat tumor incidence data is not statistically degraded in any modifications carried out for sensitivity analyses or for developing alternative model structures. The commenter also confuses the distinction between model calibration and prediction in regards considerations for sensitivity analysis. Sensitivity analysis of model predictions is now a required component of assessing model robustness; please see the section in the revised Toxicological Review on “extrapolation using a biologically based model.” (Section 5.2.1). The constraints used in these analyses are described in Section 5.2.1 and elaborated further in Appendix D.2.2. The results from alternative versions of the CIIT BBDR modeling of the rat data are presented. However, the CIIT human BBDR model developed for extrapolation of the rat model was demonstrated to be numerically unstable and could not be easily refined. The revised assessment compares the estimate derived from the CIIT model with the risk at the point of departure derived from the human epidemiology data in Section 5.2.1.

Other Public Comments on Toxicokinetics

- **Public Comment (ToxStrategies):** “U.S. EPA failed to respond to the spirit of the NRC’s comments on U.S. EPA’s 2010 draft formaldehyde assessment as it relates to toxicokinetics and MOA analysis.” *[Please see docket for details and supporting information.]*

EPA Response: Please see the 2023 NASEM consensus peer review findings on responsiveness to the NRC (2011) review above. The NASEM panel concluded that EPA was sufficiently responsive to the 2011 NRC comments on the 2010 draft formaldehyde assessment.

- **Public Comment (Natural Resource Defense Council):** “EPA’s presentation of the toxicokinetics – including the considerations of endogenous formaldehyde – is generally well-reasoned and well-presented.”

EPA Response: No response necessary.

- **Public Comment (American Chemistry Council):** “First, exogenous and endogenous formaldehyde are not similar -- they are identical. Claiming that there are no specific data indicating that they are different implies a conclusion that is not correct. While there are no data indicating that they are different, there is overwhelming evidence that formaldehyde is simply formaldehyde, regardless of where it is found or how it is generated. Secondly, stating that “the rate of cellular detoxification of exogenous formaldehyde remains unknown” ignores

the wealth of data on the half-life of formaldehyde in vivo and its non-specific binding to electrophilic centers, as well as an abundance of data on the kinetics of detoxification by glutathione and aldehyde dehydrogenase. Thirdly, one of the best characterized metabolic pathways is the one-carbon pathway that includes detoxification of formaldehyde by formaldehyde dehydrogenase (ADH5/GSNOR). Stating that “our understanding the contribution of endogenous formaldehyde is minimal” ignores the extensive database demonstrating that formaldehyde is an essential anabolic building block needed for normal metabolism. Simply, one cannot live in the absence of formaldehyde. Ignoring the essentiality of formaldehyde and the equivalence of formaldehyde regardless of its origin is a fundamental flaw of the Draft Assessment. When in a state of homeostasis, there is no added risk. As such, assessing added risk in the context of homeostasis must be a critical component of the Draft Assessment and must not be put aside. Key to understanding and managing exogenous inhaled formaldehyde is that at concentrations that do not upset normal variability (i.e., $\leq 1\text{--}2$ ppm) in metabolic processes, there is no added risk. The Draft Assessment must be revised to integrate relative dosimetry and define concentrations at which inhaled formaldehyde does not upset normal homeostasis. This topic was also the focus of the “bottom up” approach published by Drs. Swenberg, Starr and Lu in several peer-reviewed publications, which were incorrectly characterized in the Draft Assessment.”

“The Draft Assessment concludes that an understanding of homeostasis is not important when considering if there is added risk from exogenous exposure to formaldehyde. However, it is critical to understand the normal role of formaldehyde in the body, such as its central role in one carbon metabolism when determining adversity. The Draft Assessment also speculates that inhaled formaldehyde is somehow different than endogenously produced formaldehyde, an assumption that challenges our basic understanding of chemistry. Formaldehyde is a simple molecule; it is simply formaldehyde and should not be assessed differently or separately, depending on whether it is generated in the body or derived from an external source.”

“While the previous assessment and the current assessment provide a discussion of formaldehyde toxicokinetics, the 2022 Draft Assessment does not provide a discussion or consideration of when exogenous formaldehyde exposure appreciably alters normal endogenous formaldehyde concentrations, as recommended by NASEM.⁷⁹ This evaluation is critical in drawing conclusions regarding the potential for health effects following inhalation exposure of formaldehyde...Formaldehyde is a classic example of a chemical for which the beneficial and adverse effects can only be considered in the context of dose-dependent transitions. Endogenous formaldehyde is essential to life while high exogenous concentrations result in adverse effects. There are numerous studies that provide evidence that exogenous formaldehyde does not move past the portal of entry in multiple species (i.e., rats and non-human primates) following both acute and subchronic inhalation exposure across a range of air concentrations (0.001 ppm to 15 ppm) Kleinnijenhuis et al. 201382; Lu et al. 201083, Lu et al.

201184; Edrissi et al. 201385; Moeller et al. 201186; Lai et al. 201687; Yu et al. 201588; Leng et al. 201989)... Despite decades of research using highly sensitive methods, EPA continues to derive a risk for myeloid leukemia in the absence of even a hypothesized MOA and with clear toxicokinetic evidence that formaldehyde does not move beyond the portal of entry.”

“It is clear that toxicokinetic considerations are key to integrating the multiple streams of evidence necessary to make a sound data-driven conclusion. However, for leukemia, the Draft Assessment disregarded the wealth of information available that demonstrates that inhaled formaldehyde is not systemically distributed as well as dosimetry related to the naturally occurring background tissue concentrations of formaldehyde. Instead, the Draft Assessment relied on pure speculation to support what was apparently a pre-determined outcome. Notably, there is not one place in the Draft Assessment where the evidence against there being a mechanism of LHP carcinogenesis following inhalation exposure to formaldehyde is objectively discussed.” *[Please see docket for details and supporting information.]*

EPA Response: The IRIS assessment conclusions are consistent with the comment that “inhaled formaldehyde is not distributed to an appreciable extent beyond the respiratory tract to systemic sites; thus, inhaled formaldehyde is not directly interacting with tissues distal to the portal of entry to elicit effects.” The assessment likewise agrees that there is no experimentally supported, hypothesized MOA for formaldehyde induced-leukemia (Section 4.3). However, the EPA conclusions are consistent with EPA’s cancer guidelines ([U.S. EPA, 2005](#)) that lack of understanding of MOA does not provide grounds for discounting the observed epidemiologic data, which was considered *robust*. The document is in agreement with most of the scientific statements made by the commenter. However, EPA disagrees with certain perspectives on endogenous formaldehyde. As discussed in Section 2.7, “... the role of endogenously generated formaldehyde in human diseases is largely unknown... The focus ... is to estimate the risk over background that results from only the exogenous exposure, ... incidence of cancer or other health hazards that may potentially be attributed to endogenous formaldehyde is already accounted for in the background cancer incidence. Endogenous formaldehyde might be responsible for some portion of background risks for some health outcomes, particularly when normal detoxification pathways are deficient (e.g., [Pontel et al., 2015](#))); but that possibility is not the purpose of this review.”

F.3. NASEM REPORT CHAPTER 4: NONCANCER HEALTH EFFECTS; CHARGE QUESTION 3: RESPIRATORY SYSTEM (PORTAL-OF-ENTRY) HEALTH EFFECTS, CHARGE QUESTION 4: SYSTEMIC HEALTH EFFECTS, AND CHARGE QUESTION 5: NONCANCER RFC

F.3.1. Charge Questions on Noncancer Health Effects and the Noncancer RfC

This section discusses comments related to Charge Question 3 (Sections F.3.2 to F.3.5 of these responses), Charge Question 4 (Sections F.3.6 and F.3.7 of these responses), and Charge Question 5 (Section F.3.8 of these response).

Charge Question 3. Respiratory System Health Effects (Noncancer).

For each noncancer POE health effect considered in the assessment and outlined in (a) to (e), below (Note: descriptions of 3a to 3e are detailed within each health effect-specific section below), please comment on whether the evidence integration decisions for hazard identification are clearly described and scientifically justified (considering the extent to which the available data have been appropriately synthesized to describe the strengths and limitations). In addition, please separately comment on whether the dose-response decisions are transparent and scientifically justified, including study selection for dose-response analyses; point of departure (POD) estimates, including modeling choices and assumptions, and dosimetric adjustments; selection of uncertainty factors and derivation of candidate values; selection of organ- or system-specific RfCs (osRfCs); and confidence in the calculated values. For these well-studied health effects, confidence was consistently judged as either medium or high.

Charge Question 4. Systemic (i.e., nonrespiratory) Health Effects (Noncancer).

For each noncancer systemic health effect considered in the assessment and outlined in (a) to (c), below (Note: descriptions of 4a to 4c are detailed within each health effect-specific section below), please comment on whether the evidence integration decisions for hazard identification are clearly described and scientifically justified (considering the extent to which the available data have been appropriately synthesized to describe the strengths and limitations). In addition, please separately comment on whether the dose-response decisions are transparent and scientifically justified, including study selection for dose-response analyses; POD estimates, including modeling choices and assumptions, and dosimetric adjustments; selection of uncertainty factors and derivation of candidate values; selection of osRfCs; and confidence in the calculated values. Confidence was consistently lower for these effects as compared with POE effects.

Charge Question 5. Noncancer RfC.

See Section F.3.8 for the description of the charge question.

F.3.2. Sensory Irritation

Charge Question and Peer Review Comment Summary

Charge Question 3a. Sensory Irritation

- The assessment concludes that the **evidence demonstrates** that inhalation of formaldehyde causes increased sensory irritation in humans, given appropriate exposure circumstances.⁴⁵ Well-conducted studies in humans and animals support this hazard conclusion, and strong mechanistic evidence provides a plausible mode of action (MOA) for the identified outcome.
- A POD from Hanrahan et al. (1984), a human study, was ultimately selected to calculate an osRfC of 0.009 mg/m³ for eye irritation. A composite uncertainty factor (UF_C) of 10 was applied to address intraspecies uncertainty (UF_H). The assessment also considers PODs from controlled human exposure studies and discusses their utility for developing an RfC for lifetime exposure as well as their potential increased utility for purposes outside the scope of the current assessment (e.g., derivation of an acute RfC).

Peer Review Comment Summary

- 0 Tier 1 Recommended Revisions
- 5 Tier 2 Suggested Revisions
- 1 Tier 3 Future Consideration
- 10 Consensus Panel Findings (numbered below)

Literature Identification

Peer Review Findings and Comments

- **Tier 2 Suggested Revision (Recommendation 4.1):** To maximize transparency and facilitate replication, EPA should clarify the Medical Subject Headings (MeSH) terms used, list and justify any MeSH terms that were excluded (e.g., eye, ear, nose, or skin), provide the list of national and international reviews and assessments used to identify additional references, and provide more specific links to the Health & Environmental Research Online (HERO) database where the screening decisions are documented (see Appendix A, Section A-232, line 4).
 - *Related text from Appendix E:* “For sensory irritation, it is unclear if other MeSH terms like eye, ear, nose, or skin were used in the search because they were not reported in Table A-31. The number of studies (38 observational and 20 controlled trials) included in the 2022 Draft Assessment, as presented in Figure A-22, does not

⁴⁵ For all non-cancer health effects for which the evidence demonstrates or indicates an effect is caused (or likely to be caused) in humans by formaldehyde inhalation, the exposure circumstances that may be necessary for this to occur are estimated through subsequent dose-response analysis. This phrase is included to signify the linkage between hazard identification and dose-response analysis.

match the number of studies presented in Tables A-34 (13 residential studies), A-35 (1 school study), and A-36 (21 controlled trials). Neither of the numbers match with Tables 1-1 (14 controlled trials) and 1-2 (6 residential studies) in the Main Assessment.”

- *Related committee text:* "EPA used four databases, and curated reference lists in published reviews and other national or international health assessments of formaldehyde (see Appendix A, Section A-231), although the flow diagram shows that only two databases were used (Figures A-22 and A-263)."

EPA Response to Tier 2 Suggested Revision 4.1: The following revisions were made in response to this recommendation:

- The specific search terms (and MeSH terms, where applicable) were and are documented in the Supplemental Materials (i.e., Appendix B.2 in the revised assessment). No MeSH terms were excluded for sensory irritation; however, PECO criteria were used to screen studies. As described in the assessment methods section, all search strings were submitted as keyword searches, which in the case of PubMed includes MeSH terms by default, except as specified with tags like [majr] which limited the search to only when the term (and any selected subheadings) are indexed as a major topic heading (not all MeSH terms). Only the terms for “formaldehyde” were tagged with [majr]. In addition, as described in the assessment methods, additional references were identified from the 2010 draft Toxicological Review and from the reference lists in the identified journal articles.
- The rationale for use of certain PECO criteria has been expanded in the methods section of the revised assessment (see Section 2.2). A footnote has been added to the PECO table for sensory irritation to specify exclusion of dermal effects.
- The revised assessment has been corrected to reflect the two databases searched for sensory irritation (not four).
- As noted in Section 2.2, for sensory irritation, the only assessment used to search for missed studies was the prior 2010 public comment draft developed by the IRIS Program. This is noted in the description of search strategies in the assessment methods section
- Studies deemed *not informative* and thus not extracted or discussed in the evidence synthesis are now listed in the introduction to each health effect-specific section. This was not done for mechanistic study evaluations given the larger numbers of such studies and the linked discussions of overlapping mechanistic evidence across the different health effect sections. The mechanistic studies identified as *not informative* are documented in Appendix B.

- The number of sensory irritation studies identified in the literature flow diagram corresponds to the number of studies in the study evaluation tables (although there was one correction made, see below) and the main toxicological review. Specifically, 58 total sensory irritation studies met the PECO criteria prior to the SEM update, including 38 observational studies and 20 controlled exposure studies (the literature flow diagram in the 2022 draft indicated 38 observational and 20 controlled exposure studies). One citation, a description of formaldehyde concentrations, was included within a cell with the PECO reference, but has now been included in the exposure column. The SEM update identified an additional five references the supplemental appendices; one was *possibly impactful* but was already incorporated based on previous searches. The 20 unique HERO IDs for the identified controlled exposure studies are evaluated and documented in the 17 rows of the controlled exposure table representing the different experiments. Likewise, the evaluation of the 38 unique observational studies is documented in the following in tables for residential studies, school studies, anatomy course studies (this table was not counted in NASEM review), and occupational studies (it appears this table was not counted in the NASEM review).

Separately, for all health effects, the numbers of studies in the literature flow diagrams and the study evaluation tables do not match the number of studies in the main assessment text or tables because studies classified as *not informative* in the study evaluations (documented in the Appendices) were not discussed in the Toxicological review in the 2022 draft. However, for transparency in the revised draft, the studies identified as not informative are now noted in the introduction to each health effect section (which also aligns better with more recent EPA-IRIS methods).

- **Finding (1):** EPA excluded outdoor exposure studies (Table A-32) without providing adequate justification [see report for expanded discussion].
- **Tier 2 Suggested Revision (Recommendation 4.2):** EPA should include the body of evidence from outdoor exposure studies at the preliminary stage to derive a more holistic and objective assessment of the scientific literature.
 - *Additional comments related to this topic:* "...found unconvincing EPA's argument that studies with lower exposure levels may have a limited ability to detect associations between formaldehyde exposure and health effects." "The committee notes further that the assertion that outdoor levels of formaldehyde are universally below 0.005 mg/m3 may not correctly represent true population exposures..." See **TABLE 4-2** Formaldehyde Levels in Outdoor Air Reported in Selected Studies."

EPA Response to Tier 2 Suggested Revision 4.2: The revised assessment clarifies this rationale to highlight that this decision was not only about low exposure levels but also that the exposure measurements in these studies were poor surrogates for individual-level health effects (see Section 2.2.1). For example, the eight studies tagged as excluded because of outdoor exposure in the formaldehyde HERO page all rely on stationary sensors from which individuals assessed for health effects reside some distance from (typically within several miles). These sensors are generally only collecting data for a short period of time and involve collection of multiple pollutants (several studies do attempt to consider this source of confounding). On top of that uncertainty, ambient levels of formaldehyde in outdoor air are significantly lower than those measured in the indoor air of workplaces or residences, and the exposure range was narrow in many epidemiological studies of ambient exposure (<0.005 mg/m³), limiting their sensitivity to find any associations with health outcomes even if they existed. Temporal (seasonal and diurnal) and spatial variation in formaldehyde concentration is strongly influenced by photochemical interactions and traffic emissions. Consequently, the potential for exposure misclassification for estimates of individual exposure using mean formaldehyde concentrations from central outside monitors is greater than from indoor formaldehyde measurements. Therefore, the few studies examining health effects in relation to outdoor formaldehyde concentrations remain excluded in the revised assessment.

- **Finding (2):** In the Main Assessment (see Figure 2-3), the box representing typical formaldehyde levels depicts a sharp delineation between lower outdoor and higher indoor levels. In real-world exposure scenarios, that is unlikely to be the case; rather, a continuum of concentrations with overlapping outdoor and indoor levels is a more likely scenario (see Table 4-2 [of study report]). Figure 2-3 could show representative ranges of outdoor and indoor formaldehyde concentrations levels more accurately (see Appendix E).

EPA Response to Finding 2: The range of outdoor and indoor formaldehyde levels depicted in the figure (in Section 5.1 of the revised assessment) have been removed from the figure. In revising the assessment, it was decided that including such information in the figure is inappropriate, since IRIS assessments do not include an exposure assessment. For context on the RfC and study-specific PODs, a footnote describes one estimate of formaldehyde levels in outdoor and indoor air, and recommends the reader consult authoritative sources on exposure assessment.

Related Public Comments

No related public comments were submitted.

Study Evaluation

Peer Review Findings and Comments

- **Finding (3):** The outcome-specific criteria EPA used to evaluate the human studies were generally appropriate but the application of the specified criteria across studies appears inconsistent and it is not clear if the same set of quality criteria was applied uniformly across studies. The potential for selection bias is addressed for each of the studies. Determining the potential for selection bias in a study is challenging as the specific selection fractions that lead to potential bias are generally unknown. Cross-sectional studies are generally at risk for selection bias. The potential for selection bias may also be signaled by a low response rate and non-representativeness of a study population on exposure and outcome frequency. The committee could not find a consistent approach in how EPA evaluated the potential for selection bias, for example, across the range of observed response rates.
 - *Additional comments related to this topic:* “[The] committee found some inconsistencies between the presented human sensory irritation outcome– specific evaluation criteria and how they were applied to the Hanrahan et al. study, as well as how study limitations were presented for other sensory irritation studies...”
 - Additional comments on this topic: “descriptions include some of the quality characteristics listed in Appendix A.5.1 and in the text of A.5.2, but not others. For example, none of the criteria in the Selection Bias domain (recruitment, selection into study, participation independent of exposure) are included in Table A-33. ..., such as the use of a validated data collection instrument, which is included in the high and medium descriptions, but not those for low. ... [and] sample size is introduced as a study quality characteristic, but without discussion to inform when the sample size would be considered “large,” “small,” or “may be a limitation.”

EPA Response to Finding 3: The tables describing the outcome-specific criteria for sensory irritation (and all of the other health effect categories) were revised to include criteria for all of the evaluation domains including population selection, exposure assessment, outcome ascertainment, confounding, etc (see Section 2.3 of the revised Toxicological Review). Criteria are described for domain ratings of good, adequate and deficient. For example, for the outcome domain, a rating of “deficient” is assigned if the data collection instrument was not described and there is uncertainty whether symptoms were reported without knowledge of exposure status and concern with regard to a bias toward an overestimate of risk. For the analysis /other domain, while a specific number of participants is not given, a “deficient” rating is assigned if the sample size was small enough to result in an increased potential for unreliable results. Occupational studies with a cross-sectional design and potential for a healthy worker survival bias are considered to be adequate or good for population selection because the bias is toward the null hypothesis and would not be a concern if health risks were observed. However, the bias is noted for that domain. The application of the

criteria to individual studies, including the Hanrahan et al. study, was reviewed to ensure that the evaluations included each of the domains, any limitations were noted and were consistently applied across studies. Table cells for domains considered “deficient” are shaded to highlight that the limitation is expected to result in bias or results that are unreliable.

The review of the study evaluations resulted in changing the confidence for some studies. For sensory irritation, confidence in ([Alexandersson and Hedenstierna, 1989](#)) was changed from *low* to *medium* and confidence in ([Berglund et al., 2012](#)) was changed from *high* to *medium*.

- **Finding (4):** The 2022 Draft Assessment described specific aspects of exposure assessment that EPA considered when evaluating individual studies, such as having a measurement protocol, duration of exposure measurement period, number of samples obtained, consideration of temperature and humidity, and percentage of measurements below the limit of detection (Appendix A, Section A-236). The text does not clarify whether systematic or random contributors to inaccuracy were assessed in evaluating information bias in individual studies, nor does it describe how the assessment took account of multiple sources of information bias.

EPA Response to Finding 4: EPA developed evaluation criteria that assessed whether any identified information bias had the potential to attenuate the measurement of risk or exert a bias away from the null toward an overestimate of risk. Systematic contributors were of more concern because the resulting bias could result in an overestimate of the measure of association. If a study reported an association of an endpoint measure with exposure, confidence was not necessarily decreased if a potential bias was identified that was expected to attenuate the resulting risk estimate. If both exposure and outcome were found to be limited by bias, specifically away from the null, (e.g., found to be deficient), then the confidence in the study was decreased. This was clarified in the revised assessment (see Section 2.3).

- **Finding (5):** The 2022 Draft Assessment lacks clarity as to how the availability and characteristics of outcome assessment questionnaires were used to rate the confidence level of a study. For example, Mueller and colleagues (2013) provide a reference for their questionnaire, and EPA assessed this study as having a high confidence level. However, Green and colleagues (1989) do not provide a reference for their outcome assessment questionnaire, yet EPA categorized it as well as having a high confidence level (Main Assessment: Table 1-1, pp. 1–18). EPA categorized several studies (Hanrahan et al., 1984; Liu et al., 1991; Sexton et al., 1986) as having medium confidence because of concerns regarding the questionnaires used to assess symptoms without adequate explanation of their deficiencies.

EPA Response to Finding 5: EPA generally considered that the use of a questionnaire for outcome assessment was an adequate measure of outcome assessment given that respondents were blinded

to exposure status. Use of a validated, or widely used outcome assessment methodology increased confidence in the outcome measure. Use of unspecified questionnaires increased uncertainty with respect to misclassification of outcome status but was not expected to greatly change the effect estimate. The evaluation criteria for outcome ascertainment using questionnaires have been clarified to indicate that if the questionnaire was less well described, the domain is classified as “adequate” if there is information in the study to conclude that the responses to the questionnaire were not influenced by knowledge of the respondent’s exposure status. Both Hanrahan et al. and Liu et al. did not describe the questionnaire but stated that respondents were blind to their formaldehyde levels. The controlled exposure studies by Green et al. ([Green et al., 1987](#); [Green et al., 1989](#)) did not provide a reference for the questionnaire, but described the symptoms assessed including two nonirritant “sham” symptoms to allow an assessment of information bias. In addition, an index of severity also was assessed. While the sensitivity and specificity of the questionnaire was not described, EPA concluded that the misidentification of irritation symptoms by respondents during the experiments was low and the information provided was considered “adequate” to reduce concern regarding information bias. This has been clarified in the revised assessment.

EPA found that “questionnaire” is mentioned only once in the Handbook, and in the Participant Selection section. This is a useful future consideration for EPA to more fully include the occasional use of outcome questionnaires under “outcome ascertainment.”

- **Tier 2 Suggested Revision (Recommendation 4.3):** EPA should explicitly state what constitutes an adequate assessment of outcomes when a questionnaire is not cited, and explicitly provide the criteria used to determine the adequacy of a questionnaire. Information on these aspects of outcome assessment would facilitate replication of the EPA approach. It would be preferable for EPA to use age categories generally instead of ambiguous descriptors.
 - **Additional comments on this topic:** “therefore, the exposure assessment was not concurrent with the symptoms”... “does not describe the data collection instrument at all, which is seemingly inconsistent with a medium quality rating.”

EPA Response to Tier 2 Suggested Revision 4.3: In the revised assessment, EPA has expanded its description of the study evaluation considerations with information or considerations that would result in a study’s methods for each domain being rated as high, adequate or deficient. For a symptom questionnaire, a validated tool is in the high category; however, an unspecified tool is considered adequate if responses were given without knowledge about the exposure levels experienced. This clarification has been added to the tables in the methods section (Section 2).

- **Finding (6):** Some studies are potentially affected by multiple limitations. The approach used to classify the level of confidence for such studies is unclear. If there are three sources of potential bias with no apparent directionality (i.e., either over- or underestimation) for each bias, how

will the quality of that study be determined? The committee considered as an example the evaluation of the study by Main and Hogan (1983). In Table 1-2 [see report], two problems with this study are identified, with one box fully colored. The classification scheme in Figure II would suggest this study is of medium confidence, but it is classified as having low confidence, presumably because the fully colored box for “other” signifies a greater “degree of limitation.” Additional explanations related to Figure II would make it easier to understand individual study confidence determinations, such as when there is only one potential source of bias, and that bias is likely to overestimate the exposure effect.

- **Tier 2 Suggested Revision (Recommendation 4.4):** To increase transparency, EPA should document how it assessed the potential for different types of biases, the directionality of resulting biases, and the number of biases, and state how each combination should be interpreted in terms of high, medium, low, or not informative study confidence.
 - *Related committee comments:* “For sensory irritation, EPA needs to clarify how concurrent assessment ensures that exposure preceded health effects for studies in which the outcomes are considered to be acute effects.” “In Table 1-2, where the entry for the Main and Hogan (1983) study shows two sources of biases with one box fully colored. The classification scheme in Figure II would suggest this study is of medium confidence, but a fully colored box likely means something more, which needs to be explained.”

EPA Response to Tier 2 Suggested Revision 4.4: The tables describing the evaluation criteria for each health effect (including specific outcomes) that result in good, adequate or deficient classifications for each domain now clarify how study limitations were evaluated and which were most influential (Section 2.3). The direction of bias was important and is noted if possible. While the classification of a study as *low* or *not informative* was not based on a specific number of deficient classifications, generally a bias toward an inflated measure of association would result in *low* confidence and additional study-specific limitations would result in *not informative*. The pictorial displays are no longer used to convey the evaluation conclusions, rather, the influential limitations are noted in the summary column with the confidence classification of *high*, *medium* or *low*. Regarding Main and Hogan ([Main and Hogan, 1983](#)), the exposed group (administrative employees working in mobile home offices) and the referent group (police officers) were likely dissimilar for a number of attributes associated with the experience of irritation symptoms in their jobs. In addition, the nature of the exposure setting (working in mobile homes versus in the field) raised the potential for the exposed respondents to be aware of their exposure to formaldehyde. These limitations are noted in the study evaluation tables for this study (Appendix B.3).

- **Finding (7):** The considerations listed for classification of study confidence and evaluation of each study by at least two independent experts is consistent with EPA's state-of-practice methods.
- **Tier 3 Future Consideration (Recommendation 4.5):** In the population selection criteria, the potential for selection bias could be assessed by considering the proportion of the eligible population invited to participate in the study and the proportion of the eligible population that was ultimately included in the analysis. EPA should state the criteria used to assess selection bias in the text, tables, and figures.

EPA Response to Tier 3 Suggested Revision 4.5: EPA appreciates this future consideration for potential methods development in evaluating selection bias in individual studies to inform future assessments.

- *Un-Tiered Committee Comment* on specific study confidence determinations (Appendix E):
 - [Liu et al., 1991] "On page A-267 column 2, EPA compared the reporting of asthma prevalence in Liu et al. (1991) (~4 percent) to the national prevalence of asthma at that time and concluded there was "minimal concern for selection bias" in that study. The committee has some concern that this conclusion may not be fully supported. For example, there are several sets of selection factors between the general U.S. population and the study population that need to be considered... At best, the comparison that EPA described in page A-267 for the Liu et al. (1991) study makes a case for the generalizability (external validity) of the findings, if it is assumed that the Liu study sample is representative of the U.S. general population. It does not make a case against selection bias. One argument against selection bias would be to demonstrate that those who participated have comparable characteristics with those who were invited but did not participate. It is also not known how the randomly selected sample compares with the target to the accessible population. If it is not a true random sample, there could be additional potential for selection bias."

EPA Response to Un-tiered Committee: EPA has revised the evaluation of Liu et al., 1991 to reflect uncertainty regarding differential or nondifferential selection based on knowledge of exposure or symptoms. The conclusion based on asthma prevalence noted above is no longer included in the revised assessment.

Related Public Comments

No related public comments were submitted.

Evidence Synthesis and Synthesis Judgments

Peer Review Findings and Comments

- **Finding (8):** EPA provides a reasonable basis, one that is consistent with its state-of-practice methods, for labeling as robust the evidence relating formaldehyde exposure and sensory irritation.
 - *Related committee comments* (Appendix C): "For Steps 5 and 6 (synthesis of results and synthesis judgments), EPA was clear about the basis for the strength-of-evidence determination, and it was applied in a way that was consistent with the stated framework. For evidence integration (Step 7), the conclusion was supported by the evidence presented." AND "The text did not address the strength and precision of the estimates (although this category is not a required criteria to qualify as a robust study, as per Table IV), or the criteria from Table VI that there is "reasonable confidence that chance, bias, confounding, can be ruled out." Although these criteria are not discussed..." AND "However, the table mistakenly states that there were four high- and medium-confidence residential studies, when there were no high-confidence residential studies (there were four medium-confidence studies). In addition, EPA notes that there were numerous high- and medium-confidence longitudinal (occupational and anatomy lab) studies, but these studies receive limited discussion in the main text. It is unclear why EPA appears to have de-emphasized these studies when evaluating sensory irritation, but then used them as a basis for the robust determination..."

EPA Response to Finding 8: The errors in the evidence integration table have been corrected.

Related Public Comments

No related public comments were submitted.

Overall Conclusions about the Hazard Descriptor

Peer Review Findings and Comments

- **Finding (9):** EPA's overall hazard conclusions are supported by the scientific evidence and are consistent with EPA's state-of-practice methods.

Related Public Comments

- **Public Comment (American Chemistry Council):** "The 2022 Draft Assessment gives equal weight to all the study designs, and simply attributes the variation seen as part of human variability. EPA does not appear to acknowledge that subjective measures can easily be influenced by other factors such as odor perception (Cometto-Muñiz and Cain 1991; Brüning et al., 2014). Six studies were considered for derivation of Points of Departure (PODs) (Hanrahan

et al., 1984; Kulle et al 1987; Anderson and Molhave 1983; Liu et al, 1991; Mueller et al., 2013; Lang et al 2008), but only three (Andersen and Molhave, 1983; Hanrahan et al., 1984; Kulle et al 1987 –all medium quality as assessed by EPA, where Andersen and Molhave 1983 was a chapter as opposed to a peer-reviewed publication) were considered for POD derivation in spite of higher quality studies (Lang et al., 2008; Mueller et al., 2013) being available. (Lui et al 1991 was also considered medium quality.)...given the stronger quality of these studies [Mueller et al. 2013 and Lang et al. 2008], RfCs could have been derived from NOELs.”

“EPA inappropriately dismisses carefully designed chamber studies [Mueller et al. 2013 and Lang et al. 2008].”

“From the authoritative assessments and reviews published prior to 2019 (ANSES 2018, 2019; Danish Ministry of the Environment 2014; ECHA 2016, 2019; SCOEL 2016; EPA 2008, 2010; WHO 2010), there is a consistent identification of the Lang et al. (2008) and Mueller et al. (2013) studies as key for defining the most sensitive endpoints from which to derive acceptable concentrations of formaldehyde following acute/short-term inhalation exposures. The authoritative bodies identified these two studies because they are high-quality studies, where exposure concentrations were known and controlled, exposures were randomized and double-blinded, and individuals served as their own controls. They noted the studies included both subjective and objective measures, and individuals who were identified as being hyper- or hyposensitive to the effects of formaldehyde. Importantly, there is no evidence indicating an increased sensitivity to sensory irritation to formaldehyde among people often regarded as susceptible (asthmatics, children, and older people) (WHO 2010).”

“The Draft Assessment sets aside the controlled human chamber studies, in conflict with NAS (2011) recommendations.” *[Please see docket for additional details and supporting information.]*

EPA Response: To clarify, the IRIS assessment does not apply a reduced UF_H for sensory irritation based on subjects being children or asthmatics. In fact, the populations noted as potentially more susceptible to sensory irritation in the UF selection section were smokers, women, those with chronic allergic diseases, and the elderly. The assessment cites specific data from the observational studies modeled to calculate a POD for this endpoint that show such apparent increases in susceptibility.

The assessment makes careful consideration of the controlled exposure studies in human volunteers and derives several candidate values considered for the RfC based on these studies. For the controlled exposure studies, the fact that these experiments only included a very small number of volunteers with quite limited heterogeneity (e.g., all healthy and middle-aged) led to application of the full UF_H . It is noted that the commenter- indicated studies of healthy, middle-aged (approximately 20–40 year old) volunteers by Lang et al. (2008) and Mueller et al. (2013), the former of 21 men and women and the latter of 42 men with or without nasal hypersensitivity to CO_2

(not formaldehyde), were considered and not advanced for dose-response analysis. Since release of the external review draft, the IRIS assessment was revised to include tables in Section 5 explaining, study-by-study, why individual studies were or were not advanced for dose-response analysis. The two intentional exposure studies by Lang and Mueller are included in those tables in Section 5.1.1, which now outlines why those studies were not preferred for use in quantitative analyses. Although not advanced, these studies possess similar limitations to the other intentional human exposure studies advanced by EPA (Kulle et al., 1993 and Andersen and Molhave, 1983), and thus identical modeling approaches and UF selections would likely be applied.

Notably, although the PODs are much higher, after applying larger uncertainty factors (e.g., to address interindividual human variability), the cRfCs from the two controlled exposure studies advanced to dose-response analysis for this outcome were generally in agreement with the cRfC based on the residential epidemiology studies (see Section 5.1 in the revised assessment). The decision to select the residential cRfC over cRfCs based on the controlled exposure studies is presented in Section 5.1.4 and aligns with the consensus peer review finding (10).

- **Public Comment (Monell Chemical Senses Center):** “Formaldehyde has a pungent odor that can be sensed at low ppb levels in air and which at higher concentrations can elicit the onset of sensory irritation; both serve as a warning system of exposure. The lowest potentially undesirable effect levels in humans are generally recognized as odor detection via the olfactory nerve (CN I), resulting in decreased air quality, and following that, eye and nasal tract chemosensory stimulation (i.e., chemesthesis)¹. Chemesthetic detection of formaldehyde occurs via cutaneous nerve fibers in the eye and mucus membranes in the nose, via the trigeminal nerve (CN V). This ability to detect formaldehyde or any pungent chemical in the ocular and nasal mucosa represents a normal sensory physiological process and is not tissue irritation (i.e., an inflammatory response) nor an adverse health effect.”

“Olfactory and chemesthetic detection of formaldehyde can prompt adaptive responses such as preference for mouth vs nasal breathing to reduce unpleasant odor or responses such as eye blinking. However, these sensory responses occur from neuronal stimulation, and are therefore not precursors to an adverse health effect. In contrast, tissue irritation that is related to formaldehyde binding to macromolecules, including protein-protein cross-linking, in nasal epithelial cells, resulting in tissue damage and inflammation is adverse. The differing mode of action for nasal chemesthesis vs nasal tissue irritation represents the difference between a sentinel vs a precursor effect.”

“From a regulatory viewpoint, the continuum of effects associated with increasing formaldehyde air concentrations can be broken down into a definable sequence: 1) at low concentrations, odor detection and/or sensory chemesthesis or other sensory detection (NOAEL); 2) at higher concentrations, uncompensated upper respiratory tract tissue irritation (the first adverse or critical effect, LOAEL); 3) at yet higher concentrations, cytotoxicity and

regenerative hyperplasia (AEL); and 4) at the highest concentrations, potentially nasal tumors (FEL). Consequently, it is important to determine the concentration(s) of formaldehyde that can reliably be associated with non-adverse warning properties such as odor detection and chemesthesis, as well as concentrations objectively causing adverse tissue effects such as cytotoxicity and inflammatory responses.”

“There is substantial inconsistency in how participant demographics are assessed. The PODs which could be derived based on two considered controlled human exposure studies were rated with less confidence because the study participants were young, healthy volunteers. The more robust studies by Lang et al and Mueller et al (Lang, Bruckner et al. 2008, Mueller, Bruckner et al. 2013) were dismissed for these same reasons and because the USEPA found it ‘difficult to define an adverse response level cutoff for either objective or subjective symptom scores’ (Table 11, p35). However, according to the Hanrahan et al study, “negative coefficients for respondents age were found to be significant for both ocular irritation models, indicating a higher report prevalence in younger persons”(Hanrahan, Dally et al. 1984). The well-established declines in olfactory and trigeminal (chemesthetic) sensitivity with age and age-related diseases means a younger, healthier population (which is typically the demographics of participants in controlled exposure studies) will be most sensitive to the odor and irritancy of formaldehyde (Doty, Shaman et al. 1984, Doty 1989, Wysocki, Cowart et al. 2003).”

“The Draft Document also marginalizes controlled exposure studies for excluding smokers, perhaps assuming that this is a sensitive subpopulation that should be included. However, smoking decreases the sensitivity of the cornea and conjunctiva (Thomas, Jacob et al. 2012) and smokers’ show reduced nasal/ocular sensitivity to many irritant chemicals (Cometto-Muñiz and Cain 1982, Dunn, Cometto-Muñiz et al. 1982), thus it is appropriate to exclude them in a controlled exposure study where the critical endpoint is sensory irritation. As well, asthma and other health conditions are not considered to predispose individuals to be more sensitive to formaldehyde. Thus, the concerns raised about the populations tested in these well-conducted controlled exposure studies are without merit.” *[Please see docket for details and supporting information.]*

- **Public Comment (American Chemistry Council):** “Sensory irritation and other annoyance endpoints are inappropriate endpoints for developing RfCs. Sensory irritation of the eyes and upper airways are not considered *adverse* by authoritative bodies and reviews (WHO, 2010; Nielsen et al., 2017; ECHA, 2019a). Perception of, or sensory stimulation by, an irritating substance would not be considered adverse as perceptions of, or stimulations by, an unpleasant smell do not affect the form or function of the tissue or organism. Eye and nasal tract chemosensory detection and nerve stimulation that results from inhalation exposure to formaldehyde is an example of chemesthesis. Chemesthesis is a normal physiological response without functional impairment or pathological change. Thus, sensory recognition of

formaldehyde is not adverse and is similar to sensory responses to other chemicals or environmental stimuli such as tearing when exposed to fumes from cut onions or blinking when suddenly exposed to sunlight.”

EPA Response (to both public comments above): EPA disagrees that sensory irritation is an inappropriate response for developing an RfC, and this conclusion was supported by NASEM. EPA has retained the same hazard conclusions regarding sensory irritation in the revised assessment. As described in Sections 3.2.1 and 5.1, sensory irritation (e.g., “burning eyes”), at the magnitude and severity reported in most studies, is considered a minimally adverse outcome. However, the quantification of sensory irritation and the noncancer toxicity value for this effect has changed (i.e., the toxicity value is now higher and is no longer one of the health effects supporting the RfC). In accordance with EPA guidelines, once a hazard has been identified, the goal of dose-response analysis is to calculate an RfC that is reasonably protective against those identified hazards, including in susceptible groups. Thus, when selecting studies to support an RfC, in addition to evaluating the study methods, the evaluation also considers the ability of the tested population to address human variability as well as the study-specific findings (which may differ because of features such as the specific population examined or the study size). This is particularly true for an endpoint such as sensory irritation, which exhibits a large degree of inter-individual variability in sensitivity, and it is even more important when the considered studies are small.

As noted previously, the assessment makes careful consideration of the controlled exposure studies in human volunteers and derives several candidate values considered for the RfC based on these studies. For the controlled exposure studies, the fact that these experiments only included a very small number of volunteers with quite limited heterogeneity (e.g., all healthy and middle-aged) led to application of the full UF_H . It is noted that the commenter- indicated studies of healthy, middle-aged (approximately 20–40 years old) volunteers by Lang et al. (2008) and Mueller et al. (2013), the former of 21 men and women and the latter of 42 men with or without nasal hypersensitivity to CO_2 (not formaldehyde), were considered and not advanced for dose-response analysis. Since release of the external review draft, the IRIS assessment was revised to include tables in Section 5 explaining, study-by-study, why individual studies were or were not advanced for dose-response analysis. The two intentional exposure studies by Lang and Mueller are included in those tables in Section 5.1.1, which now outlines why those studies were not preferred for use in quantitative analyses. Although not advanced, these studies possess similar limitations to the other intentional human exposure studies advanced by EPA (Kulle et al., 1993 and Andersen and Molhave, 1983), and thus identical modeling approaches and UF selections would likely be applied.

With few exceptions (i.e., large population studies encompassing a wide range of demographics, including reasonable representation of known or expected susceptible groups), the available studies are judged as unlikely to sufficiently address the identified differences in susceptibility described in Section 4.1 of the Toxicological Review. Also, as outlined in greater detail

in other EPA responses, characterizing sensory irritation as a non-adverse “warning property” occurring at lower formaldehyde levels than those causing other health effects is not supported by the data. Thus, protecting against this response, especially based on acute exposure in healthy volunteers, would not be expected to protect against other chronic health effects, including cancer (particularly when considering human variability).

However, although the PODs are much higher, after applying larger uncertainty factors (e.g., to address interindividual human variability), the cRfCs from the two controlled exposure studies advanced to dose-response analysis for this outcome were generally in agreement with the cRfC based on the residential epidemiology studies (see Section 5.1 in the revised assessment). The decision to select the residential cRfC over cRfCs based on the controlled exposure studies is presented in Section 5.1.4 and aligns with the consensus peer review finding (10).

Dose-Response Evaluation

Peer Review Findings and Comments

- **Tier 2 Suggested Revision (Recommendation 4.6):** EPA should clarify and clearly state the criteria used to select the studies for dose-response analysis of noncancer endpoints.
 - *Related committee comments* (Appendix C): "the recommendation of the 2014 National Research Council (NRC) report to use data from multiple studies when possible." ... "The committee notes the complementarity of the two studies [Liu and Hanrahan] and the broadening of the range of exposures considered in the combined analysis." [Note: an initial approach to this estimation for sensory irritation specifically was outlined by the committee in Appendix D of the report].
 - *Related committee comments* (Appendix C): "categories of Overall Confidence Conclusion, Study Confidence, Population, and Exposure Information [are described; see also Table C-3],... although EPA did not provide further descriptions of those considerations to aid in interpretation of how they were applied." ... "The text of the Preface provided further considerations for study selection ...stating that EPA puts particular emphasis on the accuracy of formaldehyde exposure, the severity of the observed effects, and the exposure levels analyzed." ... "For sensory irritation, it stated that studies that conducted the exposure assessment concurrently with the outcome assessment were the most informative for RfC derivation because formaldehyde induces a rapid irritant response." ... "EPA concluded that the Hanrahan exposure assessment “reflect[ed] the usual, relatively constant formaldehyde concentrations in the residences” (p. 2-9), which is not supported by the short duration and the single occasion when measurements were taken.”
 - *Related committee comments* (Appendix C): "The committee finds that the 2022 Draft Assessment does not adequately acknowledge the full scope of uncertainty associated with the Hanrahan et al. dose-response relationship."

- *Related committee comments* (Appendix E): “Regarding sensory irritation, in Tables 1-1 and 1-2, several studies have been identified as high or medium confidence, but only six were included in the sensory irritation dose-response analysis (Table 2-1). It is unclear why only these six studies were chosen.”

EPA Response to Tier 2 Suggested Revision 4.6: EPA has expanded the description in the assessment methods in Section 2.7 to detail how studies are evaluated against multiple considerations to determine which studies are best suited for advancement to dose-response analyses. In addition, expanded tables on study selection for each health outcome have been added to the dose-response section (Section 5 of the revised assessment) to reflect study performance against those considerations. [Note: The BMD Technical Guidance (Section 2.1.3) states: “Following a complete review of the toxicity data, the risk assessor selects the studies for BMD analysis, based on the human exposure situation being addressed, the quality of the studies, the reporting adequacy, and the relevance of the endpoints. The process of selecting studies for BMD analysis is intended to identify those studies for which modeling is feasible, so that BMDs can be calculated. All relevant studies should be considered for modeling. In some cases, the selection process will identify a single study or very few studies for which calculations are appropriate. In other cases, there may be a number of studies, or studies with a number of endpoints reported, which may require a large number of BMD calculations. In these latter cases, it may be possible to select a subset of endpoints as representative of the effects in a target organ or study. This selection can be made on the basis of sensitivity or severity, which may be more easily compared within a single study in the same target organ than across studies. Sometimes combining several datasets may be an option.”]

In Section 5.1.1, EPA documents the strengths and concerns with using each of the *high* and *medium* confidence studies of sensory irritation in dose-response analysis, including Hanrahan et al. (1984) and Liu et al. (1991). There were some concerns noted for both of these studies, including that neither provided the regression parameter details in their publications. The two studies used different methods for exposure assessment, had different LODs, and had different age distributions. However, these differences complemented each other.

The 2014 National Research Council (NRC) report recommended that dose-response estimation be based on data from compatible studies of similar exposure and outcome when feasible and the specific idea to estimate the dose-response function from the combination of these two studies was recommended by NASEM. Following NASEM’s example, EPA now derives a toxicity value from the combination of these two studies. Using this approach, EPA was able to derive a POD for sensory irritation and considered that osRfC in place of the draft osRfC based on Hanrahan et al. (1984) alone.

- **Finding (10):** EPA's favoring of well-conducted and -reported epidemiologic studies over controlled human exposure studies was justifiable for sensory irritation.

Related Public Comments

- **Public Comment (American Forest and Paper Association):** "EPA's reliance on residential or home studies is misguided and is not the Best Available Science." "EPA should rely on human volunteer chamber studies that have proper controls to reduce or eliminate confounding factors and false positives." "EPA fails to rely on Mueller et al. 2013 and Lang et al. 2008, that are considered by many countries as 'critical studies' (e.g., France, Netherlands, 2019)" "EPA's representation of the sensory irritation studies as an adverse health effect cannot be considered the best available science." *[Please see docket for details and supporting information.]*

EPA Response: Please see the consensus peer review Finding 10. Consistent with this recommendation, the revised assessment continues to emphasize the epidemiologic studies over the human volunteer chamber studies for this health effect and in deriving candidate lifetime toxicity values. Expected differences in susceptibility and interindividual variability in the sensory irritation response (described in Sections 3.2.1 and 4.1) were impactful to the dose-response study selection uncertainty factor decisions (see also responses to related public comments earlier in this section, Appendix F.3.2). The dose-response decisions and the supporting rationale for these decisions are described in Section 5.1, with the reasons for not advancing the studies by Mueller et al. 2013 and Lang et al. 2008 articulated therein (in Section 5.1.1). However, two chamber studies (Kulle et al., 1993 and Andersen and Molhave, 1983) were modeled and discussed in comparison to the results from modeling of the two residential studies (Liu et al., 1991 and Hanrahan et al., 1984) advanced for dose-response analysis. Specifically, regarding support for the identification of sensory irritation as a health effect of concern for formaldehyde inhalation exposure (Section 3.2.1), Finding (9) from the NASEM report indicates, "EPA's overall hazard conclusions are supported by the scientific evidence and are consistent with EPA's state-of-practice methods."

F.3.3. Pulmonary Function

Charge Question and Peer Review Comment Summary

Charge Question 3b. Pulmonary Function

- The assessment concludes that the available **evidence indicates** that long-term formaldehyde inhalation likely causes decreased pulmonary function in humans given appropriate exposure circumstances. This conclusion was supported primarily by evidence in exposed humans, with supportive mechanistic evidence indicating that formaldehyde inhalation results in biological changes related to this outcome.

Supplemental Information—Formaldehyde (Inhalation)

- The assessment concludes that the available evidence is inadequate to determine whether acute or short-term (hours to weeks) formaldehyde inhalation might cause decreased pulmonary function in humans.
- A POD from Krzyzanowski et al. (1990), a human study, was ultimately selected to calculate an osRfC of 0.007 mg/m³ for pulmonary function. A composite UF_C of 3 was applied to address UF_H. This UF_H value was selected using available evidence on potential susceptibility including consideration of children's health.

Peer Review Comment Summary

- 0 Tier 1 Recommended Revisions
- 3 Tier 2 Suggested Revisions
- 0 Tier 3 Future Considerations
- 8 Consensus Panel Findings (numbered below)

Literature Identification

Peer Review Findings and Comments

- **Finding (1):** The inclusion and exclusion criteria listed in Appendix A, Table A-42 are consistent with EPA's state-of-practice methods.

Related Public Comments

No related public comments were submitted.

Study Evaluation

Peer Review Findings and Comments

No Findings or Recommendations specified.

Related Public Comments

No related public comments were submitted.

Evidence Synthesis and Synthesis Judgments

Peer Review Findings and Comments

- **Finding (2):** Within study groupings, EPA equates a lack of statistical significance with a lack of decline in pulmonary function (e.g., reference to Lofstedt PDF p. 98, line 19), and makes statements that are poorly supported by the evidence (e.g., reference to the size of differences and lack of precision on PDF p. 101, line 7).
- **Finding (3):** Evidence is presented inconsistently across the six sections describing results from different exposure durations and study populations.

- **Finding (4):** Overall, while EPA's distillation and synthesis of the pulmonary function evidence is challenging to follow, the judgments presented in the Main Assessment are supported by the scientific evidence and consistent with EPA's state-of-practice methods.
 - *Related committee comments :* "Small declines in pulmonary function can have a large impact on public health, regardless of whether individual declines are clinically significant."
- **Finding (5):** Given the wording in Section 3.2.3, it is difficult to understand the basis for some of the evidence synthesis judgments as presented in the Assessment Overview. For example, EPA does not provide the specific evidence and decision frameworks used to support the following statement: "overall the longitudinal analyses appear to be inconsistent, but while hindered by a lack of sensitivity, seem to support a conclusion that occupational exposure may result in declines of FEV1 and FEF over time" (p. 53, line 17). Further, some aspects of the study synthesis discussion are not clearly defined. For example, what does it mean that studies observed "inconsistent responses" (Assessment Overview p. 52, line 10)? Does this mean that reported results are in different directions, or that some results are statistically significant, while others are not?
- **Tier 2 Suggested Revision (Recommendation 4.7):** EPA should clarify the basis for its synthesis judgments and provide additional information about the studies on which they are based, such as the formaldehyde levels observed, as well as the exposure ranges or other measure of variability. The study summary tables (Tables 1-6 to 1-9) should be updated to provide an organized distillation of the points made in the evidence synthesis text.
 - *Related committee comments :* "The synthesis discussion is blended with the study evaluation tables (Tables 1-6 to 1-9) also presented in this section, and the subsection is organized by exposure duration..." AND, [in reference to acute exposure]: "studies that did include a referent group showed a change in pulmonary function on average in that group, although studies varied with respect to the direction observed, and this additional potentially insightful detail is not provided." AND [in reference to intermediate duration exposure in pathology students]: "These studies are not mentioned further in any of the summaries (i.e., t...the integrated summary of evidence in the Main Assessment)" AND [in reference to long-term occupational exposures]: "Long-term occupational: "reference to cross-sectional studies (Main Assessment, p. 102) points to studies under the "prevalence studies" heading in Table 1-7; it is left to the reader to make this connection." "Figure 1-6... would benefit from more in-depth discussion."

Supplemental Information—Formaldehyde (Inhalation)

- *Related committee comments* : “[The evidence profile table mentions] several studies of workers with long-term exposure to >0.2 mg/m³” without giving references. EPA also concluded that the evidence is inadequate to determine the causal effect of formaldehyde exposure on acute and intermediate-term time scales. This judgment does not give much weight to the evidence that pulmonary function decrements occurred in controlled human exposure settings among participants who exercised at least 30 minutes (Green et al., 1987, 1989), or that some individuals exhibited clinically significant deficits (Green et al., 1987).”
- *Related committee comments* (Appendix E): “Regarding pulmonary function, Table 1-11 indicates there are multiple additional studies included in the evidence judgment for long-term effects, although none of these are referenced. The evidence cited in the table and narrative need to be revised to provide more informative statements. For instance, “concentration-related associations” would preferably be rephrased as “concentration-related decrements in lung function.”
- *Related committee comments* (regarding Figure 1-5): EPA needs to order the studies within industry by formaldehyde exposure levels, or the exposure difference between groups; EPA needs to improve the labeling of the figure to provide clarity, such as by indicating that the numbers before the plotted results refer to sample sizes; The text refers to Figure 1-5 summarizing ten studies, while the figure caption notes there are eight; Clarity would be improved by ensuring that the comparison being reported (i.e., pre-shift pulmonary function differences in prevalence studies) is clearly stated in the caption and accompanying text. Reference information is needed for the study discussed on page 1-45, lines 11-15, and the study referred to as “this study” on line 15.
- *Related committee comments*: The pulmonary function section would benefit from a summary table that refers to all summarized studies and provides an organized distillation of the points made in the text.
- *Related committee comments*: *Tables* need to be formatted clearly to correspond to the information highlighted in the synthesis discussion (e.g., for pulmonary function, which occupational studies had employees that worked at least 5 or at least 10 years—see the Assessment Overview, p. 52, line 33; or indicate the main conclusions EPA reached for each group of studies).

EPA Response to Tier 2 Suggested Revision 4.7: The revised assessment includes summary sections on the evidence synthesis conclusions for each health effect, which are supported by evidence tables (note: these are distinct from the study evaluation tables in the Appendices). This includes context on formaldehyde exposure levels, if it was deemed appropriate to include such statements prior to performing dose-response analyses.

The study selection criteria (PECO) included studies of pulmonary function responses to any exposure duration. EPA recognizes that presentations were somewhat inconsistent across the discussion by exposure setting and duration, reflecting the differences in relevance to the development of a RfC for chronic health effects. In the revised assessment, EPA has refocused the synthesis on exposure settings most relevant to the dose-response assessment and derivation of a RfC for chronic duration exposure scenarios. These studies include the occupational studies of workers with long-term exposure, residential or school exposures to adults or children, and episodic (weekly) exposures to medical students in anatomy classes followed over a period of months. Results of studies of other exposure scenarios involving short-term exposure durations are not directly relevant to a chronic duration RfC. Moreover, these results did not provide a clear indication of effects on pulmonary function and EPA has removed the hazard evaluation for short-term/acute effects. However, these results are summarized in the appendix (see Appendix C.5 for studies of workers or medical students and pulmonary function changes across a work shift or over a dissection lab). For each exposure setting (i.e., occupational, residential, anatomy class) the synthesis discussion is oriented around the consistency of evidence across studies, evidence from different study designs (e.g., cross-sectional studies, longitudinal studies) and the interpretation of results in light of potential biases, strength of results and evidence for dose-response.

The synthesis discussion has been edited for clarity and references have been added to support specific synthesis statements. Statements were edited to improve clarity. The levels of formaldehyde experienced in each occupational exposure setting are described. The proposed draft provided overall exposure levels reported for the occupational studies, but EPA has edited the synthesis to provide more detail regarding levels in each of the three industry groupings studied: wood products, chemical manufacture, and embalming. Figure 3-5 (in the revised assessment) was not altered to order the studies by average exposure levels because the time weighted averages reported by many of the occupational studies did not characterize well the exposure levels experienced. There were wide ranges of levels reported within each study so ordering the studies by average levels would not have clarified the analysis. The study results are grouped by industry.

- **Tier 2 Suggested Revision (Recommendation 4.8):** If the Assessment Overview is retained (see Recommendations 2.1 and 2.2 in Chapter 2), EPA should harmonize its presentation of evidence synthesis with the presentation in the Main Assessment. In particular, the evidence synthesis section of the Assessment Overview could be updated to build upon the first three paragraphs of the “Integrated Summary of Evidence for Pulmonary Function” section in the Main Assessment (PDF p. 134).

EPA Response to Tier 2 Suggested Revision 4.8: The Assessment Overview was not intended as a separate documentation of EPA’s analyses. Rather, the Overview was included to provide a summary of the primary conclusions in the Toxicological Review and Appendices for audiences not

interested or able to consult the lengthy Toxicological Review. The Overview has not been retained as part of the revised assessment.

- **Finding (6):** The MOA considerations and mechanistic evidence are clearly presented and appropriately documented.

Related Public Comments

No related public comments were submitted.

Overall Conclusions About the Hazard Descriptor

Peer Review Findings and Comments

- **Finding (7):** EPA's evidence integration judgments are supported by the scientific evidence and are consistent with EPA's state-of-practice methods.

Related Public Comments

No related public comments were submitted.

Dose-Response Evaluation

Peer Review Findings and Comments

- **Finding (8):** EPA's derivation of BMCL was based on nonasthmatic children and nonmorning exposures. This practice was inconsistent with EPA's state of the practice of using more vulnerable subpopulations for risk estimation.
- **Tier 2 Suggested Revision (Recommendation 4.9):** EPA should provide additional justification for why the most vulnerable subpopulations were not used for risk estimation, and should consider using the data from children with asthma that are provided in Krzyzanowski et al. (1990).

EPA Response to Tier 2 Suggested Revision 4.9: EPA estimated the BMC and BMCL for all children and noted that Krzyzanowski et al. (1990) found that asthmatic children showed a steeper decline in morning peak expiratory flow rate (PEFR) compared with nonasthmatic children. The model shown in Table 5 of Krzyzanowski et al. (1990) did not include a main effect parameter for 'asthma' because it was not statistically significant. The model did show statistically significant parameter estimates for a quadratic function of (Bedroom HCHO)*'morning'*'asthma' and EPA was able to estimate a BMC for asthmatic children's PEFR measured in the morning after assuming that Bedroom and Household HCHO were equal and that the main effect parameter for 'asthma' was zero. The BMC was lower for asthmatic children's PEFR measured in the morning than for all children. In order to estimate a BMCL for these children, EPA would need to know the standard

error of the summary effect estimate for HCHO in these children. Summing only the standard errors terms in the model ignores that complexities of the covariances between the several related variables. EPA contacted Dr. Krzyzanowski regarding further details of the regression model, but no additional documentation is available (cite email). Thus, the POD derived from Krzyzanowski et al. (1990) for decrements in pulmonary function is based on data for all children in the study, not asthmatic children alone. In applying UFs to this POD to calculate a cRfC, because it was demonstrated that the effect of formaldehyde exposure on asthmatic children's PEFR was more sensitive than all children, an uncertainty factor for human variability of 3 was applied to address the increased sensitivity of asthmatic children.

Related Public Comments

- **Public Comment (American Chemistry Council):** "The Draft Assessment did not present an alternative to the RfC based on Krzyzanowski et al. (1990), as directed by the NAS (2011) and does not address the inherently limited reliability of the cross-sectional study design of the study and classified the study confidence as 'high.'"
"These contradictions and "plausible" speculations do not inform an evidence-based assessment. If there is no dose-response, not time concordance, and no mode of action for the observed effects, no pathological basis for the effects, and no distribution to the [lower respiratory tract], there is no support for any cause-and-effect relationship. The Draft Assessment would be improved by simply stating the evidence for and against causal association, without speculation about plausibility of undefined causes." *[Please see docket for details and supporting information.]*

EPA Response: Consistent with recommendations from the consensus peer review panel, the revised assessment continues to retain the same hazard judgment for pulmonary function (Section 3.2.2) and continues to rely on dose-response analysis based on the study by Krzyzanowski et al. (1990), noting that the RfC is not based on this study alone (Section 5.1.5). Attempts to incorporate modeling for a sensitive subgroup in this study (i.e., asthmatic children), as recommended by the NASEM panel and which would have resulted in a lower point-of-departure, were unsuccessful as the required information to estimate a BMCL were not available in ([Krzyzanowski et al., 1990](#); [Krzyzanowski and Bateson, 2023](#)).

As described in the Assessment Methods (Section 2) and consistent with EPA guidelines, understanding of MOA is not required to identify a hazard (or to support subsequent dose-response analysis for identified hazards). It is noted that for pulmonary function, however, a partial MOA was in fact identified and, although inhaled formaldehyde is unlikely to reach the lower respiratory tract in most exposure scenarios, inflammation and other changes in the lower respiratory tract are well supported (see MOA summary in Section 3.2.2).

EPA notes that varied epidemiological study designs, not only cross-sectional studies, were available to support the hazard determinations for pulmonary function as well as the other

identified hazards. It is important to emphasize, however, that well-conducted studies with a cross-sectional design can address causation, including the temporality concern raised by the commenter above. This is the case if the study's measurement(s) of exposure address the etiologically relevant time-period for the outcome under review. Additional discussion and explanation on this well-established epidemiological principle has been added to Sections 2 and 3 of the Toxicological Review. The IRIS assessment paid significant attention to the exposure measurement design and quality during study evaluation. Although this was already discussed in Section 2 and detailed in Appendix B.3 in the public comment/external peer review draft, the final assessment now includes an expanded discussion and rationale on this point in Section 2. Namely, through the extensive analyses of the available epidemiology studies meeting the PECO criteria as well as numerous supplemental resources, the assessment focuses on epidemiological studies with measures of formaldehyde exposure of 5–7 days or more as a high-quality exposure assessment representative of exposure levels over the preceding weeks and months. Studies such as Krzyzanowski et al. (1990) with the additional attribute of including formaldehyde measures across multiple seasons provides additional confidence in the interpretation of the measures as representing longer term past exposures. These measurements reflecting long-term, ongoing formaldehyde exposure levels are most useful in developing the chronic RfC and IUR. Exposure assessments of longer duration and in multiple periods provide more precise estimates of longer-term exposure and there is greater confidence in PODs based on them. Overall, 3 of 6 cross-sectional studies on pulmonary function among adults and children had high quality exposure measures and two studies had adequate exposure assessments; these 5 studies were interpreted with high or medium confidence. One study had deficient exposure quality and was interpreted with low confidence.

Specifically, for Krzyzanowski et al. (1990), the assessment now includes more frequent highlighting of the details of the study that support the high confidence rating. The study used a strong design and analysis, including two daily measurements of pulmonary function over a 12-day period and a high-quality exposure assessment. Since there was not a trend in the average daily peak expiratory flow rate (PEFR) measurements over the 12 days of testing, the 12-day average of PEFR in an individual reasonably represented the current average pulmonary function for the children and adults at the time of the study. The association observed in children with formaldehyde concentrations averaged over two 1-week sampling periods (with a subset of samples collected across different seasons) indicates a persistent effect of formaldehyde exposure. The assessment has added details from the supporting study, (Quackenboss et al., 1989), which shows that average formaldehyde measurements in the homes during the first and second sample weeks were highly correlated, and that this pattern was consistent within each of the sampling locations, with none of the week-to-week differences being significantly different. This shows that the exposure indices based on one-week samples or by averaging over two, one-week samples to obtain a single HCHO concentration index (i.e., an index for each household average and separately for each person's bedroom) were stable over time, and this supports that these indices reasonably

reflect past exposures. Thus, these exposure measurements reliably represent average formaldehyde levels relevant to both the short-term timeframe of the pulmonary function measures as well as the months prior to those outcome measurements. These exposure measurements are of clear etiological relevance.

F.3.4. Respiratory Pathology

Charge Question and Peer Review Comment Summary

Charge Question 3e. Respiratory Tract Pathology

- The assessment concludes the ***evidence demonstrates*** that inhalation of formaldehyde causes respiratory tract pathology in humans, given sufficient exposure circumstances. Well-conducted studies in humans and animals support this hazard conclusion, and strong mechanistic evidence in animals provides a plausible MOA for the identified outcomes.
- PODs from the Kerns et al. (1983) and Woutersen et al. (1989) rat studies were ultimately selected to calculate an osRfC of 0.003 mg/m³ for squamous metaplasia. The PODs were estimated using dosimetric simulations of formaldehyde flux to the nasal lining using a computational fluid dynamics model to extrapolate from results in rats to humans. A composite UF_C of 30 or 100 was applied to address UF_H, subchronic (UF_S) and interspecies (UF_A) uncertainties.

Peer Review Comment Summary

- 0 Tier 1 Recommended Revisions
- 2 Tier 2 Suggested Revisions
- 0 Tier 3 Future Considerations
- 6 Consensus Panel Findings (numbered below)

Literature Identification

Peer Review Findings and Comments

- **Finding (1):** For human studies, the comparator of the PECO is defined as evaluated outcome associations with formaldehyde exposure; it is unclear what specific comparisons were made. The comparator in the PECO statement used for screening the animal studies is undefined.
- **Finding (2):** It is unclear why EPA did not consider more distal effects in the respiratory tract since studies performed in nonhuman primates have reported changes in the respiratory epithelium of the trachea and major bronchi (Monticello et al., 1989). The exclusion criteria for human respiratory tract pathology included exclusion of studies reporting rhinitis. This exclusion is surprising since rhinitis was a search term used to find human studies. Moreover, it

is unclear whether this exclusion criterion resulted in exclusion of studies with histologic evidence of inflammation.

- **Tier 2 Suggested Revision (Recommendation 4.10):** EPA should provide an explicit description of the comparator used in screening human and animal studies, and resolve discrepancies between search terms and inclusion and exclusion criteria.
 - Related committee comments (Appendix E): “For respiratory pathology, it is unclear how search terms were used (e.g., controlled vocabulary [MeSH] and keyword terms).”

EPA Response to Tier 2 Suggested Revision 4.10: In reference to the comparator, EPA has provided a revised definition consistent with the IRIS Handbook in the revised draft: “evaluated outcome associations with formaldehyde exposure risk in relation to variation in exposure based on level, duration, or other parameter.” In reference to the outcome domain (presumed to be in reference to the search of human studies as the animal search explicitly included distal sites), the assessment methods have been clarified to explain the focus on proximal effects on the respiratory system in humans as more distal evaluations of pathology are highly invasive and thus not well-studied (note: BAL is a noninvasive marker of potential distal/pulmonary injury; however, BAL endpoints were captured in the mechanistic search related to inflammation and immune responses). Signs such as rhinitis or mucous flow rate were included in the discussion of mechanisms of inflammation and immune system responses; this has been clarified in the revised assessment.

All search strings were submitted as keyword searches, which in the case of PubMed includes MeSH terms by default, except as specified with tags like [majr] which limited the search to only when the term (and any selected subheadings) are indexed as a major topic heading (not all MeSH terms); as defined by PubMed, a MeSH Major Topic is one of the main topics discussed in an article.

Related Public Comments

No related public comments were submitted.

Study Evaluation

Peer Review Findings and Comments

- **Finding (3):** EPA’s outcome-specific criteria are generally appropriate and consistent with its state-of-practice methods. It is unclear whether the downgrade for cross-sectional studies among occupational cohorts is relevant for histopathologic outcomes. Evidence supporting consideration of gender as a confounder is not provided. For animal studies, sample size

limitations were applied inconsistently. In general, EPA was also inconsistent in providing a rationale for each study quality domain (Appendix A, Table A-59), reducing transparency.

- **Tier 2 Suggested Revision (Recommendation 4.11):** EPA should provide a consistent rationale for each study quality domain used in the assessment.
 - *Related committee comments:* "EPA downgraded cross-sectional studies among occupational cohorts since workers may have been less sensitive to the irritant properties... EPA did not critically evaluate whether a health worker survivor effect was present in these studies" Note also: "sample size of less than 10 was considered a significant limitation"; however, this criterion was applied to the Holmstrom et al. (1989) study even though this study had 16 animals"

EPA Response to Tier 2 Suggested Revision 4.11: The evaluation criteria for occupational studies of formaldehyde exposure and respiratory outcomes have been revised so that a potential “healthy worker” (survival) effect does not automatically result in a *low* study confidence. Since any bias, if present, would result in an underestimate of effect estimates, it was noted in the study evaluation tables, but did not result in *low* confidence based on that limitation alone. While critical evaluation of the studies of respiratory pathology for evidence of survival bias was not possible, information was provided in studies of other health effects that allowed a more specific evaluation of the evidence for survival bias, which was noted in the study evaluation tables. As is shown in these evidence tables within Section 3.2.4, for the small number of human studies of respiratory pathology (all *medium* confidence studies of occupational formaldehyde exposure), this change did not influence the evidence synthesis conclusions or associated uncertainties. It is noted that this potential bias attenuates, or underestimates, effect estimates and yet the majority of available studies still observed effects of formaldehyde exposure. Also, gender was removed as a potential confounder for effects on nasal pathology, as no support was found.

For animal studies, NASEM noted N = 10 was considered a limitation and used the downgrade of Holmstrom (1989) with N = 16 animals as a source of inconsistency. However, to clarify, the sample size consideration based on the OECD guideline study design differs between chronic and subchronic studies, requiring N > 20 for a chronic study and N > 10 for a subchronic study. As Holmstrom (1989) was a chronic study, N = 16 animals was appropriately noted as a limitation. However, the study evaluations were reviewed and revised for consistency.

Note that for animal studies throughout the assessment, when the study methods met the domain-specific criteria the symbol ‘++’ for ‘Good’ was used and no further domain-specific explanation was provided. Study-specific details that resulted in a lower rating ‘Adequate’ (shading of ‘+’) are italicized within each domain-specific cell in the table. When a rating of ‘Deficient’ (symbolized by a gray-shaded cell) was noted, the study-specific detail that caused that rating is bolded within each domain-specific cell. Notes about study details that did not alter the domain-

specific ratings but which may be informative to the evidence synthesis are sometimes included. This has been made clearer in the revised assessment.

Related Public Comments

No related public comments were submitted.

Evidence Synthesis and Synthesis Judgments

Peer Review Findings and Comments

- **Finding (4):** The judgments presented in the Main Assessment are supported by the scientific evidence and consistent with EPA's state-of-practice methods.

Related Public Comments

No related public comments were submitted.

Overall Conclusions About the Hazard Descriptor

Peer Review Findings and Comments

- **Finding (5):** The evidence integration judgments made by EPA are supported by the scientific evidence and consistent with EPA's state-of-practice methods.

Related Public Comments

No related public comments were submitted.

Dose-Response Evaluation

Peer Review Findings and Comments

- **Finding (6):** EPA's dose-response evaluation was well documented and transparent. Steps used are consistent with EPA's state-of-the-practice.
 - *Related committee comments:* "[EPA] did not explain why its confidence in the POD based on the Woutersen et al. (1989) study was medium."

EPA Response to committee comments related to Finding 6: The confidence classification for this candidate toxicity value is "Medium-high" in the revised assessment. As now documented in Section 5.1.4, the following concerns relating specifically to the POD have been added: Smaller study (as compared to Kerns et al., 1983) can introduce increased variability; no exposure levels between 1.2 and 11.2 mg/m³ (which introduces uncertainty regarding the shape of the curve between those levels), and default dosimetric extrapolation (as compared to the ability to apply dosimetric modeling approaches for Kerns et al., 1983). However, Woutersen et al. (1989) remains the selected cRfC for this health effect-specific toxicity value (the osRfC) in the revised assessment.

Related Public Comments

No related public comments were submitted.

F.3.5. Allergy and Asthma

Charge Question and Peer Review Comment Summary

Charge Question 3c. Allergy-related Conditions

- The assessment concludes that the available ***evidence indicates*** that formaldehyde inhalation likely causes increased allergic responses in humans, given appropriate exposure circumstances. This conclusion was supported primarily by evidence in exposed humans, with supportive mechanistic evidence indicating that formaldehyde inhalation results in biological changes related to this outcome.
- A POD from Annesi-Maesano et al. (2012), a human study, was ultimately selected to calculate an osRfC of 0.008 mg/m³ for allergy-related conditions. A composite UF_C of 3 was applied to address UF_H. This UF_H value was selected using available evidence on potential susceptibility including consideration of children's health.

Charge Question 3d. Prevalence of Current Asthma and Degree of Asthma Control

- The assessment concludes that the available ***evidence indicates*** that formaldehyde inhalation likely causes an increased frequency of current asthma symptoms or difficulty controlling asthma, given appropriate exposure circumstances. This conclusion was supported primarily by evidence in exposed humans, with supportive mechanistic evidence indicating that formaldehyde inhalation results in biological changes related to these outcomes.
- PODs from the Annesi-Maesano et al. (2012), Krzyzanowski et al. (1990), and Venn et al. (2003) human studies were ultimately selected to calculate an osRfC of 0.006 mg/m³ for current asthma or degree of asthma control. A composite UF_C of 3 or 10 was applied to address UF_H. The UF_H value applied to the POD from Annesi-Maesano et al. (2012) was selected using available evidence on potential susceptibility including consideration of children's health.

Peer Review Comment Summary

- 0 Tier 1 Recommended Revisions
- 3 Tier 2 Suggested Revisions
- 0 Tier 3 Future Considerations
- 1 Un-tiered Committee Comment
- 11 Consensus Panel Findings (numbered below)

General Scope and Approach

Peer Review Findings and Comments

- **Finding (1):** Allergies and asthma as systemic immunopathologies related to inhaled formaldehyde are well documented in the 2022 Draft Assessment. It is unclear whether additional systemic immune-mediated diseases (e.g., contact allergy, immunodeficiencies, lupus, rheumatoid arthritis) were also considered and rejected because of a lack of relevance or information.
 - *Related committee comments:* "Although it is a portal-of-entry effect, the committee's discussion concerning asthma is included in the evaluation of systemic effects, reflecting the broader inflammatory consequences of asthma."

EPA Response to committee comments related to Finding 1: Acknowledging the systemic inflammatory consequences, EPA continues to discuss allergy in asthma as effects on the respiratory system, consistent with the committee's statement that these are POE effects. EPA did not include search terms relating to autoimmunity, autoimmune conditions such as systemic lupus erythematosus and rheumatoid arthritis or conditions relating to immunodeficiency because these had not been discussed in any of the reviews or previous risk assessments that were consulted. It is likely that the broad net used by EPA would have collected studies on these topics, since a variety of immune-related terms were used. However, no research articles on these topics in relation to formaldehyde exposure were noted by the screeners.

- **Tier 2 Suggested Revision (Recommendation 4.12):** EPA should provide a comprehensive description or listing of immunopathologies that were considered as potentially related to formaldehyde before the decision was made to limit the focus to prevalent allergies and prevalent asthma.

EPA Response to Tier 2 Suggested Revision 4.12: As discussed and now more specifically documented in the Assessment Methods (Section 2), the outcomes were selected based on prior risk assessments of formaldehyde and the NRC (2011) review of a prior EPA draft assessment. Note that, in addition to the more narrow focus of the search for epidemiology studies, a broad search of information relevant to inflammation or immune responses was conducted and documented. No additional immunopathologies of potential concern were highlighted based on that broader review.

Related Public Comments

No related public comments were submitted.

Literature Identification

Peer Review Findings and Comments

- **Finding (2):** The considerations and methods adopted by EPA were appropriate given the often poor specificity of diagnostic classifications in the published literature on these conditions. Study choice decision metrics are adequately described.
- **Finding (3):** The terms “very young” and “very old” (as exclusion criteria) are not defined, and several studies of school-age children were used to inform the strength-of-evidence determination. While age cutoffs of <5 and >75 years are cited in the Assessment Overview and Appendices as exclusion criteria, the Main Assessment does not describe age cutoffs, and it is unclear how these cutoffs were made actionable in study choice as some studies of infants and children <5 feature in asthma evaluations in particular. “Infants” (again, a specified age needs to be given each time this word is used) were deemed to have nonspecific symptoms (wheeze and infection) compared with adults and were not considered in the formulation of PODs or synthesis judgments. Likewise, the “elderly” (assumed to mean >75, but again, age needs to be specified each time the word is used) were not considered because of the potential interference of alternative mechanism diseases such as chronic obstructive pulmonary disease (COPD). In general, EPA divided evaluations into “child” and “adult” studies, but age cutoffs for these categories need to be stated and applied consistently.
- **Finding (4):** EPA’s decision to exclude food allergy in response to airborne formaldehyde while including eczema is not explained. The rationale for excluding formaldehyde-specific immunoglobulin E (IgE) as an endpoint (because of its rarity in evaluation) is explained appropriately.
- **Finding (5):** The inclusion/exclusion criteria for animal studies are described in confusing terms. While the search terms emphasize biological mechanisms as relevant (biomarkers such as immunoglobulin G (IgG), IgE, cytokines, hypersensitive reactions), EPA also states that studies “describing the development of immunological or allergy animal models” were not included (Appendix A, p. A-337). The 2022 Draft Assessment documents that animal models are generally helpful only in immediate pathological response to formaldehyde rather than the development of disease states that recapitulate human diseases, but a clear reason for excluding animal models of “the development of immunological or allergy” outcomes is not provided.
- **Tier 2 Suggested Revision (Recommendation 4.13):** EPA should explicitly state its rationale for age-based exclusions and define the terms “very young” and “very old,” better justify the rationale for excluding allergic contact dermatitis and food allergy as outcomes of interest, and

provide the rationale for excluding animal models of “the development of immunological or allergy” outcomes (unless such studies do not exist or are inadequate).

EPA Response to Tier 2 Suggested Revision 4.13: EPA implemented the following five decisions in the revised assessment in response to the five main suggestions above:

1. EPA does not include studies in the evaluation of asthma, asthma symptoms, and asthma control in children younger than 5 years of age or in adults older than 75 years of age. This is because the specificity of symptoms is reduced in the very young (i.e., <5 years) since wheezing can occur with respiratory infections in infants and young children. Specificity is also reduced at older ages (i.e., >75 years) because of the similarities in symptoms and medication use for chronic obstructive pulmonary disease, which occurs in the elderly, and asthma ([Taffet et al., 2014](#); [Abramson et al., 2014](#)). Studies examining wheezing or other related symptoms in infants and children < 5 years of age were evaluated within a separate category of “lower respiratory tract conditions in infants and toddlers.” [Raaschou-Nielsen et al. \(2010\)](#) [Roda et al. \(2011\)](#) [Rumchev et al. \(2002\)](#). [Li et al. \(2019\)](#) [Yu et al. \(2017\)](#). However, studies of asthma or asthma symptoms that included ages 3–4 within a larger cohort of older children were included if the proportion of the study group in the age range was likely to be relatively small (e.g., if the mean age was > 5 years); similarly if the age range extended over 75 years the study was included in the asthma evaluation if the mean age was younger, e.g. < 65 years.
2. EPA has replaced the terms “very young” and “very old” with specific age ranges.
3. Allergic contact dermatitis is excluded as an outcome because it is a well-established effect from dermal exposure, and the effects of dermal exposure are not a focus of this review. In contrast, eczema is not an effect of dermal exposure to an allergen but can be triggered by reactions to respiratory and other types of allergens (as well as by other factors); therefore, eczema, but not allergic contact dermatitis, is included as an outcome.
4. Unlike eczema that can be triggered by reactions to respiratory and other types of allergens (as well as other factors), food allergies do not result from exposure to respiratory allergens or other inhaled substances. Because this assessment focuses on inhalation exposures only, food allergies are excluded from consideration.
5. There are currently no universally accepted animal models applicable to humans for determining dose-response relationships or the potency of low molecular weight chemicals to induce allergic symptoms via the inhalation route ([IPCS, 2012](#)). Most of the experimental animal formaldehyde studies that are relevant to interpreting these respiratory immune-mediated conditions used the ovalbumin (OVA) murine model, the best studied animal model of asthma. However, the OVA mouse model has several limitations relative to human data for hazard characterization [for details, see immune-mediated conditions synthesis]. Because of these limitations, EPA concluded for this assessment that the OVA model was

more appropriate for examining mechanistic questions in support of hazard identification, based in part on the reasonably large number of well-conducted human studies on these endpoints.

Related Public Comments

No related public comments were submitted.

Study Evaluation

Peer Review Findings and Comments

- **Finding (6):** The outcome-specific criteria used to evaluate human studies were generally appropriate and in line with expert recommendations. However, EPA's approach appears to contradict the expert panel's advice that children's allergic symptoms are more liable to misdiagnosis than those of adults, particularly for asthma in infants and young children.
- **Tier 2 Suggested Revision (Recommendation 4.14):** EPA should include a specific statement on the age at which asthma diagnosis is considered valid to justify the age exclusions for young children, as well as the category of "the elderly".
 - *Related committee comments* (Appendix E): "EPA's approach appears to contradict the expert panel's advice for asthma. Several studies in infants and young children (<3 years) are examined (p. 1-17 and Table 1-20), where the EPA considers diagnoses in young children to likely be exacerbations of respiratory tract infections rather than representing a true "asthma" phenotype."

EPA Response to Tier 2 Suggested Revision 4.14: Because of the reduced specificity of symptoms in the very young (<5 years of age) and in the elderly (> 75 years of age), an asthma diagnosis is only considered valid for people who are 5 to 75 years old. Relevant text in the supplemental material that states that the eligible population for asthma is now "humans, age ≥4 years" has been revised to "humans who are 5 to 75 years old." As noted above, however, studies that included a small proportion of children < 5 are included. This change in definition had no impact on decisions related to dose-response analysis (i.e., the studies selected, the PODs derived, the uncertainties applied, or the values selected as cRfCs, osRfCs, or the RfC).

- *Un-Tiered Committee Comment* on specific study confidence determinations (Appendix E):
 - "Annesi-Maesano (2012) is rated *high* confidence, despite the vague description of the outcome in Table 1-12. EPA needs to indicate whether this study used an appropriate questionnaire, which would support the high confidence rating."
 - "The study by Smedje and Norback (2001) is rated as *low* confidence in Table 1-12, page 1-91, contradictory to the text on pages 1-81 and 1-83, which states it is

medium. This discrepancy needs to be rectified. The *low* confidence rating is justified by the large proportion of exposures below the limit of detection, and possible information bias and confounding; however, the “alternative evaluation” of *medium* confidence features strongly in recommendations and the confidence judgment needs to be consistent throughout the document to avoid confusion...”.

- “It would be helpful to check all the reference table confidence statements with their citations in the text of the documents. Additionally, the tabulated data in the main document need to be checked for consistency with the longer descriptions located in Appendix A.5.4 regarding confidence judgments.”

EPA Response to Un-tiered Committee Comment:

- The questionnaire used in Annesi-Maesano (2012) was the ISAAC questionnaire, which had been identified by the experts consulted by EPA to be a validated, high quality tool. This detail had been included in the Appendix table summarizing the Annesi-Maesano (2012) methods. EPA has added this detail to the table in the Synthesis and Integration in Section 3.2.3 of the revised Toxicological Review.
- EPA considered Smedje and Norback (2001) to be a *low* confidence study, and it was not used to form conclusions regarding allergy and asthma risk. However, because it was one of only two studies that presented data on effect modification by atopy status, EPA had included it in its discussion of that issue. To avoid confusion, we have removed the discussion of this study from that section. Regarding the confidence classification, a *low* confidence rating because has been retained because although the study is prospective, there were only two exposure measurements taken, each 1 day (4 hours) over a 4-year period, and 2/3 of the students had left the school more than a year before the health assessment, which further increases the uncertainty regarding the relevance of the exposure measure.
- The details and confidence rating in the study evaluation table (Appendix B.3.4) and the main text and tables (Section 3.2.3) of the revised assessment have been checked for consistency.

Related Public Comments

No related public comments were submitted.

Evidence Synthesis and Synthesis Judgments

Peer Review Findings and Comments

- **Finding (7):** While EPA accorded some studies of acute exposures high confidence, those studies were not deemed generalizable to other populations (p. 1-114) because of brief exposures not relevant to the chronic-exposure scenario. A short and useful section (p. 1- 122)

considers effect modification by tobacco smoke, family history of atopy, and known atopy status.

- **Finding (8):** EPA's use of animal data and the resulting synthesis judgments are appropriate, described thoroughly, and consistent with EPA's state-of-practice methods. The relative risk size of 1.2 for exposures of around 0.04–0.06 mg/m³ is small, but these conditions are common, making this an important outcome. A specific study (Matsunaga, 2008) suggests higher risks, including a two-fold higher risk for eczema, and a three-fold higher risk for allergy-like symptoms from a childhood classroom study that is helpful for determining a POD.
- **Finding (9):** EPA provides a properly nuanced discussion of the complex MOA for immune-related conditions and identifies many gaps in knowledge, consistent with its state-of-practice methods.
 - *Related committee comments:* "These immune mechanisms are complex and largely beyond the scope of the 2022 Draft."

Related Public Comments

No related public comments were submitted.

Overall Conclusions About the Hazard Descriptor

Peer Review Findings and Comments

- **Finding (10):** EPA's hazard determination judgments are appropriate given the scientific evidence described and are consistent with EPA's state-of-practice methods.

Related Public Comments

- **Public Comment (American Chemistry Council):** "EPA's integration of evidence is overly simplistic. Asthma is widely recognized as a heterogeneous group of diseases based on clinical features, physiological characteristics, and varying outcomes ranging from mild to severe. Variation in response to different asthma treatments also suggests that there are multiple mechanisms and pathways that are relevant to the development and exacerbation of asthma. It is not a single disease entity. EPA does not adequately address potential differences in asthma phenotypes that likely represent different underlying biological mechanisms for asthma development. EPA does not describe how the postulated mechanistic pathways are potentially related to different asthma phenotypes. There is a large body of ongoing research focused on improving the understanding of the specific biological mechanisms (or endotypes) that are associated with phenotypes (for example, see Akar-Gibril et al. 2020; Berdine et al. 2020; Carr et al. 2018; Kuruvilla et al. 2019; Kaur and Chupp 2019; Conrad et al. 2021; Schoettler and Strek, 2020; McDowell and Heaney 2020; Lötvall et al. 2011; Wenzel et al. 2012). As a result of

differences in phenotypes and endotypes, there are likely different mechanisms (and different modes of action) by which formaldehyde could potentially increase risk. At the very least, the evidence related to potential mode of action should be synthesized separately for occupational asthma (a phenotype that is generally not related to atopic or allergic asthma). Differences in potential mechanisms and the pathogenesis for early onset/atopic asthma versus early onset/non-atopic asthma could also be described (even if these groups cannot be distinguished in the existing epidemiologic studies). Collectively, these differences, which are not related to differences in exposure concentrations, potentially explain inconsistencies in results.”

“Two studies were selected to derive a point of departure (POD) for current asthma (Annesi-Maesano et al. 2012; Krzyzanowski et al. 1990); however, neither study showed clear associations between current asthma and formaldehyde exposure...Two studies were selected to derive a POD for poorly controlled asthma (Dannemiller et al. 2013) and Venn et al. (2003). These studies only reported correlations between formaldehyde concentrations and poorly controlled asthma. Both studies were cross-sectional studies that relied on response to questionnaires for the identification of poorly controlled asthma. Neither study controlled for potential confounding by indoor allergens that trigger asthma.”

“EPA’s misrepresentation of the two studies (Krzyzanowski et al., 1990; Annesi-Maesano et al., 2012) it relied on to derive a POD cannot be ignored. EPA’s bias against the chemical to even use a negative study in support of an association does not represent the best available science.”

“Not only did EPA ignore the two relevant publications by Drs. Golden and Holm, the 2022 Draft Assessment also ignored evidence on the key substances [acrolein] that are considered causally related to asthma. In doing so, the 2022 Draft Assessment presents conclusions that are at complete odds with NASEM (2000) (an authoritative review for the endpoint of asthma) and Kanchongkittiphon et al. (2015) (a comprehensive update involving 69 additional studies focused on indoor environmental exposures and exacerbation of asthma), both of which demonstrated a practical weight of evidence approach...EPA must therefore revise the 2022 Draft Assessment to include a practical Weight of the Evidence evaluation on asthma using the best available science and must account for the contributing role of acrolein.”

“The revised assessment does not provide clear criteria for evaluating evidence on asthma and provides insufficient support for the classification of formaldehyde as a asthmatoxin. The Draft Assessment then calculates several RfCs based on asthma effects (Table 2-10) without addressing the fundamental issues raised during the NAS (2011) review.”

“The 2022 Draft Assessment also appears to contain errors in the text:

- The 2022 Draft Assessment states that the EPA conducted a meta-analysis to calculate a summary effect estimate for similar results; there is no further information, and no summary effect estimates are reported.
- Figure 1-11, Panel C: Effect Modification of Prevalence of Current Asthma in Children by Atopy Status) shows increased risks (odds ratios > 3.0) for both atopic

and non-atopic asthma in the highest exposed group ($>0.06 \text{ mg/m}^3$, which also appears to be mislabeled as $\mu\text{g/m}^3$). These odds ratios do not match the results presented in Annesi-Maesano et al (2012). Annessi-Maesano et al. (2012) reported odds ratios of 0.96 and 0.82 for the prevalence of current asthma in the highest exposed group for atopic children and nonatopic children, respectively.”

[Please see docket for details and supporting information.]

EPA Response: EPA has retained the same hazard conclusions regarding allergic conditions and prevalence of current asthma or control of asthma symptoms in the revised assessment. These hazard conclusions were supported by the NASEM peer review panel. The studies identified by the commenter that were advanced for POD derivation were Krzyzanowski et al., 1990; Annesi-Maesano et al., 2012; and Venn et al., 2003, noting that Dannemiller et al., 2013 is not advanced for POD derivation. These *high* (Annesi-Maesano and Venn) or *medium* (Krzyzanowski, due primarily to small numbers of asthma cases, which is a limitation expected to bias results towards the null) confidence studies are thoroughly reviewed in the assessment, with formal study evaluations documented in Appendix B.3.4, synthesis of their strengths and uncertainties in Section 3.2.3, and re-evaluation of their reliability specifically for use in dose-response in Sections 5.1.1 through 5.1.4.

It is important to emphasize that well-conducted studies with a cross-sectional design can address causation, including the temporality concern raised by the commenter above (see Section 2.3 of the Toxicological Review). This is the case if the study’s measurement(s) of exposure address the etiologically relevant time-period for the outcome under review. Additional discussion and explanation on this well-established epidemiological principle has been added to Sections 2 and 3 of the Toxicological Review. The IRIS assessment paid significant attention to the exposure measurement design and quality during study evaluation. This was discussed in Section 2 and detailed in Appendix B.3 in the public comment/external peer review draft, the final assessment now includes an expanded discussion and rationale on this point in Section 2. Namely, through the extensive analyses of the available epidemiology studies meeting the PECO criteria as well as numerous supplemental resources, the assessment focuses on epidemiological studies with measures of formaldehyde exposure of 5–7 days or more as a high quality exposure assessment representative of exposure levels over the preceding weeks and months. Studies such as Krzyzanowski et al. (1990) with the additional attribute of including formaldehyde measures across multiple seasons provides additional confidence in the interpretation of the measures as representing longer term past exposures. These measurements reflecting long-term, ongoing formaldehyde exposure levels are most useful in developing the chronic RfC and IUR. Exposure assessments of longer duration and in multiple periods provide more precise estimates of longer-term exposure and there is greater confidence in PODs based on them.

The strengths of the study by Krzyzanowski et al. (1990) in this regard have been elaborated in response to a prior comment in Appendix F.3.3. For Annesi-Maesano et al. (2012) and

Venn et al. (2003), the assessment now includes a more visible discussion and explanation of the expert panel consultations on allergic conditions and asthma that previously were primarily discussed in the Appendices (mentions and callouts have now been added to Sections 2.3, 3.2.3, and 3.2.4, with a few related notes added to Section 5.1). The two expert panels were each comprised of five international experts convened through an external contractor and vetted for potential COI to consult to EPA on the design, analysis, and interpretation of observational studies of allergic conditions and, separately, asthma. These expert panel consultations were initiated by EPA to provide more in-depth subject matter expertise on immune-mediated conditions. Most observational studies on these outcomes were cross-sectional with concurrent assessment of exposure and of symptoms over a preceding period ranging from 4 weeks to 12 months. For allergies, advice from the five-member expert panel on allergic conditions led to the identification of cross-sectional designs using questionnaires for rhinitis or rhinoconjunctivitis as an adequate basis for case ascertainment in studies in Europe and the United States; in studies in other areas (i.e., areas that have not been included in the International Study of Arthritis and Allergies in Children: ISAAC), specific mention of validation of the questionnaire was needed to receive a high confidence rating. Although the specificity of questions pertaining to rhinitis may be lower than the specificity of questions pertaining to rhinoconjunctivitis (see Kim et al., 2012, HERO ID 2333960), based on the expert feedback received, this difference was interpreted as insufficient to conclude that the rhinitis questions should be viewed with lower confidence. For asthma, again based on consultation with the expert panel, the questionnaire-based approaches used in the available studies were interpreted to have an adequate level of specificity and positive predictive value for use in etiologic research (see Burney et al., 1989, HERO ID 2342689; Jenkins et al., 1996, HERO ID 2919120; and Ravault et al., 2001, HERO ID 2138435) that focuses on the occurrence of episodes of asthma, as opposed to the first occurrence of asthma. As described in the assessment (Section 3.2.3), the design of the study by Venn et al. (2003), a nested case-control study that included a 4-week symptom diary for assessment of asthma control among cases is particularly powerful for the purposes of hazard and dose response assessment. The reliability of the exposure measures in these studies to represent average formaldehyde concentrations over, in the case of Annessi-Maesano (2012), the previous 12 months, and in the case of Venn et al. (2003), the 4 weeks subsequent to the exposure assessment, is discussed in the assessment methods (Section 2.3.1) and evidence synthesis (Section 3.2.3); namely, the sampling periods were considered and justified as reflective of usual average formaldehyde exposure levels experienced by the occupants.

Quantitative use of a NOAEL is an example of a POD that can be based on a study that does not show an effect for a hazard identified based on the available evidence base as a whole. This is a foundational practice in risk assessment. Section 3.2.3 demonstrates, across multiple well-conducted studies, that very low formaldehyde exposures are unlikely to affect the prevalence of current asthma. The evidence synthesis also documents that the exposure levels in Annessi-Maesano were reliably measured and not far below the levels shown to be related to increases in

the prevalence of current asthma in the multiple other well-conducted studies used to support the hazard judgment. Thus, use of the NOAEL from Annesi-Maesano et al. to approximate a level below which effects on this outcome are unlikely to occur is reasonable, appropriate, and consistent with EPA's BMD technical guidance.

For the study by Venn et al., the synthesis text in Section 3.2.3 describes that Venn assessed the potential effects of indoor allergens and did not find that evidence of an adverse effect of NO₂ or VOCs other than formaldehyde on children's respiratory health and thus there was no evidence that these indoor allergens were confounders of the observed association between formaldehyde and prevalence of current asthma or control of asthma symptoms. Section 3.2.3 also describes that Venn was a stronger study for this effect than the study by Dannemiller et al. Section 5.1.1 documents the decision and rationale for not advancing Dannemiller et al. to POD derivation.

Overall, the results from these studies were determined to be reliable and robust for use in dose-response analysis. The details of the study results pointed out as potential errors by the commenter have been reviewed and corrected as needed.

- **Public Comment (American Forest and Paper Association):** "...For the endpoint of asthma there is an authoritative review (NAS 2000) and a comprehensive update (Kanchongkittiphon et al. 2015) involving 69 additional studies focused on indoor environmental exposures and exacerbation of asthma...Having ignored these two relevant and knowledgeable publications, EPA has ignored evidence on the key substances that are considered causally related to asthma. In doing so, the Review presents conclusions that are at complete odds with NAS and Kanchongkittiphon, both of which demonstrated a practical Weight of the Evidence approach...Importantly, EPA provides a review of a subset of the relevant literature instead of a full Weight of the Evidence evaluation on asthma and allergy, when compared to the two reviews mentioned above and reaches a very different conclusion on the role of FA and asthma. EPA concludes that the "evidence indicates" a role of FA with "allergic conditions and current asthma symptoms or degree of asthma control" while the National Academy of Sciences and Kanchongkittiphon et al. (2015) find only limited or suggestive evidence of an association between FA exposure and exacerbations of asthma...Due to its incomplete review of the literature, this Review does not raise to the level of "Weight of the Evidence" using the "Best Available Science" as required by the Lautenberg amendments to the Toxic Substances Control Act (TSCA) (2016). As TSCA will rely on the Review to inform the Risk Evaluation we feel it should be required that EPA conduct a suitable review at this stage in the TSCA Risk Evaluation process. We also urge EPA to revise the document to reflect appropriately the conclusions of the current literature. Of note, the evidence provided in a recent paper (Golden and Holm 2017) that was not cited or integrated into the Review supplies a roadmap of why unrecognized exposure to acrolein is an important confounding factor in many indoor air-related studies focused on FA."

“The above noted substances [acrolein] potentially present in indoor air that can exacerbate childhood asthma, many with different levels of certainty, present a substantial challenge with respect to designing and interpreting studies. The discovery that acrolein, an aldehyde that is 200 times more potent as a sensory irritant than FA (Fowles and Dybing 2003) and ubiquitous in indoor air, was significantly associated with asthma, whereas FA was not (Annesi-Maesano et al. 2012). Since it is plausible that FA has served as an unrecognized proxy for acrolein in studies conducted to date, our review (Golden & Holm, 2017) summarizes the well-established dose–response aspects of FA-induced irritation and its potential to exacerbate asthma symptoms. This is followed by a discussion of the available data on acrolein in sufficient detail to document its likely role in exacerbating asthma due to its irritant properties. The implications of acrolein as a previously unrecognized confounder are that indoor air studies, which report associations between FA and childhood asthma, should be interpreted with caution unless/until potential contributions and/or associations with acrolein are also considered. Even Annesi-Maesano et al, 2012, who EPA uses their data to provide a POD for “current asthma prevalence” stated, “Although small, the significant correlations between EIA and PM2.5 and acrolein represent an important message for public health as between 40% and 90% of people with asthma usually have EIA and could be at even higher risk when exposed to air pollution.” In addition, as noted by Leikauf (2002) due to ever-increasing acrolein emissions into the environment, acrolein as a direct irritant may increasingly become a health hazard in individuals with respiratory diseases such as asthma.”

“Acrolein sources in indoor air are similar to FA...Therefore, it is reasonable to conclude that there is a sound exposure-based rationale for better understanding the potential health impacts of acrolein, particularly as they might relate to sensory irritation and asthma. EPA must incorporate this rational information into their Review.”

“It is well documented (i.e., Garrett et al., 1998, 1999; Rumchev et al., 2002, 2004) that other substances in indoor air (e.g., VOCs and fungal spores) can cause and/ or exacerbate respiratory symptoms quite apart from FA. Consequently, it is likely inappropriate to conclude that the results reported by Krzyzanowski et al. (1990) can be unequivocally attributed to FA alone in indoor air. EPA has used this study published 32 years ago as “eligible for POD derivation for current asthma prevalence.” Findings of this study (i.e., Peak Expiratory Flow Rate or PEFs) are questionable in view of the low levels of FA found in the homes and at odds with controlled human studies where FA was the only variable (e.g., Lang et al., 2009). In addition, as in most studies of this kind, the lack of measurements of allergens or other chemical agents that may have been present in indoor air and possibly contributed to reported symptoms is a major confounder. Although the authors did report greater changes in PEFs in children than in adults, the use of this measure does not confirm the presence or absence of asthma or bronchitis or that FA (or something else) was responsible for this finding. This is the only study suggesting

differential effects in children versus adults, hardly a convincing basis for concluding that children are more sensitive to FA.”

“EPA also states in Krzyzanowski et al. (1990), (a study used by EPA to develop a POD for “current asthma prevalence”) that an “increased prevalence of current asthma was seen in the highest exposure group in a categorical analysis.” It is unclear what data EPA are relying on in making this statement. However, as noted in Table 4 of this paper there are three exposure ranges reported, <40 ppb; 41-60 ppb; >60 ppb. There is no dose response exhibited in the data presented and the prevalence rate for asthma in the highest concentration group for children exposed to FA but not to environmental tobacco smoke was 0. The authors report, “...the prevalence rates of current asthma diagnosed by a doctor were significantly increased in children living in houses with high FA levels in the kitchen, but only in those also exposed to ETS.” (Emphasis added). ETS is considered in the Kanchongkittiphon et al., 2015, review to be causally related to exacerbations of asthma in children. This incomplete presentation of the available data even in the studies EPA relies on is inherent in the Review and shows EPA’s bias in the presentation of data.”

“EPA stated that Annesi-Maesano et al. (2012), reported that FA was significantly associated with rhinoconjunctivitis at median reported FA levels of 21 ppb. However, these levels at which rhinoconjunctivitis (i.e., eye/nose irritation) occurred appear inconsistent with substantial data on dose–response aspects of FA-induced sensory irritation from controlled human studies reported by others. This endpoint is likely confounded by other causally associated substances in indoor air that were not measured in this study and not considered by EPA. This endpoint should not be considered causally related with FA and the study should not be used to derive a POD.”

“Importantly, Annesi-Maesano et al. (2012) reported that FA was not associated with asthma in this study. Acrolein was the only exposure significantly associated with both asthma (OR: 1.37, 95% CI: 1.14-1.66) and allergic asthma (OR: 1.41, 95% CI: 1.16-1.73) whereas NO₂ was statistically significantly associated with asthma (OR: 1.18, 95% CI: 1.01-1.39). FA was statistically significantly associated only with rhinoconjunctivitis (OR: 1.41, 95% CI: 1.08-1.85) but not with asthma.”

“Comparing the actual data in the [Annesi-Maesano et al. 2012] paper show that for the high dose of FA there is an increase in rhinoconjunctivitis (1.19), a decrease in asthma in the entire sample population (0.90), a decrease in asthma in atopic children (0.96) and a significant decrease in asthma among non-atopic children (0.82). Yet, EPA has used this study to incorrectly include a FA POD for current asthma prevalence. The use of this study to derive or support a Reference Concentration for FA is not consistent with the Best Available Science.”
[Please see docket for details and supporting information.]

EPA Response: As noted in the response above and in the revised assessment, Annesi-Maesano and several other supporting studies were used because they represented a set of studies with no effect for asthma at the low levels of formaldehyde that were present. A separate set of studies at higher levels consistently observed effects, providing support for the identified hazard and evidence of a dose-response across studies (rather than within each study). Confounding was explicitly considered during study evaluation. That documentation has been revised (Appendix B.3.4). Note that Billionnet et al. examined acrolein specifically and it wasn't associated with asthma in their study. But, more broadly, consistent with the consensus peer review finding, "EPA's hazard determination judgments are appropriate," EPA has retained the same hazard conclusions regarding allergic conditions and prevalence of current asthma or control of asthma symptoms in the revised assessment. The NASEM committee did not have any Tier 1 recommendations on this topic and EPA has revised the document to address the committee's findings and other comments.

Dose-Response Evaluation

Peer Review Findings and Comments

- **Finding (11):** EPA's conclusions and its choice of UFs are consistent with its state-of-practice methods, given the confidence levels and quality of the chosen studies. It is appropriate that asthma control PODs pertain to asthma in children, as no studies of asthma control in adults were of high enough quality.

Related Public Comments

No related public comments were submitted.

F.3.6. Reproductive and Developmental Toxicity

Charge Question and Peer Review Comment Summary

Charge Question 4a. Female Reproductive or Developmental Toxicity

- The assessment concludes that the ***evidence indicates*** that inhalation of formaldehyde likely causes female reproductive or developmental toxicity, given appropriate exposure circumstances. The conclusion for female reproductive or developmental toxicity is supported by evidence in humans, specifically, increases in time-to-pregnancy (TTP) and spontaneous abortion risk; mechanistic evidence explaining such effects without systemic distribution of formaldehyde is lacking.
- A POD from Taskinen et al. (1999), a human study, was ultimately selected to calculate an osRfC of 0.01 mg/m³ for TTP. A composite UF_C of 10 was applied to address UF_H and UF_S.

Charge Question 4b. Male Reproductive Toxicity.

- The assessment concludes that the ***evidence indicates*** that inhalation of formaldehyde likely causes reproductive toxicity in men, given appropriate exposure circumstances. The conclusion for male reproductive toxicity is supported primarily by coherent evidence of several alterations to the male reproductive system in animals exposed to very high levels of formaldehyde ($>6 \text{ mg/m}^3$), with some corroborative changes in an occupational epidemiological study; although no MOA is available, some relevant mechanistic changes have been observed in well-conducted studies.
- A POD from Özen et al. (2002), a rat study, was ultimately selected to calculate an osRfC of 0.01 mg/m^3 for testis weight. A composite UF_C of 3,000 was applied to address UF_H , LOAEL (UF_L), UF_S , and UF_A uncertainties.

Peer Review Comment Summary

- 0 Tier 1 Recommended Revisions
- 0 Tier 2 Suggested Revisions
- 0 Tier 3 Future Considerations
- 12 Consensus Panel Findings (numbered below)

Literature Identification

Peer Review Findings and Comments

- **Finding (1):** EPA's process for literature identification, PECO assessment, and study inclusion/exclusion was generally transparent and in line with EPA's state-of-practice methods for the female and male reproductive and developmental outcomes. However, search terms related to birth defects and teratology, such as congenital anomalies, were not included in the search.

EPA Response to Finding 1: The PECO criteria (Section 2.2) make it clear that birth defects and malformations were included within the scope of the review. While search terms of "birth defects" or "teratology" or "congenital anomalies" were not specifically used, the broader search terms applied in the assessment encompass such health outcomes.

Related Public Comments

No related public comments were submitted.

Study Evaluation

Peer Review Findings and Comments

- **Finding (2):** EPA's outcome-specific criteria for evaluating human studies are consistent with EPA's state-of-practice methods. A summary of key assessment factors specific to human reproductive and developmental outcomes is not provided.
- **Finding (3):** For human studies, EPA applied the appropriate general criteria for study quality (e.g., epidemiologic biases), consistent with its state-of-practice methods, as were the conclusions for individual studies (e.g., one medium-confidence study for effects on time to pregnancy and two medium-confidence studies for effects on spontaneous abortion).

EPA Response to Findings 2 and 3: EPA applied evaluation considerations specific to the reproductive and developmental outcomes in the epidemiological studies that met the PECO criteria. These considerations were previously discussed in the appendix in the study evaluation section for developmental and reproductive effects. In the revised assessment, tables of the evaluation criteria have been revised and added to the assessment methods section of the Toxicological Review (Section 2.3). These tables outline the criteria used for evaluating each specific outcomes, including time to pregnancy, spontaneous abortion, and postnatal effects.

- **Finding (4):** For animal studies, EPA applied the appropriate criteria for study quality (e.g., test substance, dose(s), test animals, evaluation endpoints), consistent with its state-of-practice methods, as were the conclusions for individual studies (e.g., two medium confidence studies and three high-confidence studies for male reproductive effects). Potential updates for two high-confidence studies (Ozen, 2015; Ozen et al., 2002, 2005, 2012) and one medium-confidence study (Sapmaz et al., 2018) are detailed in Appendix E. However, the potential reassignment of these three studies would not affect synthesis judgments and the next assessment steps since all *medium*- and *high*-confidence studies were considered in the next steps.
 - *Related committee comments* (Appendix E): "Two high-confidence studies (Ozen, 2012, and Ozen, 2015) could be reassessed based on small sample size ($N < 10$) for reassignment to medium confidence. One medium-confidence study (Sapmaz, 2018) could be reevaluated for consideration as high confidence because the test article box was flagged gray, when it likely ought to be clear."

EPA Response to Finding 4: The following decisions were implemented regarding the three studies identified by the peer reviewers:

- Sapmaz et al., 2018: The reviewers are correct that the exposure quality domain was incorrectly shaded and that the conduct of exposure was in fact 'good'; this has been

corrected. However, other notable study limitations remain within the test animals and study design domains that warrant keeping the confidence classification as *medium* rather than increasing it to *high* confidence. Thus, a rating of *medium* confidence was retained for this study in the revised assessment.

- Ozen et al., 2005 [note: not 2015] and Ozen et al., 2002: It is acknowledged that a sample size of 10 or greater is generally preferable for the assays performed in these studies (testes toxicity), with these two studies by Ozen et al. including 6–7 males per exposure group. It is important to note, however, that a lower sample size alone does not necessarily warrant reducing confidence. Upon revisiting these study evaluations in comparison with ratings for other animal toxicity studies, it was determined that the limited nature of the study design (i.e., a narrow focus on a few endpoints) and the other identified limitations, when considered in combination with the smaller sample sizes, do warrant decreasing study confidence from *high* to *medium* for these studies. Importantly, the identified limitations would be expected to reduce study sensitivity, or the ability of these studies to identify adverse effects on male reproduction (e.g., the 1996 EPA reproductive toxicity guidelines highlight that small sample size typically increases variability and thus reduces the ability to detect effects; this also has implications for dose-response analysis, as discussed in the assessment). In summary, these studies are now identified as *medium* confidence in the revised assessment.

Related Public Comments

No related public comments were submitted.

Evidence Synthesis and Synthesis Judgments

Peer Review Findings and Comments

- **Finding (5):** Within EPA’s framework for synthesizing results from human studies and making judgments, the conclusion of moderate evidence for female reproductive or developmental toxicity is justified. However, combining all reproductive and developmental outcomes in a single group is an oversimplification. EPA could consider separating its evidence synthesis section into early and late events.
 - Related committee comments: "The justification for combining outcomes was that it would be difficult to distinguish underlying pathogenic events that could yield a delayed recognized pregnancy or fetal loss. Although this rationale may apply generally for delayed time to pregnancy and spontaneous abortion, the broader category of female reproductive and developmental toxicity also encompasses human studies of other “later” developmental outcomes, such as congenital malformations and birthweight."

EPA Response to Finding 5: EPA did not separate the evidence synthesis section into early and late development events in the revised assessment (see Section 3.3.2). The available human studies were limited by design and they did not allow for such a distinction, e.g., delayed time to pregnancy or spontaneous abortion. The human evidence for events that might more clearly be “later” events was weak and ultimately did not contribute to the assessment conclusions (and the corresponding animal studies were all of low confidence). Without additional health effects evidence or mechanistic understanding about whether the different events are unlikely to be linked, it was not considered appropriate to attempt to draw separate judgments.

- **Finding (6):** The conclusion of indeterminate evidence for developmental toxicity and female reproductive toxicity in animals is consistent with EPA’s state-of-practice methods.
- **Finding (7):** The conclusion of slight evidence for male reproductive toxicity in humans is consistent with EPA’s state-of-practice methods.
- **Finding (8):** Information provided in the text and tables for animal studies of male reproductive toxicity is inconsistent (see Appendix E).
 - *Related committee comments* (Appendix E): “EPA needs to reconcile information provided in the text and in the tables for animal studies of male reproductive toxicity.”

EPA Response to Finding 8: The specific study details have been reviewed and revised as appropriate. Note that the Assessment Overview, which contained some of these discrepancies, has not been retained in the revised assessment.

Related Public Comments

No related public comments were submitted.

Overall Conclusions About the Hazard Descriptor

Peer Review Findings and Comments

- **Finding (9):** The conclusion that the evidence indicates that inhalation of formaldehyde likely causes increased risk of developmental or female reproductive toxicity in humans is consistent with EPA’s state-of-practice methods.
- **Finding (10):** The conclusion that the evidence indicates that inhalation of formaldehyde likely causes increased risk of reproductive toxicity in men is consistent with EPA’s state-of-practice methods.

Related Public Comments

- **Public Comment (Natural Resources Defense Council):** "NRDC supports EPA's determination that inhalation of formaldehyde "likely causes" female reproductive or developmental toxicity in women – EPA's conclusion is supported by evidence in humans, including time-to-pregnancy (TTP) and spontaneous abortion risk. As NIEHS noted in its comments, "it is reasonable to conclude that female reproductive toxicity based on TTP (Taskinen et al. 1999) and spontaneous abortion (John et al. 1994, Taskinen 1994, 1999) is supported by epidemiological evidence" (NIEHS p. 6) EPA notes that "mechanistic evidence explaining such effects without systemic distribution of formaldehyde is lacking" (p. 54). However, NIEHS comments noted that, "it has been clearly demonstrated that formaldehyde is metabolized to formic acid" which is demonstrated to impair embryo viability and is linked to adverse developmental outcomes (NIEHS p. 6). NRDC agrees with NIEHS that the addition of this scientific evidence and proposed mechanism would strengthen EPA's evaluation. NIEHS identified this as a "Tier 1" necessary revision. Nonetheless, NRDC concurs with EPA that mechanistic information is not necessary, and a data gap or the absence of knowledge should not be used as a basis for downgrading the strength of evidence. (p. 40)"

EPA Response: The mechanism proposed by the commenter has not been included in the revised assessment given the assumption that inhaled formaldehyde and its byproducts do not reach systemic tissues. EPA Note: Interagency reviewer (e.g., NIEHS) comments were on a draft prior to revision for public release. Please consult the full interagency review comments in the EPA docket.

- **Public Comment (American Chemistry Council):** "The best available science does not support EPA's conclusions regarding formaldehyde and reproductive and developmental effects. The NASEM (2011) Committee stated that EPA did not provide a clear weight of evidence discussion to support its classification for reproductive and developmental toxicity and did not agree with EPA's conclusions...As discussed further below, EPA maintains a similarly strong conclusion regarding the reproductive and developmental evidence, despite the absence of new supportive sentinel studies published in the last decade or any plausible MOA."

"The Draft Assessment incorrectly concludes that 'evidence indicates' that inhalation of formaldehyde causes increased risk of reproductive and developmental toxicity, and classifies it as a reproductive and development hazard. This is despite the statement (p. 1-414) that 'The primary basis for this conclusion is based on bioassays in rodents testing formaldehyde concentrations above 6 mg/m3.' In drawing this conclusion, the Draft Assessment excludes both toxicokinetic considerations and relative dosimetry. This is in direct opposition to Globally Harmonized Classification and Labeling (GHS) guidance as well as EPA guidance on evaluating reproductive and developmental toxicity. First, inhaled formaldehyde is not distributed

systemically, thus, it does not affect normal formaldehyde concentrations in reproductive or developmental organs/systems, so it cannot be a direct hazard. Secondly, any reproductive effects were only observed under conditions of high toxicity in the dams, which does not trigger a Hazard Classification for reproductive effects, according to EPA guidelines or GHS. As such, it must not be classified as presenting a reproductive and developmental hazard.” *[Please see docket for details and supporting information.]*

EPA Response: To clarify, only the conclusion about male reproductive toxicity is based on studies in rodents using high formaldehyde exposure levels. The conclusion that the evidence indicates that formaldehyde exposure is likely to cause female reproductive and developmental effects is based on studies in human workers (see Section 3.3.2). While it is unknown how inhaled formaldehyde may cause these effects without systemic distribution, EPA guidelines do not require such understanding to draw hazard conclusions.

The consideration of confounding effects due to possible overt toxicity in the rodent studies examining male reproductive effects has been revised. In particular, confidence in the candidate RfC based on decreased serum testosterone is now decreased due to the revised interpretation that the responses in the selected study could be amplified due to indirect systemic toxicity (see Section 5.1). However, the remaining key decisions on male reproductive toxicity have been retained.

Overall, consistent with the consensus peer review findings, EPA has retained the same hazard conclusions regarding reproductive and developmental toxicity in the revised assessment. The NASEM committee did not have any Tier 1 recommendations on this topic and EPA has revised the document to address the committee’s findings and other comments.

Dose-Response Evaluation

Peer Review Findings and Comments

- **Finding (11):** The rationale for study selection, the POD determination, uncertainty factors, and cRfC derivation, including confidence levels, were consistent with EPA’s state-of-practice methods.
 - *Related committee comments* (Appendix E): “Regarding reproductive and developmental toxicity, EPA needs to double check the publication year for references in Tables 30 and 31. The testes endpoint is likely incorrectly citing, linking to Ozen (2005) when it ought to be Ozen (2002). For animal male reproductive toxicity studies, Ozen (2002) was appropriately considered to be the stronger of the two studies and was therefore used to derive the RfC.”

EPA Response to Finding 11: Tables 30 and 31 of the Assessment Overview in the external review draft did indeed incorrectly cite the 2005 study by Ozen et al. for both sets of male reproductive endpoints considered for dose-response analyses. The Assessment Overview has not been retained

in the revised assessment. The revised Toxicological Review correctly cites Ozen (2002) for the testes endpoint and Ozen (2005) for the testosterone endpoint, with the 2002 result considered to be the stronger of the two and that used to derive the candidate toxicity value for male reproductive toxicity (Section 5.1).

- **Finding (12):** The rationale for exclusion of studies from the dose-response assessment is consistent with EPA's state-of-practice methods and includes analysis of pooled tissues, short exposure duration, single exposure level, and nonpreferred endpoint assessment.

Related Public Comments

- **Public Comment (ToxStrategies):** "The U.S. EPA's hypothesize MOA for male reproductive effects misses a critical alternative explanation. Modeling the serum testosterone data from Ozen et al. (2005) resulted in good fitting models with a BMDL_{1SD} value of 0.21 mg/m³ in agreement with U.S. EPA (data not shown). Interestingly, this POD is similar to that derived for bodyweight gain changes and testes (see above). A very quick literature search reveals that food restriction affects testes weight and serum testosterone levels in rats (Rehm et al. 2008). If the bodyweight gain changes reported in Ozen et al. (2002) were due to reduced feed intake, then the changes in testes weight and serum testosterone might be related to changes in bodyweight as opposed to formaldehyde toxicity per se. Notably, these effects all occur at about the same concentration—a possible indicator that they are in fact related to one another. This hypothesis seems as likely, if not more likely, than U.S. EPA's hypotheses about indirect mechanisms like inflammation, oxidative stress, and neuroendocrine disruption (see section 1.3.2 of the Toxicological Review). That the linkage between feed intake and these same markers of toxicity are not even considered by EPA is problematic. Notably, the Ozen et al. (2002) study that EPA scored as high makes no mention of feed or water consumption despite the significant decreases in bodyweight gain. This calls into question the overall thoroughness and quality of this study."

EPA Response: The possible indirect effects of reduced food and water consumption (and presumably decreased body weight) on serum testosterone measurements by Ozen et al. (2005), was highlighted as an uncertainty in the revised assessment. The study authors indicate an effect of exposure on food and water consumption, but do not report the specific magnitude of these effects, or possible effects on body weight, for comparative purposes in interpreting the serum testosterone decreases. In the revised assessment, this uncertainty is now highlighted in the study evaluation, evidence synthesis, MOA, and dose-response sections on this study (the latter contributing to a lower level of confidence in the candidate toxicity value based on this finding; see Section 5.1). However, these revisions did not affect any key assessment decisions or conclusions.

Separately, after additional consideration, the observation of reduced body weight gain (note: food intake was not specifically measured in this study) after formaldehyde exposure continues to be interpreted as not affecting EPA's interpretations regarding potential effects on testes weight (Ozen et al., 2002). As noted in the assessment, in EPA guidelines, and in the study cited by the commenter above (Rehm et al., 2008), toxicity to the testes (e.g., decreased testes weight) is generally independent from effects on body weight leading to a preference outlined in the assessment for absolute testes weight measures. In fact, in the cited study (Rehm et al., 2008), food restriction-induced effects on body weight of nearly 30% (i.e., larger than the body weight % change at 12.3 mg/m³ in Ozen et al., 2002) had no effect on absolute testes weights. A limitation noted for Ozen et al. (2002) is that only relative, not absolute, testes weights were reported. However, because relative weight reflects the ratio of the organ weight to body weight, a decrease in body weight without a parallel decrease in the organ weight would increase the relative weight; the primary concern with relative testes weight is that the body weight decrease (artificially increasing the relative organ weight result) would mask (reduce the magnitude) of the specific formaldehyde-induced decreases. Thus, the decreases in relative testes weight in Ozen et al. (2002) are considered real and are likely an underestimate of the true effect magnitude in that study, were absolute testes weights available. The discussion of Ozen et al. (2002) was not changed.

- **Public Comment (American Chemistry Council):** "Neurotoxicity and reproductive and developmental toxicity studies used for dose-response and/or hazard classification did not meet the criteria in USEPA guidance for the confidence ratings proposed in the Draft Assessment yet were advanced (sometimes even all of the way to RfC development)... The Ozen et al. (2002) and Ozen et al. (2005) subchronic rat studies were rated 'high confidence' studies and used to derive osRfCs for potential male reproductive toxicity in the Draft Assessment. Both studies had numerous deficiencies that would lead to a grade of 'uninformative' and thus not used for either hazard classification or dose-response assessment, as follows." *[Please see docket for details and supporting information.]*

EPA Response: The male reproductive toxicity studies highlighted by the commenter were evaluated and synthesized in accordance with the criteria in Section 2.3 and EPA's guidelines. While study limitations that affect the utility of these studies for dose-response analysis were identified (and which are highlighted in the assessment and lead to low confidence in the derived values), these limitations did not affect the study evaluation ratings. However, it is noted that these studies were reevaluated based on peer input and now are classified as *medium* rather than *high* confidence (see documentation in Appendix B.3). EPA Note: neurotoxicity was not identified as a hazard and no neurotoxicity studies were advanced for dose-response analysis in either the external review draft assessment or the revised assessment.

F.3.7. Nervous System Effects

Charge Question and Peer Review Comment Summary

Charge Question 4c. Nervous System Toxicity

- While many studies reporting evidence of potential neurotoxic effects were available, due to limitations identified in the database (e.g., poor methodology, lack of consistency), it was ultimately determined that the *evidence suggests*, but is not sufficient to infer, that formaldehyde inhalation might pose a human nervous system hazard. This judgment was separately arrived at for three different manifestations of potential neurotoxicity, namely developmental neurotoxicity, altered neurobehavior, and an increased incidence of, or mortality from, the motor neuron disease amyotrophic lateral sclerosis. The evidence integration narrative emphasizes that additional study is warranted.
- The available data on potential nervous system effects were considered insufficient for developing quantitative toxicity estimates.

Peer Review Comment Summary

- 0 Tier 1 Recommended Revisions
- 0 Tier 2 Suggested Revisions
- 1 Tier 3 Future Consideration
- 9 Consensus Panel Findings (numbered below)

Literature Identification

Peer Review Findings and Comments

- **Finding (1):** The process for literature identification and inclusion/exclusion criteria for the review of nervous system effects was transparent.
 - *Related committee comments* on literature identification (Appendix E): “Regarding the nervous system, Figure A-35 of the Supplemental Information indicates that 40 human studies were considered, but only 12 are described in the Main Assessment in (Tables 1-44 and 1-45), and 15 were included in the Appendices (Tables A-84 and A-85). The narrative indicates that studies that were evaluated as *not informative* were excluded from these tables; however, both the Kilburn (2000) and Schenker et al. (1982) studies are described despite their overall confidence evaluation of *not informative*. Thus, it is not clear why these studies were included in the table. Clear reasoning for exclusion from tables need to be provided or the literature described in Tables A-84 and A-85 need to be updated to be consistent with the information provided at the end of Figure A-35.”

“Table A-84 incorrectly describes the sample for the Bellavia et al. (2021) paper as “cancer cases”; however, they were amyotrophic lateral sclerosis (ALS) studies from registry data. The job exposure matrices (JEMs) for this study was developed for a previous cancer study; ALS cases were identified through Danish medical records without consideration of cancer status. The details in Table A-84 need to be reviewed for accuracy, and minor typographical errors need to be addressed.”

EPA Response to Finding 1: The clarifications noted by the committee have been incorporated.

- **Finding (2):** EPA elected to exclude headache as a human health outcome for evaluation because of the subjectivity of outcome reporting (Appendix A, Table A-83). Considering that other endpoints included in this assessment, such as sensory irritation, depend on subjective self-reporting, excluding headache based on this criterion is inconsistent.
- **Tier 3 Future Consideration (Recommendation 4.15):** EPA should include studies with headache as an outcome to maintain consistency with other health effect categories. Alternatively, a stronger rationale should be provided for exclusion of headache other than its perceived subjectivity. Headache could be combined with other self-reported neurotoxicity outcomes.

EPA Response to Tier 3 Future Consideration 4.15: In addition to being subjectively reported, headaches were typically not rated by severity and were often pooled with non-nervous system-specific complaints (e.g., related to irritation, such as dry eyes). Further, questions remain regarding the specificity of the complaints to nervous system effects, as they could be byproducts of other health symptoms (e.g., irritation-related complaints were frequently correlated). These additional explanatory considerations have been added to the assessment methods. It is also noted that, as was generally not the case for sensory irritation, studies of objective measures of nervous system effects that involved long-term formaldehyde exposures were available, and thus were preferred. Separately, it is noted that in many of the available studies, individuals were included in the study due to complaints about symptoms attributed to exposure to chemicals in the air, and thus these studies would generally involve critical concerns about selection bias. This has been clarified in the revised assessment.

Related Public Comments

No related public comments were submitted.

Study Evaluation

Peer Review Findings and Comments

- **Finding (3):** Overall, criteria for evaluating human studies were consistent with EPA's state-of-practice methods. However, it is not clear whether the study quality criteria were applied uniformly across studies.

EPA Response to Finding 3: The study evaluation criteria were reviewed and revised to address each of the evaluation domains assessed in the studies of human exposure. These criteria were cross-checked with the evaluation tables for studies of nervous system effects in the appendix. The review resulted in changing the confidence conclusions for two of the studies based on the criteria for exposure assessment ([Bellavia et al., 2021](#)) (*medium to low*) and other limitations including small number cases ([Pinkerton et al., 2013](#)) (*high to medium*).

- **Finding (4):** Where explanations are provided for confidence assessments for more recently published literature, EPA gives no details about the basis for confidence decisions for controlled exposure studies in humans (Appendix A, Table A-85).

EPA Response to Finding 4: "Table A-85" (Appendix Table B-50 in the revised assessment) documents the evaluations of the three controlled human exposure studies of neurobehavioral outcomes. Confidence in two of the studies ([Bach et al., 1990](#); [Andersen and Molhave, 1983](#)) was *low* because test batteries were performed during exposure (irritation anticipated to result in distracted responses), an incomplete set of tests were conducted, authors did not report whether subjects or administrators were blinded to exposure, and deficiencies in reporting were noted. Methods were not as limited in the third study ([Lang et al., 2008](#)).

- **Finding (5):** Regarding human studies, study quality criteria (i.e., selection bias, sample size, exposure assessment, and confounding) were consistent with EPA's state-of-practice methods. However, the reasons for final conclusions on study confidence are not always clear, and there are inconsistencies in the explanations for the confidence ratings. Potential updates to specific studies (e.g., Bellavia et al., 2021; Kilburn, 2000; Pinkerton et al., 2013; Schenker et al., 1982; see also Seals et al., 2017 and Peters et al., 2017) are detailed in Appendix E.
 - *Related committee comments* (Appendix E): The following comments were made:
 - "Pinkerton et al. (2013) was rated with high confidence, with a comment of "small number of cases" in reference to 8 ALS deaths. However, other studies with a small number of cases were rated as medium."
 - "Kilburn (2000) and Schenker et al. (1982) were assessed as not informative with comments about limited or no exposure measures. However, it is important to note that two of the included manuscripts used some version of

the same set of Danish registry data (Seals et al. [2017] and Bellavia et al. [2020]), both used JEMs derived from the Swedish JEM used in the Peters et al. (2017) manuscript as part of the Nordic Occupation Cancer Study, and each of these JEMs estimate exposure based on previous studies of biological samples, air and dust monitoring, and expert opinion, and are not direct exposure measures. Furthermore, the occupational codes used to determine exposures are not based on job title, but only on tax-recorded industry codes. Therefore, exposure assessment provided in these studies is not significantly better than that provided in the studies ranked with low confidence."

EPA Response to Finding 5: The study evaluation criteria were reviewed and revised to address each of the evaluation domains assessed in the studies of human exposure (Section 2.3.7 in the revised Toxicological Review). These criteria were cross-checked with the evaluation tables for studies of nervous system effects in Appendix B.3.7. The review resulted in changing the confidence conclusions for two of the studies based on the criteria for exposure assessment ([Bellavia et al., 2021](#)) (*medium to low*) and other limitations including small number cases ([Pinkerton et al., 2013](#)) (*high to medium*). Kilburn (2000) and Schenker et al. (1982) were rated as *not informative* primarily because of concern that the exposed group was selected on the basis of their exposure to formaldehyde. The selection bias would be expected toward an inflated estimate of risk. Limitations in the other *low* confidence studies included deficiencies in reporting study methods and results and a lack of information on variation or intensity of exposure ([Kilburn et al., 1985b](#); [Kilburn et al., 1987](#); [Kilburn et al., 1989b](#); [Kilburn and Warshaw, 1992](#)). The studies with exposure assessments based on the Nordic Occupation Cancer Study assigned formaldehyde levels to individuals based on industry and occupation codes. The Danish studies used the Danish Pension Fund for occupational histories while the Swedish studies used the national census. While these databases record jobs at the industry/occupation level, the job-exposure-matrix uses monitoring data specific to each country developed by industrial hygienists with expertise in the industries for each country. Exposure misclassification is expected, however this method provides information on the variation in levels and intensity of exposure in the study population. [Bellavia et al. \(2021\)](#) assigned exposure to formaldehyde as "ever" versus "never" and therefore was reclassified as *low* confidence.

- **Finding (6):** Regarding animal studies, study quality criteria were consistent with EPA's state-of-practice methods.

EPA Response to Finding 6: In ensuring consistency in evaluations across studies of a particular type (e.g., in vivo experimental animal studies), three in vivo studies examining mechanistic

changes received a revised rating of *medium*, from *high*, confidence: Sorg, 2001, 626595, Tsukahara, 2006, 626805, and Hayashi, 2004, 626503

Related Public Comments

No related public comments were submitted.

Evidence Synthesis and Synthesis Judgments

Peer Review Findings and Comments

- **Finding (7):** With human studies having medium and high confidence for ALS and low or not informative confidence for neurobehavioral outcomes (e.g., memory, mood changes), along with medium confidence for animal studies on developmental neurotoxicity, the basis for the overall evidence judgment is not well articulated.

EPA Response to Finding 6: The three separate evidence integration conclusions regarding the potential for (1) changes in ALS incidence, (2) neurobehavioral alterations, and (3) structural changes in the developing brain across the available human, animal, and mechanistic studies relevant to each are outlined in Section 3.3.1 and summarized separately in the evidence profile tables at the end of that section. This presentation has been revised in the final assessment. As outlined in the methods for evidence synthesis and integration (Sections 2.5 and 2.6), the conclusions are not based on confidence in the individual studies, but rather they are based on the consistency, coherence, and dose-dependence, etc. of the results of the higher confidence studies, alongside additional considerations including the human relevance of the findings in animals and the biological plausibility of the observed effects.

- **Finding (8):** The 2011 NRC committee found the discussion of the neurogenic MOA in the 2010 Draft Assessment to be vague and speculative. In the 2022 Draft Assessment, EPA provides a much more thorough overview of potential MOAs without speculative conclusions.

Related Public Comments

No related public comments were submitted.

Overall Conclusions About the Hazard Descriptor

Peer Review Findings and Comments

- **Finding (9):** The conclusion that the evidence suggests but is insufficient to infer significant long-term neurotoxicity from inhaled formaldehyde in humans is consistent with EPA's state-of-practice methods, given the number of studies with final assessments of confidence in the not informative to low range, and very few being assessed as having medium or high confidence.

Related Public Comments

- **Public Comment (American Chemistry Council):** “The 2022 Draft Assessment stated, ‘Overall, conclusive of a nervous system health hazard in humans exposed to formaldehyde was not identified (i.e., suggestive evidence).’ The finding of ‘suggestive evidence,’ however is at odds with EPA’s acknowledgement of inconclusive evidence. Indeed, a critical review of the available evidence suggests that an ‘inadequate’ descriptor would be more appropriate for this endpoint... Given the lack of data for any postulated MOA and failure to demonstrate consistent effects across any endpoint, an integration judgment of ‘evidence is inadequate’ is more appropriate rather than the proposed ‘suggestive’ integration judgment for nervous system effects.” [Please see docket for details and supporting information.]

EPA Response: Consistent with the consensus NASEM peer review finding (9, above), the conclusion of *evidence suggests* but is insufficient to infer is retained in the revised assessment. Section 3.3.1 describes the rationale for drawing a judgment of *slight* evidence of formaldehyde-induced nervous system effects in humans, including the potential for effects on amyotrophic lateral sclerosis disease incidence, neurobehavioral changes, and structural changes in the developing brain. The available studies were limited, and none were advanced for dose-response analysis. The potential for nervous system effects remains an area deserving of additional study.

F.3.8. Derivation of the RfC

Charge Question and Peer Review Comment Summary

Charge Question 5. Noncancer RfC

An RfC was selected based on the grouping of osRfCs for sensory irritation, decreased pulmonary function, allergy-related conditions, and increased prevalence of current asthma or decreased degree of asthma control. Please comment on whether the approach and selection of the proposed RfC was clear and scientifically justified, including consideration of other potentially sensitive health effects.

Peer Review Comment Summary

- 0 Tier 1 Recommended Revisions
- 1 Tier 2 Suggested Revision (multiple parts)
- 1 Tier 3 Future Consideration (multiple parts; note: not numbered in NASEM study report)
- 1 Un-Tiered Committee Comment
- 1 Consensus Panel Finding

Peer Review Findings and Comments

- **Finding (1):** The Main Assessment and Appendices reflected EPA’s efforts to follow its various guidelines and are generally consistent with its 2022 IRIS Handbook. The committee identified

concerns with the dose-response approach taken for some studies (e.g., Hanrahan et al., 1984, as detailed in Table 4-3 and footnotes). Other occasional issues concerning consistency and accuracy were sometimes the result of omissions or inadequate documentation. More than a dozen specific examples are detailed in Appendix E, which provides additional suggestions regarding EPA's consideration of the following studies: Kulle et al. (1987); Andersen and Molhave (1983); Krzyzanowski et al. (1990); Dannemiller et al. (2013); Woutersen et al. (1989); Ozen et al. (2002, 2005); and Kerns et al. (1983). [Note: EPA interpreted the Appendix suggestions on these studies as an unnumbered Tier 2 suggested revision below]

- *Relevant committee comments:* As noted in Table 4-3 of the consensus study report, "the committee had concerns about EPA's approach to deriving the POD" for the following three studies: Hanrahan et al., 1984; Kulle et al., 1987; and Andersen and Molhave, 1983. Although suggestions for improvement were noted in several cases, the committee did not note concerns about EPA's approach to deriving PODs for the remaining studies for which PODs were derived by EPA in the draft assessment.
- *Un-tiered Committee Comment* on modeling of specific studies (from Appendix E):
 - a) Kulle 1987; Andersen and Molhave 1983: "it is uncertain whether a factor of 2 is greater than the true ratio of BMC/BMCL (i.e., whether it is sufficiently large to account for the data dependence).... [this] appears inconsistent with EPA's own guidelines and EPA did not provide a justification."; "[no methods for] conversion of the Likert-scale [Kulle] ...to the dichotomous variable used to estimate prevalence." "... [for the] human studies of Kulle et al. (1987) and Andersen and Molhave (1983), EPA correctly recognized that the same group of volunteers were exposed to multiple concentrations, and the responses from individual volunteers were correlated across different exposure concentrations. EPA's benchmark dose (BMD) modeling failed to account for such data dependence, and thus likely underestimated the variation of the BMC. EPA subsequently divided the benchmark concentration (BMC) by a factor of 2 to replace the model-based benchmark concentration lower bound (Table 2-2). However, it is uncertain whether a factor of 2 is greater than the true ratio of BMC/BMCL (i.e., whether it is sufficiently large to account for the data dependence). This practice appears inconsistent with EPA's own guidelines and EPA did not provide a justification."
 - b) Kulle et al., 1987: "Kulle et al. (1987) measured eye irritation using a 4-point Likert scale for none, mild, moderate, and severe, and reported the mean score difference (standard error) between 180 minutes postexposure and baseline. EPA's dose-response modeling of Kulle et al. (1987) and Andersen and Molhave (1983) was, however, for the fraction of affected (i.e., prevalence of eye irritation). EPA did not provide information in the Main Assessment or Appendices on the conversion of the Likert-scale in Kulle et al. (1987) to the dichotomous variable used to estimate prevalence."

- c) Krzyzanowski 1990: “For pulmonary function, EPA relied on the linear mixed-effects model results reported by Krzyzanowski and colleagues (1990). The original regression model incorporated whether or not the children had asthma and whether or not the exposure measurements were taken in the morning. EPA’s derivation of the BMCL was based on children without asthma exposed at times other than the morning. This approach was inconsistent with EPA’s state-of-the-practice methods (i.e., using more vulnerable subpopulations for risk estimation). To its credit, however, EPA conducted a sensitivity analysis for children with asthma and morning exposures. The sensitivity analysis, however, was based on BMC, but would more appropriately be based on BMCL. The derivation of a BMCL for children with asthma can be achieved by first determining the standard errors of the combination of regression coefficients associated with the terms of formaldehyde concentration and concentration squared. Note that children with asthma had their own baseline prospective epidemiological risk factor (PERF) (348.09) and the corresponding 10 percent decrease of 34.8. The intercept in equation B-9 is incorrect.”
- d) Krzyzanowski 1990; Dannemiller 2013: “EPA used either a group median (e.g., current asthma, Krzyzanowski et al. [1990]) or a group mean (e.g., atopic eczema, Matsunaga et al. [2008]) as an estimate of lowest observed effect level / no observed effect level (LOAEL/NOAEL) when a range of exposure concentrations was reported within the group. EPA also seemed to have used either an arithmetic mean or a geometric mean to estimate a LOAEL/NOAEL. Dannemiller et al. (2013) reported the geometric mean of 54.0 and 34.4 ppb in the “very poor control” group and “all others” group, respectively. EPA seemed to have designated 34.4 ppb as the NOAEL (0.042 mg/m³, Table 2-4, [footnote e in this table indicating that EPA did not make this decision explicit]). (Note that the reference in the last row of Table 2-4 ought to be Dannemiller et al. [2013]). The study of Dannemiller et al. (2013) was dropped from the discussion for cRfC derivation thereafter. EPA did not give reasons for excluding this study. The text starting from page 2-16, line 19, appears corrupted.”
- e) Kerns 1983; Woutersen 1989: “EPA reanalyzed the animal data of Kerns et al. (1983) (Level I of sagittal crosssection I only) and of Woutersen et al. (1989) (anterior, Levels I and II). EPA did not disclose whether it obtained raw data or extracted secondary data from the original report. Multiple models were fit to the datasets, and the one with the smallest Akaike information criterion (AIC) was chosen. Note that AIC is a relative criterion for comparison across models. Alone, AIC does not tell how well a model fits the data... EPA did not report goodness-of-fit tests, a practice inconsistent with the reanalysis of other studies in the Assessment... “osRfC for respiratory pathology was based on Woutersen 1989. Kerns 1983) was incorrectly cited in Table 2-11.

- f) Ozen et al. (2002, 2005: “In the reanalysis of the data from Ozen et al. (2005), EPA stated that (p. B-23, lines 6–8): “If the BMDL estimates were ‘sufficiently close’, that is, differed by at most xx-fold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.” This criterion is vague and does not enhance transparency.”

“In conducting BMD modeling of Ozen et al. (2002) and Ozen et al. (2005), which had three and four distinct concentration levels (including the control), respectively, EPA fit models with three or four parameters, resulting in parameter saturation in the model or failure in model fitting.” [Note: “The various model-based tests for the Ozen et al. (2005) study were labeled as “test 1”, “test 2”, etc. (Table B-17, p. B-29) without explanation.”]

EPA Response to Un-Tiered Committee Comment on study-specific modeling decisions: The following changes were made.

- a) The assessment has been revised to select PODs from these studies based on a wider confidence interval (i.e., the 99th as compared to the typical 95th percentile lower bound) rather than the BMC divided by 2. This widened confidence interval provides a means of more transparently approximating the increased variability expected if independent data measurements were available. Perhaps more importantly, it is based on the variability within the experimental data rather than a factor of 2 applied regardless of the underlying data variability. Ultimately, use of the 99th percentile results in PODs from these two studies that are in some cases less than 2-fold below the BMC and in other cases more than 2-fold (almost 4-fold in one instance) below the BMC, depending on the underlying experimental data. The revised PODs remain similar to those in the external review draft (i.e., for the study by Kulle et al., the selected POD is now 0.44 mg/m³, as compared to 0.42 mg/m³ previously; for the study by Andersen and Molhave, the POD is now 0.12 mg/m³, as compared to 0.19 mg/m³ previously). The dose-response analysis for these studies is outlined in Appendix D.1.1.
- b) The assessment has been revised to model the data from ([Kulle et al., 1987](#); [Kulle, 1993](#)) using Categorical Regression analyses (CatReg; <https://www.epa.gov/bmds/about-catreg>.) to more fully explore both the reported severity information as well as the available data for multiple endpoints of sensory irritation. To clarify, the external review draft combined the reported symptoms of mild and moderate severity into an “affected” count (note: the published data on the mean symptom difference or symptom score difference in the 1987 publication were not modeled previously and are not modeled in the revised draft as those findings involved pooled measures across individuals with differing severity and thus are not preferred for modeling without access to the underlying data and score calculations; the modeled data used in the

external review draft and the revised assessment are reported in the 1993 reanalysis). The symptoms of differing severity are now more fully considered using CatReg. Overall, the application of CatReg in the revised assessment does not have a large influence on the selected sensory irritation POD from (Kulle et al., 1987; Kulle, 1993), but it does facilitate greater inclusion and combining of the available data. See Appendix D.1.1 for details.

- c) EPA attempted to reach the study authors for additional details; however, no additional information was available (see earlier responses). EPA contacted Dr. Krzyzanowski regarding this analysis and notes that there was no main effect for ‘asthma’ in the model and any information on the covariances between the terms was unavailable. These additional attempts are noted in the revised assessment.
 - d) The reasons for not advancing Dannemiller et al. (2013) and the details related to POD decisions on Krzyzanowski et al. (1990) have been added or clarified, respectively, in the revised assessment.
 - e) The assessment was revised to state that EPA extracted the data from the Battelle Report and from Woutersen et al. (1989). Overall goodness of fit *p*-values have been added to the revised Appendix D.1.4. The error for Woutersen was corrected as noted.
 - f) The fold-change was clarified in the revised assessment to reflect that a 3-fold difference is what is typically used. Regarding parameter saturation, if one runs a *n*-parameter model on a dataset with *n*-total dose groups (including controls), there will not remain a degree of freedom left to calculate a goodness-of-fit *p*-value and BMDS will report N/A. In that case, the model is not further considered for selection. In the revised assessment, the POD derivation for the Ozen studies was re-performed, with decisions made consistent with the committee comments (see Appendix D.1.5).
- **Tier 2 Suggested Revision (Recommendation 4.16):** EPA should carefully address the following points regarding the derivation of the RfC:
 - a) Fully disclose data extracted from original study reports using HERO or other means.
 - b) Cite relevant guidance documents regarding the use of a mean versus median and arithmetic mean versus geometric mean to estimate a lowest observed adverse effect level or no observed adverse effect level.
 - c) In reanalyzing data from published studies, the use of raw data is preferred. Aggregated data may be used when appropriate. At a minimum, group size, group mean, and a measure of variance (e.g., group standard deviation or standard error of the mean) for each exposure level are needed to capture data variation in a reanalysis of dose-response.

- d) Avoid fitting a dose-response model that has as many parameters as the number of distinct aggregated data points taken from the published literature. Report and consider only models that meet the goodness-of-fit criteria EPA accepts.
- e) To ensure that the resulting benchmark concentration lower bound is not artificially overestimated, better account for within-group variability in the dose-response analysis of Hanrahan et al. (1984) to address limitations arising from reliance on only secondary, aggregated rates per exposure group that were extracted from the plot of the originally fitted model.

Committee comments related to “e” (from Appendix E): “[The] regression model has artificially narrow standard errors for the model parameters compared with those fitted to the raw data because the model-predicted means failed to reflect the true data variation. As a result, the model-based BMDL (0.09 mg/m³) is biased. Therefore, the use of the cRfC derived from Hanrahan et al. (1984) as the osRfC for sensory irritation needs additional justification.” [Note: In Appendix D, NASEM is able to replicate EPA methods; but recommends combined modeling with Liu 1991 to result in a lower BMC by ~20%]... “EPA extracted 8 distinct model-predicted means, one for each exposure concentration, from the Hanrahan et al. (1984) by reading a plot (Figure 1), and then refitted a logistic regression model with a polynomial of order 3. The resulting logistic regression model has artificially narrow standard errors for the model parameters compared with those fitted to the raw data because the model-predicted means failed to reflect the true data variation. As a result, the model-based BMDL (0.09 mg/m³, Table 2-2) is biased.”

- f) Be more explicit as to how the final RfC was chosen (in Figure 2-2 of the 2022 Draft Assessment and elsewhere).

Committee comments related to “f” (from Appendix E): “Figure 2-2 displays variability and uncertainty across all osRfCs along three dimensions: confidence, uncertainty, and risk size. The committee found this graphic display to be an effective visual aid. The size of the plot symbol for each osRfC was determined by three factors: the level of the confidence in the study(ies) and health hazard identification; risk estimate(s) (EPA gives slightly greater weight to the risk estimate than other factors); and completeness of evidence database for each health outcome. However, EPA was not explicit about how these factors were weighted.” ... “Figure 2-3 is also interesting and informative. It graphically displays the uncertainties within a cRfC as well as variations and uncertainties between cRfCs.” ... “EPA presented a detailed discussion of uncertainties and variabilities, and noted that the osRfCs for asthma, pulmonary function, allergies, and sensory irritation were 0.006, 0.007, 0.008, and 0.009 mg/m³, respectively, reflecting the impact of formaldehyde on the respiratory system. EPA proposed the overall RfC of 0.007 mg/m³ but was not explicit about how it was chosen.”

EPA Response to Tier 2 Suggested Revision 4.16: The following revisions were implemented.

- a) This documentation has been revisited and revised as appropriate (note: one dataset provided in a memo from CIIT has been added to Appendix D.2.2 in its totality for transparency).
 - b) EPA's BMDS technical guidance defines "Central estimate" as the mean or median value. However, EPA has clarified the rationale for individual decisions on POD selection in the revised assessment.
 - c) This comment is noted and appreciated. Modeling of multiple datasets has been redone.
 - d) In the revised assessment, models are selected in accordance with this criterion.
 - e) EPA provides additional analyses building from the recommendation above in the revised assessment (see Appendix D.1.1 on sensory irritation) by combining the dose-response data from Hanrahan et al. (1984) and Liu et al. (1991). Using the data available and building on approaches outlined by the NASEM committee, EPA now estimates a BMCL from the combination of these studies. Concerns about the precision of the estimate remains; however, the value based on the combined studies is judged as more stable and appropriate. Note that, in estimating a BMCL from Hanrahan et al. (1984) alone in the external review draft, EPA used the information on lower confidence bound on the main effect. The lower bound captures information about the standard error and incorporates within-group variability in the dose-response analysis.
 - f) In the revised assessment, the midpoint of the three highest confidence values (0.006, 0.007, 0.008 mg/m³) was selected as the RfC (Section 5.1.5). The supporting rationale was revised. The figure supporting the rationale for selecting the RfC based on the three values has also been revised to be more transparent. Facilitating this transparency, greater resolution of the confidence determinations for each value is now provided, which allows for straightforward, step-wise changes in the size of the shape based on step-wise changes in confidence across the values (e.g., from low to low-medium to medium-low to medium to medium-high, etc.).
- **Tier 3 Future Consideration 4.17 [note: not numbered in the NASEM report]:**
 - a) Handle dose-response modeling of correlated data (e.g., Andersen and Molhave, 1983; Kulle et al., 1987) by standard statistical methods, employing a two-step process that involves first fitting a dose-response model for correlated data using standard statistical methods, and then deriving BMC and BMCL using the fitted model.
 - b) Develop methodology that goes beyond a qualitative display of the variability and uncertainty of cRfCs or osRfCs. The current EPA method has limited reproducibility because of the lack of detail. A meta-analysis approach offers a viable option.

EPA Response to Tier 3 Future Consideration 4.17: EPA appreciates these future considerations and the topics have been highlighted to the IRIS Program's statistical working group for consideration in modeling data from studies in future assessments.

Public Comments on the RfC

- **Public Comment (ToxStrategies):** "It is difficult to verify all the BMD modeling results as Appendix B clearly indicates that the model outputs were run nearly a decade ago in January of 2013, apparently using EPA's BMD software (BMDS) v1.9. The current version of BMDS is v3.2. U.S. EPA's BMDS website (<https://www.epa.gov/bmds>) explicitly states that BMDS 2.7 is no longer supported, and this must also be true for prior versions. It is reasonable to expect that any new U.S. EPA assessment should use the most current (and supported) version of BMDS. The U.S. EPA should re-run all the BMD modeling in the assessment with the latest version of BMDS.

Curiously, many of the BMD modeling results in this document are expressed in units of mg/kg-d as opposed to mg/m³. This is likely a result of the modeling software not knowing the units, but it is surprising that EPA did not correctly indicate the units. Moreover, all the discussion about BMD models should technically be benchmark concentration (BMC) as these are inhalation concentrations as opposed to oral doses."

"U.S. EPA's benchmark dose modeling of rat respiratory histopathology has several reporting and methodological flaws."

"U.S. EPA's benchmark dose modeling of male reproductive toxicity studies has several potential flaws." *[Please see docket for details and supporting information.]*

EPA Response: It is not EPA's practice to update modeling results because a new version of BMDS software is released (note: most of the BMDS modeling in the Step 4 draft was done using BMDS v2.2). It is also important to emphasize that the few modeling results derived using BMDS were for studies in experimental animals, and the results from the animal studies were not the drivers of the assessment conclusions. However, in the process of reviewing the PODs from these animal studies to address other peer review and public comments, multiple datasets were re-run using BMDS Online (v3.3). For example, the modeling and reporting of the rat respiratory histopathology has been revised. However, the 24-month data cannot be modeled as the incidence (0.01, 0.97, 0.99, 1.0) saturates at the first non-zero dose. Likewise, the modeling of male reproductive toxicity studies was also re-modeled, and several errors, including incorrect units, were corrected.

- **Public Comment (American Chemistry Council):** "The Draft Assessment does not present explicit rationales for selecting a particular benchmark dose (BMD) or for choosing BMD over a LOAEL/NOAEL approach. In particular it must be clarified when effects are observed at

concentrations above the range of saturable metabolism (i.e., 1-2 ppm for formaldehyde), but extrapolated to doses below the range of saturable metabolism.”

“In Table 2-10 on pages 2-30 and 2-31, NOAELs are not presented and a discussion of the dose levels used in the studies is not provided, in favor of only presenting the POD...In the derivation of the cRfC for male reproductive toxicology from Ozen et al. (2002), a POD of 2.91 is listed, with a combined uncertainty factor of 3,000, to yield a cRfC of 0.001 mg/m³. Without knowledge of the assumptions underlying this calculation, the reader cannot evaluate uncertainty and conservatism in the calculation. The lowest concentration tested in the study was 12 mg/m³, which is 12,000 times above the calculated osRfC. This highlights that there is more uncertainty in the extrapolation than presented.

Additionally, the Draft Assessment states (p. xxii): ‘... higher confidence was placed in the osRfC when the POD was identified close to the range of the observed data.’ However, almost all the RfCs and osRfCs were modeled outside of the range of the observed data which introduces substantial uncertainties, none of which appear to be recognized in the draft assessment.”

EPA Response: The commenter’s statement that almost all the noncancer toxicity values in this assessment were modeled outside of the range of the observed data is incorrect. Most notably, the PODs supporting the RfC are all within the range of the observed data, as are most of the other PODs. This feature of the noncancer evidence base was highlighted in the NASEM peer review report (consider calling out what page in that report). For the minority of PODs that are based on occupational exposure or on animal studies with higher exposure levels, this uncertainty is described. Note also that a reliable BMC-based POD is preferred over NOAEL- or LOAEL-based POD, consistent with EPA guidelines. The revised assessment clarifies the rationale for selection of NOAELs or LOAELs when those are the only PODs that can be reliably used in Section 5.1. Specifically, regarding the Ozen study, this modeling has been revised and the UF_C lowered; the osRfC based on this study in the revised assessment is 0.006 mg/m³ and interpreted with low confidence.

The available evidence on susceptibility to the health effects of formaldehyde is synthesized in Section 4.1, with the primary application of that understanding to dose-response analysis for the studies advanced for toxicity value derivation (Section 5), including in the application of uncertainty factors (Section 5.1.3). As outlined in these sections, at least for pulmonary function and asthma, and likely for other respiratory effects, there is evidence to support that children, and asthmatic children in particular, are likely to be more sensitive. However, it is important to note that the assessment does not conclude that these effects do not occur in adults exposed to formaldehyde. Section 3.2.2 describes the consistent and strong evidence for declines in pulmonary function in workers with long-term exposure to formaldehyde (note: Appendix C.5 describes studies of pulmonary function in adults with short-term exposure); however, these long-term occupational studies generally only tested higher (≥ 0.2 mg/m³) levels of formaldehyde as

compared to the residential studies, the latter of which sometimes included adults or adults and children. Section 3.2.3 highlights that there are few studies on the potential effects of formaldehyde on allergic conditions in adults, with concerns noted regarding the methods and imprecise results in those studies that prevent drawing a reliable judgment. However, Section 3.2.3 also shows that studies of workers with long-term exposure to formaldehyde collectively provide strong support for increases in asthma prevalence, but as above these findings are generally at higher formaldehyde levels ($> 0.1 \text{ mg/m}^3$) than the more sensitive residential- and school-based studies advanced for dose-response analysis for the purposes of developing an RfC. In addition to age, a host of additional factors (e.g., respiratory health; pre-existing disease; sex; smoking status; race) have been shown, sometimes with weaker evidence, to affect inter-individual variability in sensitivity for one or more of the health effects of formaldehyde. With few exceptions (i.e., large population studies encompassing a wide range of demographics, including reasonable representation of known or expected susceptible groups), the available studies are judged as unlikely to sufficiently address these differences in susceptibility, irrespective of the health outcome being considered. Consistent with EPA's intent for an RfC and its definition, the RfC is applicable to all persons, including all lifestages as well as susceptible individuals, regardless of job status. Additional demographic details (e.g., information on age and sex) for the studies advanced for candidate value derivation have been added to the POD tables (Section 5.1.2) as further information supporting the UFs applied.

- **Public Comment (Monell Chemical Senses Center):** "While the POD for chemosensory detection and adaptive physiological response is not based on an adverse effect, formaldehyde odor detection, resulting in poor air quality, could be considered undesirable for consumers in their homes and workplaces, thus, justifying a guideline value, but not a regulatory limit."

"An increase in eye blinking or conjunctival redness is a normal adaptive response to external stimuli and not an adverse health effect. As such, a guideline value may be appropriate for lower concentrations of an irritant chemical (i.e., concentrations below 1 ppm), but developing a RfC based on the potential for adverse health effects on the eyes at lower concentrations is not supported by either individual studies or a weight-of-evidence approach."

"Despite the numerous controlled exposure studies that have demonstrated the effective concentrations for preventing eye irritation from formaldehyde, in the Draft US EPA/IRIS Assessment, studies [Hanrahan, Dally et al. 1984; Liu, Huang et al. 1991; Kulle, Sauder et al. 1987; Andersen and Molhave 1983] that are critically flawed and should have been excluded from the determination for dose-response were inappropriately advanced and used to derive or support a RfC of 0.009 mg/m^3 for formaldehyde. In contrast, several controlled human exposure studies that are generally considered to be robust were not used to derive a RfC (Lang, Bruckner et al. 2008, Mueller, Bruckner et al. 2013). It is also well-acknowledged that the ability of a chemical to elicit odor occurs at (significantly) lower concentrations than its ability

to elicit sensory irritation (Doty, Cometto-Muñiz et al. 2004). Despite this, the USEPA has proposed a RFC for sensory irritation that is well below the established threshold for odor. This inconsistency arises from the inappropriate use of and support by uninformative epidemiological studies with confounding variables that produce critical flaws. Following are concerns identified among the epidemiological or controlled exposure studies judged with medium confidence [Hanrahan, Dally et al. 1984; Liu, Huang et al. 1991; Kulle, Sauder et al. 1987; Andersen and Molhave 1983] that were used to derive or support a RfC based on sensory (eye) irritation.”

“Overall, the reliance on historical symptom prevalence reports that are not obtained in conjunction with exposures raises serious concerns that an inappropriately low RFC is being derived. To date, no chemical has been identified as having an irritation threshold that is lower than its odor threshold and formaldehyde is no exception. Reliance solely on the data from the Hanrahan study has resulted in the identification of an irritation threshold that is orders of magnitude below the acknowledged odor threshold. The number of well-conducted controlled exposure trials with no evidence of ocular irritation between 0.3-0.5 ppm provide ample evidence that maintaining exposures at or below these concentrations will provide sufficient protection from sensory irritation, including eye irritation.”

[Please see docket for details and supporting information.]

- **Public Comment (Ramboll):** “We were both co-authors of the Formaldehyde section of the World Health Organization Indoor Air Quality Guidelines for Select Pollutants (WHO 2010). We are concerned that the US Environmental Protection Agency (USEPA) evaluation of noncancer endpoints in the Draft document “Toxicological Review of Formaldehyde—Inhalation” largely ignores several important studies that the formaldehyde working group considered key. These recommendations were further endorsed by peer reviewers, and then the entire set of working groups and their reviewers. The recommendations were also published in a series of peer-reviewed journal papers by two of the WHO formaldehyde working group authors, Drs. Wolkoff and Nielsen...We were disappointed to see USEPA discount use of these high-quality studies [Mueller et al. 2013 and Lang et al. 2008], in favor of a population-based study where exposure was not controlled (Hanrahan et al 1984), which USEPA states was supported by older studies (Kulle et al 1993 or Andersen and Molhave 1983). The rationale provided was that the more recent chamber studies did not find a dose-response relationship. However, we note that one of these studies did not find any effect, even at the highest exposure, and the other study only found mild, chemosensory effects. This seems to discount better conducted studies simply because no effects were observed, which seems to be biased towards finding an effect where none exists. We believe that properly controlled human exposure studies are the “gold standard” for setting safe exposure limits, as they offer the major advantage of having defined exposure conditions, carefully characterizing volunteers, and altering experimental methods to

consider potential confounding factors (Nielsen and Wolkoff, 2017). For example, people can detect the odor of formaldehyde at concentrations as low as 0.1 mg/m³ (WHO, 2010), which can interfere with subjective reporting of sensory irritation (Cometto-Muñiz and Cain 1991; Brüning et al., 2014). Controlled human exposure studies can add controls that mask these odors and their influence on subjective measures.

We hope that USEPA will reconsider the studies serving as the basis of the reference concentration for sensory effects.”

EPA Response (to both public comments above): The revised assessment retains the identification of sensory irritation as a hazard and provides a toxicity value for this effect. This is consistent with the consensus 2023 NASEM peer review recommendations and prior advice in the 2011 NRC review. The decision criteria for the studies selected to support this toxicity value as well as the uncertainties in the selected studies and the other studies considered for deriving this value are described in the revised assessment (Section 5.1). Once a hazard has been identified, the goal of dose-response analysis is to calculate an RfC that is reasonably protective against those identified hazards, including in susceptible groups (see Sections 2.7 and 5.1, and the IRIS Handbook). Thus, in addition to evaluating the methodological conduct of the studies, the specific study populations and their diversity to represent the general population including a wide range of ages and health statuses, and the exposure scenarios (constant exposure vs peaks) are influential to the decision to advance or select certain studies. This is particularly true for an endpoint such as sensory irritation, which exhibits a large degree of inter-individual variability in sensitivity, and it is even more important when the considered studies are small. The chamber studies evaluating sensory irritation possessed these important limitations, which reduces their utility for the purposes of deriving a chronic RfC that is protective of a population.

The WHO guidelines (2010) cited by the commenter above state, “The short-term guideline will also prevent effects on lung function as well as long-term health effects, including nasopharyngeal cancer and myeloid leukaemia.” The WHO (2010) short-term guideline is based on sensory irritation in acute controlled exposure studies of healthy, middle-age human volunteers. Because the WHO analysis is quite brief (1-page), the scientific basis for this conclusion is not clear. WHO (2010) appears to reason that the short-term guideline is protective of long-term effects based on the following statements: (1) “There is no indication of accumulation of effects over time with prolonged exposure”; (2) “Neither increased sensitivity nor sensitization is considered plausible at such indoor concentrations in adults and children”; (3) “Indoor air effects of formaldehyde are expected to be limited to the site of contact, generally the nasal and upper airways”; (4) “Increased cell proliferation due to cell damage is considered a key mechanism for the development of nasal malignancies following exposure to formaldehyde... The NOAEL for cell proliferation [in rats] is 1.25 mg/m³ for long-term exposures” (Note: assuming a threshold for cancer, WHO applied to this NOAEL an interspecies factor of 3 and an interindividual variation

factor of 2 to yield a value of 0.21 mg/m³); and (5) “An alternative approach was taken by several other groups, using a biologically motivated model... These two assessments (using a NOAEL/assessment factor approach and estimates from the biologically motivated models) yield similar results, with values of approximately 0.2 mg/m³.” The evidence basis supporting the above statements, the selected NOAEL, and the other decisions is not apparent in the WHO (2010) document. Because their estimate of 0.2 mg/m³ for cancer was higher than their short-term guideline, WHO (2010) concludes, “use of the short-term (30-minute) guideline of 0.1 mg/m³ will also prevent long-term health effects, including cancer.”

EPA does not consider this approach supported by the available evidence, particularly given the availability of well-conducted studies demonstrating several more sensitive health effects of formaldehyde after long-term inhalation exposure. In the available studies, these other health effects were either not evaluated after acute exposure or the effects were not observed at similar or even higher levels after acute exposure. Further, during the extensive review of the apical and mechanistic studies relevant to the portal of entry health effects of formaldehyde (hundreds of publications), EPA did not identify evidence to support the hypothesis that protecting individuals from developing sensory irritation would protect against other chronic noncancer health effects or cancer, particularly when considering human variability. Likewise, EPA did not identify evidence to support the hypothesis that sensory irritation is “upstream” of other health effects caused by formaldehyde inhalation (e.g., evidence supporting temporal- or exposure level-dependent biological linkages between key events for sensory irritation and those for the different health effects). EPA is not aware of any evidence to support the hypothesis that protecting against sensory irritation following acute exposure would be protective against chronic health effects. Biologically, such approaches would require assuming that formaldehyde levels that are too low to affect biological components (e.g., receptors) enough to cause detectable sensory irritation following an acute exposure are also too low to affect other biological components enough to cause any other effects, even with longer-term exposures. There seems to be no evidentiary basis to support this assumption.

Quantitatively, it is noted that the values (i.e., points of departure, PODs, and candidate RfCs, cRfCs, for chronic exposure) for sensory irritation are not among the most sensitive noncancer values in the IRIS assessment, and the values derived from the acute chamber studies of sensory irritation in intentionally exposed human volunteers are even less sensitive, with PODs generally an order of magnitude higher than many of the other chronic noncancer PODs. Thus, none of the sensory irritation values are protective against other sensitive noncancer health effects. In addition, based on the cancer IUR in the IRIS assessment, the selected IRIS cRfC for sensory irritation (i.e., based on the observational epidemiology studies by Hanrahan et al., 1984 and Liu et al., 1991) would be associated with a lifetime extra cancer risk of 2.2×10^{-4} (i.e. roughly 2 in 10,000 people at increased risk for developing cancer over their lifetime). Use of an IRIS cRfC for sensory irritation from the acute chamber studies (i.e., based on the preferred chamber study by Kulle et al., 1993)

would be associated with an increased cancer risk in approximately four (4.4) in 10,000 people. For context, performing the same calculation using the IRIS RfC yields an extra cancer risk of 0.77×10^{-4} (increasing the lifetime cancer risk for roughly 8 in 100,000 people). While the IRIS cRfC based on cytotoxicity-induced regenerative hyperplasia is higher (less sensitive) than the selected IRIS cRfC for sensory irritation based on the human observational studies (Hanrahan et al., 1984 and Liu et al., 1991), it is several-fold lower (more sensitive) than the cRfC derived from the preferred chamber study (Kulle et al., 1993). That is, a toxicity value based on sensory irritation from the chamber studies would not only be inadequate to protect against cancer, but it would also be inadequate to protect against cytotoxicity-induced cellular proliferation contributing to cancer development (noting that this represents only one of at least two independent pathways contributing to cancer development). In summary, none of the values (PODs or cRfCs) based on sensory irritation are protective against other noncancer health effects or cancer.

- **Public Comment (American Chemistry Council):** “In addition to these studies [Lang et al. 2008 and Mueller et al. 2013] of irritation following short-term exposures, multiple studies have also been conducted in animals focused on objective measurements of tissue irritation, cell proliferation and cytotoxicity following short-term repeated dose exposures (Swenberg et al. 1983a, 1983b; Monticello et al. 1991; Monteiro-Riviere and Popp 1986). Although some of these studies were discussed by EPA under the category “respiratory pathology” with robust evidence, they are objective evidence of an adverse effect, tissue irritation, following short-term exposures and would thus be more relevant for deriving an irritant RfC.”

“EPA should use objective measures of adverse effects for developing noncancer RfCs.”

[Please see docket for details and supporting information.]

EPA Response: EPA does not agree that the animal studies mentioned by the commenter are appropriate to represent the identified hazard of sensory irritation (separately, please see comment responses relating to Lang and Mueller in Appendix F.3.2 on sensory irritation). The available evidence also does not support that the tissue changes in these short-term studies are appropriate for derivation of a chronic RfC, as these effects clearly worsen with continued exposure. However, the assessment does consider these studies and as mentioned by the commenter, presents a toxicity value for respiratory tract pathology. The assessment also leverages related effects for derivation of an RfC-based value for one MOA contributing to the development of cancer, cytotoxicity-induced regenerative proliferation.

- **Public Comment (ToxStrategies):** “On page 1-154 of the Toxicological Review, EPA states that most of the studies reporting histopathological changes in humans had average exposures ranging from 0.05 to 0.6 mg/m³. EPA speculates that these workers are likely less sensitive to formaldehyde than the average person, characterizing them as “survivors” of long-term irritant

exposure. This appears to be complete speculation and, to this reviewer, undermines the credibility of the assessment. Notably, there were no high confidence studies in humans. Among the four medium confidence studies, one (Boysen et al. 1990) was characterized by EPA as equivocal. Importantly, all the studies found relatively mild differences in nasal histopathology when plant workers were compared to non-plant workers (see table below). Many of the plant workers were exposed to wood dust and likely other chemicals. As shown in the table below, many of the individuals in the referent groups appear to work in very different professions with different environmental settings (e.g., factory vs hospital). These comparison groups do not appear to be suitable for ascribing any observed differences to formaldehyde with any certainty.”

EPA Response: The potential for a “healthy worker” effect is an established concern (with a clear bias towards the null) for analyses of occupational populations in epidemiology studies. However, the specific wording about “survivors” has been removed in the revised assessment to focus on the specific concern for potential “survival bias” as follows: “These were cross-sectional studies of current workers who may have been less sensitive to the long-term respiratory irritant effects of formaldehyde, which would cause survival bias and an attenuation of comparisons between exposed and comparison groups.”

- **Public Comment (American Chemistry Council):** “EPA did not derive, and present for comparison, an RfC for upper respiratory tract effects, based on cytotoxicity, as directed by the NAS (2011).”

“The Draft Assessment often confuses the difference between an adverse and compensatory response, leading to the development of RfCs based on compensatory rather than adverse responses, which is prohibited by EPA guidelines for risk assessment. Hyperplasia and metaplasia are often compensatory responses, as recognized in the Draft Assessment, but then considered adverse and used for developing RfCs.”

[Please see docket for details and supporting information.]

EPA Response: The commenter is incorrect. The assessment estimates and discusses RfC-based values for one MOA contributing to the development of cancer, cytotoxicity-induced regenerative proliferation (see Section 5.2.1 in the revised assessment). It is noted that both hyperplasia and metaplasia can pre-dispose a tissue to neoplastic changes and squamous metaplasia is interpreted as an adverse change on its own (i.e., separate from its potential association with cancer) (Section 3.2.4). Peer reviewers supported these conclusions.

- **Public Comment (American Chemistry Council):** “The Draft Assessment chose to exclude available data but adds uncertainty factors to the POD to account for lack of these same data.

The Draft Assessment (p. 2-28) when justifying an uncertainty factor being applied for extrapolation of subchronic to chronic exposure for respiratory tract pathology states: ‘A factor of 3 was applied to the respiratory tract pathology POD from Kerns et al. (1983) because it was based on 18-month exposure data from that rodent study in lieu of the 24-month exposure data available in the same study.’ First, it is noteworthy that the 24-month NOAEL for respiratory tract pathology is the same as that defined for the 18-month exposures. Second, the Draft Assessment, in the same paragraph, provides a contradictory analysis by first stating: ‘...there are data to suggest that exposure concentration would be more important to the development of this lesion than duration’ and ‘a lower POD would have been expected if the 24-month data could have been modeled.’ Speculation on what would be “expected,” does not present a strong case for adding uncertainty factors for the absence of the excluded data. Thirdly, there are internal inconsistencies in the Draft Assessment when determining to ignore the 24-month data of Kerns et al. (1983)... There is no rationale for concluding that a preference for a shorter-term value from the same study is inappropriate in one study evaluation, but acceptable for another study evaluation.”

“The Draft Assessment lacks transparency in the quantitative assessments and conservatism in proposed exposure limits is introduced by the use of modeling practices and exposure concentration adjustments that are ill defined. In addition, insufficient rationale is provided and most often relegated to footnotes. Some resulting uncertainty adjustments exceed the limits defined by EPA.”

EPA Response: As discussed previously, the 24-month data could not be modeled. The statements about the data at 24-months resulting in a lower POD are not speculative but rather based on the incidence information available at 24 months versus 18 months. There is a clear increase in the severity of the response at 24 months as compared to 18 months in this dataset and thus the UF = 3 is retained in the revised assessment. It is noted, however, that due to lower confidence in the candidate value based on this study, it was not selected as the osRfC for respiratory tract pathology.

- **Public Comment (Toxicology Excellence for Risk Assessment):** “...EPA should focus on the area of critical effect and not belabor effects that are clearly well advanced in formaldehyde’s underlying mode of action. For example, estimating a Reference Concentration (RfC) for a pathological endpoint that only occurs well above a No Observed Adverse Effect Level (NOAEL) for tissue irritation is non-sensical. Furthermore, EPA has some exquisite human data in hand including data in sensitive subgroups. EPA needs to recognize that a NOAEL for such data are, in effect, the RfC, as per its own definition of RfC. In such cases, no additional uncertainty factors are needed for within-human variability, as readily demonstrated by prior EPA judgments found on its Integrated Risk Information System (IRIS).”

- **Public Comment (American Chemistry Council):** “The Draft Assessment presents RfCs for a number of non-cancer systemic effects, including reproduction/developmental effects in females and reproductive toxicity in males. NAS (2011) identified this as problematic and not supported by the evidence.”

“The Draft Assessment does not integrate available data, as required when reaching conclusions; individual RfC and Inhalation Unit Risk (IUR) factors were calculated based on individual study results with only uncertainty factors added and/or linear extrapolations. The exception is an inappropriately averaged RfC for female reproductive or developmental toxicity.” *[Please see docket for details and supporting information.]*

EPA Response (to both public comments above): All decisions and supporting justifications for the noncancer dose-response assessment are described in Section 5.1 of the revised Toxicological Review. This includes decisions for study selection, POD derivation, uncertainty factor application, candidate value calculations and confidence, and selection of the RfC. The NASEM peer review panel had no Tier 1 recommendations on the RfC, and EPA has addressed all other panel comments on this topic.

F.4. NASEM REPORT CHAPTER 5: CANCER; CHARGE QUESTION 6: CANCER AND CHARGE QUESTION 7: IUR FOR CANCER

F.4.1. Hazard Identification

Charge Question 6. Cancer.

The assessment concludes that formaldehyde is ***Carcinogenic to Humans by the Inhalation Route of Exposure***. Please comment on whether the judgments in (a) to (f), below, are clearly described and scientifically justified. Note that the three judgments in (a), (b), and (c) outline the primary evidentiary support, and that each of these judgments would independently substantiate the carcinogenicity conclusion.

- a. The **evidence demonstrates** that formaldehyde inhalation causes nasopharyngeal cancer (NPC) in humans, based primarily on observations of increased risk of NPC in groups exposed to occupational formaldehyde levels and evidence of nasal cancers in animals, with strong, reliable, and consistent mechanistic evidence in both animals and humans, including support for a mutagenic MOA.
- b. The **evidence demonstrates** that formaldehyde inhalation causes sinonasal cancer (SNC) in humans, based primarily on observations of increased risk of SNC in groups exposed to occupational formaldehyde levels and supported by the evidence of nasal cancers in animals, with consistent mechanistic evidence and support for a mutagenic MOA.

- c. The **evidence demonstrates** that formaldehyde inhalation causes an increased risk of myeloid leukemia in humans, based primarily on observations of increased risk in groups exposed to occupational formaldehyde levels. This judgment is supported by other studies of human occupational exposure that provide strong and coherent mechanistic evidence identifying clear associations with additional endpoints relevant to lymphohematopoietic (LHP) cancers, including an increased prevalence of multiple markers of mutagenicity and genotoxicity in peripheral blood cells of exposed workers, other perturbations to immune cell populations in blood (primarily from human studies). Generally, evidence supporting the development of LHP cancers after formaldehyde inhalation has not been observed in experimental animals (i.e., rodents), including a well-conducted, chronic cancer bioassay in two species, a similar lack of increased leukemias in a second rat bioassay, and multiple mechanistic evaluations of relevant biological changes such as genotoxicity in systemic tissues of exposed rodents. The lack of findings in animals does not detract from the strong human evidence.
- d. The overall conclusion on the carcinogenicity of inhaled formaldehyde was independently supported by the three sets of evidence outlined in 6a-6c above. This carcinogenicity conclusion was not influenced by the judgments for several other cancer types. Specifically, the **evidence suggests**, but is not sufficient to infer, that formaldehyde inhalation might cause oropharyngeal/hypopharyngeal cancer, Hodgkin lymphoma, or multiple myeloma in humans; there is **inadequate evidence** to determine whether formaldehyde inhalation may be capable of causing laryngeal cancer or lymphatic leukemia in humans.
- e. Formaldehyde is genotoxic in several test systems and operates, at least in part, through a mutagenic MOA. Specifically, a mutagenic MOA was identified in association with the development of nasal (i.e., nasopharyngeal and sinonasal) cancers, while no MOA was identified for other cancer types. The mechanistic evidence was sufficient to conclude that both mutations and cellular proliferation play a role in nasal carcinogenesis.
- f. The MOA(s) leading to cancer formation outside of the respiratory tract are unknown.

Peer Review Comment Summary

- 0 Tier 1 Recommended Revisions
- 3 Tier 2 Suggested Revisions
- 0 Tier 3 Future Considerations
- 13 Consensus Panel Findings (numbered below)

Organization, Literature Identification and Evaluation of Study Methods

Peer Review Findings and Comments

- **Finding (1):** The overall organization of the information on cancer effects of formaldehyde is appropriate and commensurate with prior recommendations from the NRC. The focus on respiratory tract cancers and cancer of the lymphohematopoietic system, but not other cancer types, is appropriate and justified.
- **Finding (2):** With respect to cancer hazard evaluation, EPA adequately and transparently evaluated the scientific literature using methods consistent with its state-of-practice methods. Sections of the 2022 Draft Assessment describing the methods used for identifying and evaluating studies of formaldehyde and cancer in humans and animals, as well as mechanistic evidence, are voluminous and extensive but well organized. EPA did not have the final version of the IRIS Handbook while it was developing the 2022 Draft Assessment, and also had the additional challenge of a 4-year hiatus during which no work could be performed on this assessment. Nonetheless, the overall process used and choices made are consistent with EPA's state-of-practice methods and responsive to the recommendations of the 2011 NRC committee.

Related Public Comments

- **Public Comment (Thomas B. Starr):** "These comments are directed at EPA's discussions of the collaborative research conducted by myself and Dr. James A. Swenberg on a novel "bottom-up" approach to bounding human cancer risks that could arise from chronic inhalation exposure to low levels of airborne formaldehyde. This work has been described in three peer-reviewed publications (Swenberg et al. 2011, and Starr and Swenberg 2013 and 2016). It has also been addressed critically in a "Letter to the Editor" by Crump et al. (2014), and our response to that letter (Starr and Swenberg 2014), the latter of which is not discussed or even cited in the Draft IRIS Toxicological Review, its reference list, or the Supplemental Information for Formaldehyde document. More recently, Hartwig et al. (2020) and Lu et al. (2022) have reviewed (positively) the "bottom-up" approach in two additional peer-reviewed articles, neither of which is discussed or cited in these EPA documents. EPA's failure to cite our response to Crump et al.'s 2014 letter and the two more recent peer-reviewed articles calls into question the completeness (and validity) of EPA's Systematic Review process."

EPA Response: The Starr and Swenberg (2014) response to Crump (2014) is cited in the revised assessment in Section 5.2.1. EPA Note: reviews and studies with no primary data are considered as supplemental materials and tracked in HERO.

- **Public Comment (Natural Resources Defense Council):** "Comments from NIOSH identified three genotox studies – Chebotarev et al 1986, Vasudeva and Anand 1996, Yager et al 1986 - that are not included in EPA's evaluation, that provide experimental evidence supporting a genotoxic mechanism of toxicity for formaldehyde. While the first is in Russian, an English abstract is available, and for the other two they are available in English. If EPA is excluding all studies that are not in English, this is problematic and deserves some discussion and scrutiny." "NIOSH commenters also noted the failure to include an important published meta-analysis of myeloid leukemia risks in occupationally exposed workers, focused on occupations known to have high formaldehyde exposures by Zhang et al 2009. The study shows a statistically significant 1.5X increase leukemia in 15 studies (RR=1.54, CI 1.18-2.00), and an almost 2-fold higher risk for myeloid leukemia in six studies (RR=1.90, CI 1.31-2.76). Omission of this important paper should be corrected."

EPA Response: Two of the three noted studies are cited in the Appendix C.3; the third is tagged in HERO as supplemental. To clarify, non-English studies are not excluded although they are not necessarily translated [see also IRIS Handbook ([U.S. EPA, 2022](#))]. EPA staff consider the criticality of non-English studies (e.g., based on a translated abstract or review by a native speaker) with respect to their potential impact on assessment conclusions. Non-English papers considered unlikely to have a substantial impact, such as this one on a topic for which there are many studies, were not translated.

- **Public Comment (Cardno ChemRisk [now Stantec]):** "The EPA also disregarded the Mundt et al. (2018) summary of relevant studies [Meyers et al. 2013; Coggon et al. 2014; Talibov et al. 2014; Saberi Hosnijeh et al. 2014] published in the 6 years following the NRC review of EPA's Draft IRIS Toxicological Review of Formaldehyde and addressing several gaps identified by the NRC...EPA's characterization of the literature published since the 2010 IRIS draft is limited and would have been informed by the summaries provided in Mundt et al. (2018)."

"I reviewed the recently released IRIS Toxicological Review of Formaldehyde (Inhalation) (External Review Draft, 2022) to assess the accuracy and completeness of my formaldehyde research publications. Specifically, I identified all occurrences of citations to my publications in each of the IRIS documents (Main Text, Overview document, and Supplementary Materials), then evaluated the presentation of the information (i.e., risk estimates) and the interpretation of these publications.

As detailed further below, I have published 18 formaldehyde publications, but the Draft only cites 10. Several of the omitted papers were critical contributions to the literature on the potential human health effects of formaldehyde. Additionally, in several instances, EPA has misinterpreted and mis-cited my publications in a manner that substantively affects the

interpretation of these individual studies and the overall weight of the evidence regarding formaldehyde carcinogenicity.

EPA should consider re-reviewing all 18 publications and updating the Draft to add missing studies, correct citation errors, and revise the language characterizing the findings of these studies." *[Please see docket for details and supporting information.]*

EPA Response: The primary data studies indicated by the commenter are cited in the assessment. The eight "missing" citations are reviews and were tagged as supplemental material. As described in prior responses and the assessment methods (Section 2.2), reviews and studies with no primary data are considered as supplemental materials and tracked in HERO. However, supplemental studies are not necessarily cited in the assessment, in particular if they are reviews of studies that meet the assessment PECO criteria. EPA conducts an independent analysis of the studies meeting PECO criteria, so the stance of EPA on the impact and utility of the papers to inform assessment conclusions can differ from the views and conclusions expressed by the study authors.

Study Evaluation

Peer Review Findings and Comments

- **Finding (3):** EPA followed a transparent and reasonable approach in evaluating the studies relevant to cancer in humans and in animals, and in providing mechanistic evidence to support its analysis. EPA followed the state of practice for literature review at the time the assessment was being conducted in evaluating studies of cancer outcomes in humans and in animals. For mechanistic evidence, EPA used a narrative review approach for summarizing the voluminous evidence on mechanistic events that are known to be associated with cancers of the upper respiratory tract and lymphohematopoietic cancers. Studies supporting and refuting the proposed mechanistic hypotheses were evaluated in a balanced and transparent manner.

EPA Response to Finding 3: For transparency, EPA notes that one study was downgraded from *high* to *medium* confidence for LHP cancers in the revised assessment based on analyses of consistency in study evaluations ([Kerns et al., 1983](#)).

Related Public Comments

- **Public Comment (University of North Carolina at Chapel Hill):** "The IRIS Draft significantly underestimated the impact of co-exposure on the confidence in assessing how formaldehyde may lead to leukemia and associated mechanistic events in both experimental animal models and humans...The results [Lu, Gu, et al. 2012] strongly indicate that studies confounded by methanol in exposure should be excluded from any rigorous risk assessment of formaldehyde. The EPA IRIS Draft acknowledges that methanol may influence the confidence of assessment by stating 'although results may be confounded by methanol co-exposure (low confidence).'

However, the EPA IRIS Draft still used these studies to support several hypothesized mechanistic events associated with formaldehyde induction of lymphohematopoietic cancers." "Recommendation: The EPA IRIS Draft needs to discuss how methanol co-exposure significantly impacts evidence-based risk assessment of formaldehyde. Any study, including epidemiology studies, with potential methanol co-exposure should be excluded from the integration of evidence, as methanol seriously confounds the finding and potential understanding of mechanistic events." *[Please see docket for details and supporting information.]*

EPA Response: EPA has thoroughly and transparently evaluated the published studies on LHP cancers using methods, and arriving at judgments, supported by the consensus peer review panel (Finding 3 above). Please see Appendix B.3.9 in the revised assessment for a discussion of methanol co-exposure.

- **Public Comment (University of California San Diego):** "The EPA mischaracterized the findings of the reanalysis of the NCI cohort study by Checkoway et al. (2015) and downgraded the exposure assessment without consideration of the issues with the peak exposure definition in the Beane Freeman et al. (2009) analysis of myeloid leukemia (addressed in Checkoway et al. 2019)..."

"EPA misinterpreted the impact of defining an absolute peak exposure metric on the study findings...."

"The EPA assigned low confidence to the exposure assessment in Checkoway et al. (2015) due to purported information bias and low sensitivity and suggested that the direction of expected bias would be toward the null, representing an underestimation of the actual risk. In fact, there is no such evidence of a bias toward the null in the reanalysis..."

"We also published a comprehensive review of approaches to characterizing peak exposures and deriving cancer potency values from the epidemiological literature. Our analysis included nine epidemiological studies of environmental chemicals classified as carcinogens, including formaldehyde (Checkoway et al., 2019). This analysis provides additional valuable information that EPA should have considered and incorporated when evaluating the Checkoway et al. (2015) analyses conclusions to inform its approach to quantitative cancer potency characterization in the 2022 draft IRIS. Specifically, in addition to succinctly summarizing the results of the Checkoway et al. (2015) analysis, it also identifies issues with the characterization of peak exposure in in a case-control study of embalmers (Hauptmann et al. 2009). Furthermore, Checkoway et al. (2019) identified a pattern of limitations associated with using peak exposure data from epidemiological studies of seven additional substances (benzene, trichloroethylene, acrylonitrile, ethylene oxide, methylene chloride, styrene and/or butadiene) to inform risk characterization. Thus, the lack of a uniform approach is a critical issue that is not limited to formaldehyde. These methodological challenges include variability in

peak exposure definitions across studies, lack of direct peak exposure measurements and reliance on expert judgement for exposure classifications, and potential exposure misclassification. The 2022 draft IRIS should have referred to this paper to better understand the limitations of the underlying formaldehyde epidemiological literature particularly with respect to the characterization of “peak” exposures to formaldehyde in some studies. Perhaps most importantly, the Checkoway et al. (2019) analysis substantiates the findings of Checkoway et al. (2015). The clear lack of an association with myeloid leukemia and its subtypes (AML and CML) in relation to cumulative exposure (the default exposure metric in epidemiologic studies based on its incorporation of both intensity and duration of exposure) do not support a causal association at any exposure level (Checkoway et al., 2015).”

[Please see docket for details and supporting information.]

EPA Response: EPA considered the methods of defining peaks in Checkoway et al. (2015) in Section 3.3.3 and noted that “In Beane Freeman et al. (2009), for peak exposure there were four cases of ML who were unexposed, 14 cases with peak exposure from >0 to <2 ppm, 11 cases with peak exposure from 2 to <4 ppm, and 19 cases with peak exposure ≥4 ppm. In [Checkoway et al. \(2015\)](#), the new definition of peak exposure and the recategorization resulted in 27 cases of ML with peak exposures from 0 to <2 ppm, 11 cases with peak exposure from 2 to <4 ppm, and 10 cases with peak exposure ≥4 ppm. The [Checkoway et al. \(2015\)](#) results were classified with low confidence due to information bias and low sensitivity.” A footnote in Table 4 of Checkoway et al. (2015) says that Referent group includes study subjects with peaks less than 2 ppm of hourly, daily, weekly, and monthly frequency as well as peaks 2 ppm or more if hourly or monthly frequency. Reclassification of subjects who were judged to have experienced formaldehyde exposures in excess of 2 ppm in the Beane Freeman et al. (2009) to the reference category (>0 and < 2 ppm) in Checkoway et al. (2015) because those exposures of greater than 2 ppm occurred hourly or monthly had the mathematical effect of attenuating the observed effect reported in Beane Freeman et al. (2009). No explanation was provided of why exposures greater than 2 ppm that occurred hourly or monthly should have been discounted and EPA judged there to be potential bias towards the null and an overall rating of *low confidence*.

Evidence Syntheses and Synthesis Judgments for Cancers of the Upper Respiratory Tract

Peer Review Findings and Comments

- **Finding (4):** With respect to cancer hazard identification for respiratory tract cancers in humans exposed to formaldehyde, EPA synthesized the current state of the science in a manner consistent with its state-of-practice methods and presents conclusions based on its expert judgment with the support of the available scientific evidence.
 - *Related committee text:* While there is uncertainty in the degree to which nonmutagenic processes may also contribute to the carcinogenic activity of formaldehyde inhalation at

the point-of-entry tissues, there is sufficient evidence to support the assumption that a mutagenic MOA is involved in the carcinogenesis of formaldehyde in the upper aerodigestive tract in humans.

- **Tier 2 Suggested Revision (Recommendation 5.1):** While the narrative describing the application of criteria for each site is well done, EPA should enhance clarity by providing explicit statements in Section 1.2.5 summarizing synthesis judgments for each criterion (consistency, strength, temporal relationship, exposure-response relationship, etc.).

EPA Response to Tier 2 Suggested Revision 5.1: Brief synthesis judgments have been added following each criterion judgment in the respiratory cancer section (now Section 3.2.5) and in the lymphohematopoietic cancer section (now Section 3.3.3).

- **Tier 2 Suggested Revision (Recommendation 5.2):** For consistency, EPA should add a summary of the data and evidence synthesis for laryngeal cancer after page 103 of the Assessment Overview.

EPA Response to Tier 2 Suggested Revision 5.2: The Overview has not been retained in the revised assessment.

- **Finding (5):** With respect to cancer hazard identification for respiratory tract cancers in animals exposed to formaldehyde, EPA synthesized the current state of the science in a manner consistent with its state-of-practice methods and presents conclusions based on its expert judgment with the support of the available scientific evidence.
- **Finding (6):** With respect to evidence on MOA for upper respiratory tract cancers, EPA used its state-of-practice methods to synthesize the evidence.
- **Finding (7):** EPA presents an overall clear and succinct evidence integration summary for effects of formaldehyde inhalation on upper respiratory tract cancers. EPA followed its state of practice for evidence integration at the time the 2022 Draft Assessment was being developed in evaluating studies of cancer outcomes in humans and animals, as well as mechanistic evidence.
- **Finding (8):** EPA integrated the mechanistic evidence in considering biological plausibility for the human or animal evidence. However, it separately presents MOA considerations in the “other inferences” section of the evidence integration table (for this endpoint and for other endpoints throughout the 2022 Draft Assessment). The IRIS Handbook calls out “other critical inferences” as a factor to address in evidence integration (EPA, 2022, pp. 6-1 and 6-2 in the

Assessment Overview; Chapter 6 of the Main Assessment). This concept is applied in the evidence integration sections throughout the Draft Assessment, but the term “other inferences” is not explained. See Chapter 2 of the present report, Recommendation 2.5.

EPA Response to Finding 8: As noted previously, the “other inferences” are now better defined in the assessment methods and the specific inferences relevant to each health effect-specific decision more clearly documented within each evidence integration section (and supporting table). This has also been clarified in the methods (primarily Section 2.5 of the revised Toxicological Review).

Related Public Comments

- **Public Comment (American Chemistry Council):** “EPA should evaluate carcinogenicity entirely under its 2005 Guidelines for Cancer Risk Assessment (2005 Guidelines). The 2005 Guidelines and descriptors have not been harmonized with the re-purposed GRADE framework and descriptors used in the IRIS Handbook.”

“The 2022 Draft Assessment must comply with EPA’s guidelines for carcinogenic risk assessment...In implementing its Information Guidelines, EPA relies on existing guidelines and policies, which includes EPA’s Guidelines for Carcinogenic Risk Assessment (Cancer Guidelines). The Cancer guidelines explicitly note that ‘when judging and considering the use of any data, the basic standard of quality, as defined by the EPA Information Quality Guidelines, should be satisfied.”

“Thus, the 2022 Draft Assessment fails to rely on the best available science and apply a transparent and systematic review incorporating a weight-of-evidence approach that properly integrates evidence streams in assessing potential non-cancer and cancer risks of formaldehyde exposures, especially at human relevant, low levels of exposure. Moreover, EPA repeatedly cites its Cancer Guidelines but fails to properly apply them. As a result, EPA’s linear unit risk estimate for NPC is essentially unchanged from the value derived over 30 years ago, despite the prodigious growth in the scientific database on formaldehyde indicating that formaldehyde is a threshold carcinogen.”

“The Draft Assessment often states the assessment followed EPA’s Cancer Guidelines (U.S. EPA 2005), without citation. Likewise, there is no discussion of rationale for departing from the Guidelines when it did.”

"Regarding evidence synthesis and integration within and across lines of evidence, the 2022 draft Formaldehyde Review does not follow best practices for systematic review. Perhaps most importantly, there is a greater reliance on "strength" rather than "weight" of evidence. For example, the draft concludes that the strength of the human evidence for myeloid leukemia is "robust" based on "several" studies with consistent findings. EPA's conclusion is based largely on the Beane Freeman analyses of the NCI cohort (pg. 1-542). EPA did not appropriately integrate apparently conflicting findings in the older epidemiological studies (Hauptman et al. (2009) funeral workers study, Beane Freeman et al. (2009) analysis of the NCI cohort) with the

analyses published more recently, which demonstrate no excess in cancer risk (e.g., Checkoway et al. (2015) re-analysis of the NCI cohort). EPA should re-examine its evaluations of study confidence (and risk of bias, especially including statistical analyses) and more fully integrate and interpret the earlier studies in the context of the broader body of more informative and updated studies."

"Strength and weight of the evidence determinations in animal studies are often subjective, unfounded, and contradictory."

"EPA's treatment of mechanistic data continues to be problematic. As EPA notes on page xxxviii, "the lack of mechanistic data explaining an association did not discount results from human or animal health effect studies." While very strong, consistent and positive evidence in animals and humans, indicative of an effect, is often sufficient to make some conclusions on the likelihood of human health risk, in most cases, there is some inconsistency both within and across the animal and human streams of literature, which makes mechanistic information exceedingly important. EPA should give more weight to mechanistic information, particularly when it exists and does not support a human-relevant mechanism. The Agency for Toxic Substances and Disease Registry (ATSDR) echoed this concern in their interagency review comments."

"The 2022 Draft Assessment fails to incorporate a weight of evidence approach or properly integrate evidence streams in assessing non-cancer and cancer endpoints."

"The 2022 Draft Assessment does not incorporate best practices for evidence synthesis...The process of evidence synthesis (within lines of evidence) and evidence integration (across lines of evidence) are critical but still somewhat underdeveloped areas of systematic review. In the 2022 Draft Assessment, the procedure is described, but only in general terms, as depicted in the figure below (a figure that is found in the 2022 Draft Assessment but does not appear in the IRIS Handbook). Further, it is notable that there is no discussion of mechanistic studies or information on MOA."

"The 2022 Draft Assessment does not incorporate best practices for evidence integration. In 2011, NASEM gave the following recommendation (in this case, in regard to the lymphohematopoietic malignancies): As stated in EPA's cancer guidelines, EPA's approach to weight of evidence should include 'a single integrative step after assessing all of the individual lines of evidence.' Although a synthesis and summary are provided, the process that EPA used to weigh different lines of evidence and how that evidence was integrated into a final conclusion are not apparent in the draft assessment and should be made clear in the final version."

"NASEM (2011) went on to criticize the 2010 Draft Assessment for the lack of a clearly articulated framework for establishing causation on the 'basis of the weight and strength of the evidence.' EPA reports that it implemented NASEM's recommendations by evaluating studies related to each endpoint using "a common evidence integration framework for determinations

of causality” with rationales described in the integrated summaries of evidence (Appendix D of the 2022 Draft Assessment). Indeed, EPA has developed a framework for weighing evidence, informed by Bradford Hill postulates (as recommended by NASEM 2011) as presented in the IRIS Handbook and the 2022 Draft Assessment. However, as applied, the integration process requires substantial scientific judgment, and the 2022 Draft Assessment does not clearly describe the way in which evidence was synthesized and integrated to reach its determinations of causality.” *[Please see docket for details and supporting information.]*

- **Public Comment (Louisiana Chemical Association):** “The classification of ‘evidence demonstrates’ that formaldehyde causes nasopharyngeal cancer (NPC) and sinonasal cancers is not fully supported and should be reevaluated based on the growing amount of information which argues against a causal association. Based on EPA guidelines (Table 8 of EPA 2022a), the classifications of ‘evidence indicates’ is more appropriate given the conflicting and inconsistent findings in human epidemiological studies, the indications of chance, bias and confounders in key positive epidemiological studies, and the stronger but limited evidence in high exposure rat studies.”

EPA Response (to both public comments above): The assessment continues to adhere to EPA’s 2005 cancer guidelines ([U.S. EPA, 2005](#)) in evaluating and judging the available scientific evidence on cancer. The assessment decisions on cancer were supported by the consensus peer review panel (see findings above). The NASEM panel provided no Tier 1 recommendations on this topic and EPA has addressed the other panel comments on this topic.

Specifically, regarding NPC, the judgment of evidence demonstrates is based on robust human evidence of increased risk in groups exposed to occupational formaldehyde levels, and robust animal evidence of nasal cancers in rats and mice that exhibits steeply increasing incidence at high formaldehyde levels. Strong mechanistic support is provided across species (primarily rats, but also mice, monkeys, and humans), including genotoxicity, epithelial damage or remodeling, and cellular proliferation that are consistent with neoplastic development in a regional, temporal, and dose-related fashion. Mutagenicity- and cell proliferation-based MOAs both contribute. This is described in Section 2.3.5.

The judgment of evidence demonstrates for myeloid leukemia is based primarily on observations of increased risk in groups exposed to occupational formaldehyde levels. This judgment is supported by other studies of human occupational exposure that provide strong and coherent mechanistic evidence identifying clear associations with additional endpoints relevant to lymphohematopoietic (LHP) cancers, including an increased prevalence of multiple markers of mutagenicity and genotoxicity in peripheral blood cells of exposed workers, other perturbations to immune cell populations in blood (primarily from human studies). The few animal studies on LHP cancers do not contribute to this judgment. The MOA is unknown. This is discussed in Section 3.3.3.

Supplemental Information—Formaldehyde (Inhalation)

EPA Note: Interagency reviewer (e.g., ATSDR) comments were on a draft prior to revision for public release. Please consult the full interagency review comments in the EPA docket.

Although not influential to the independent evaluations of all identified, pertinent, primary information performed in the IRIS assessment, the conclusions on cancer by other federal and international agencies are summarized in Appendix G. Specifically, the hazard conclusion for human carcinogenicity in the IRIS assessment is consistent with those drawn by other expert review panels. Formaldehyde was classified as a known carcinogen by the ([NTP, 2011a](#)) and a Group 1 carcinogen by ([IARC, 2006, 2012](#)), both based on evidence for nasal cancers in humans and animals and myeloid leukemia in humans, with supporting data on mechanisms of carcinogenesis. In addition, an expert committee convened by the NAS confirmed the conclusions of the NTP 12th Report on Carcinogens (RoC) and conducted an independent review of the literature through 2013, concluding that formaldehyde is a known carcinogen. The European Union and Health Canada concluded that formaldehyde is a genotoxic carcinogen with a cytotoxic MOA ([SCOEL, 2017](#); [Health Canada, 2001, 2006](#); [ECHA, 2012](#)). Contrasting this, the conclusions reached in the IRIS assessment are consistent with those concluded by ([IARC, 2012](#));([NTP, 2014](#)), and the ([NRC, 2014](#)). Notably, in these latter assessments, there was widespread, general agreement that the available genotoxicity evidence is largely consistent and strong, particularly in circulating blood cells. From IARC 2012: “Mechanistic evidence supporting a causal relation between inhalation of formaldehyde and induction of cancer of the nasopharynx and nasal sinuses is based on the chemical reactivity of formaldehyde in producing DNA–protein crosslinks, and its genotoxicity in vitro and in vivo, including in the nasal cells of exposed humans... Local effects in the nasal passages, genotoxicity, and cell-proliferation rate appear to be the major determinants of nasal carcinogenicity after exposure to formaldehyde... The current data strongly indicate that genotoxicity plays an important role in the carcinogenicity of formaldehyde in nasal tissues in humans, and that cellular replication in response to formaldehyde-induced cytotoxicity promotes the carcinogenic response.” From NTP RoC 15th: “Formaldehyde causes genetic damage to the nasal tissues of both experimental animals and humans exposed by inhalation... Inhalation-exposure studies in experimental animals have shown that airway deposition and cytotoxicity-induced cellular proliferation also are important factors in the carcinogenicity of formaldehyde to nasal cells... Formaldehyde exposure also causes cytotoxicity and cellular proliferation at anatomical sites that are not thought to be the origin of the squamous-cell carcinoma, suggesting that factors other than cellular proliferation play a role in formaldehyde-induced nasal cancers.” From NTP RoC background document (2010): “Formaldehyde is a direct-acting genotoxic compound that affects multiple gene expression pathways, including those involved in DNA synthesis and repair and regulation of cell proliferation.”

- **Public Comment (Formacare):** “Formaldehyde is a very well-known chemical. It is one of the first registered under the EU REACH regulation (EC) No 1907/2006 and benefits from decades of extensive scientific research.

Formaldehyde is already highly regulated: consumer and worker safety are ensured in the European Union via harmonised classifications under the (EC) No 1272/2008 Regulation, existing and forthcoming REACH restrictions and the implementation of an EU-wide Binding Occupational Exposure Limit (BOEL). This BOEL is based on the evaluation of the European Scientific Committee on Occupational Exposure Levels (SCOEL) which derived an occupational exposure limit (OEL) of 0.3 ppm TWA and a short-term exposure limit (STEL) of 0.6 ppm. The opinion of the SCOEL was joint to this submission.

An important prerequisite for the derivation of these exposure limits was the categorization as a so-called group C carcinogen, i.e. a genotoxic carcinogen with a mode-of-action based threshold. For all relevant steps of local carcinogenesis, clear No-Observed-Adverse-Effect-Concentrations (NOAECs) could be identified (cf. page 9 of the [submitted] joint SCOEL document):

‘Experimental studies support that the local carcinogenesis at the portal-of-entry is pivotal. In the sensitive rat species, the apparent LOAEC was 6 ppm, and the apparent NOAEC was 2 ppm for nasal cancer. Experimentally, the histopathological NOAEC for nasal effects of FA in rats and monkeys is 1 ppm and the NOAEC for regenerative cell replication is 2 ppm. At these NOAECs, the FA-DNA adducts were less in monkeys than in rats as was the relationship of exogenous/endogenous DNA adducts, which is in line with the assumption that humans should be a less sensitive species. The new studies confirm that local FA-DNA adducts show a highly non-linear relationship with external FA exposures. At ≤ 2 ppm FA, the FA DNA-adducts induced by external exposures comprise a minor portion of the total FA-DNA adducts, which were driven mainly by internal (naturally generated) FA. This is supported by considerations on toxicokinetics, concluding that the intracellular FA concentration increases only slightly, and the intracellular glutathione concentration decreases only slightly in this range and that the homeostasis within the epithelial cells would not be affected. Therefore, the apparent NOAEC of 1 ppm can be considered a mode-of-action based NOAEC for carcinogenic effects at the portal-of-entry.’”

EPA Response: Please see prior responses on this topic (including in Section F.2.3 of these responses above), although EPA notes that the considerations mentioned by the commenter do not necessarily indicate a threshold for formaldehyde’s mutagenic action. As previously described, reviews and studies with no primary data are considered as supplemental materials and tracked in HERO. EPA conducts an independent evaluation of the available evidence.

The IRIS assessment carefully evaluated the temporal and dose-dependence of mutagenicity and cytotoxicity-induced regenerative cell proliferation as key events in the MOA in Section 3.2.5.

Ultimately, the available evidence does not support a conclusion that cytotoxicity-driven regenerative cell proliferation is the sole driver of formaldehyde-induced carcinogenicity, nor do the data support that a significant contribution of mutagenicity at lower doses is unlikely.

- **Public Comment (American Chemistry Council):** “EPA failed to consider Thompson et al. (2020), which provides important data integration related to the MOA for formaldehyde-induced nasal tumors.”

“The Draft Assessment does not follow the EPA Framework in presenting alternative MOAs and including the empirical support for each. This is directly relevant to the discussion of the cytotoxicity and regenerative hyperplasia MOA for nasal tumors related to inhaled formaldehyde. The Draft Assessment does not reference the Thompson et al. (2020) IPCS MOA analysis for formaldehyde-related nasal tumors that updated the McGregor et al. (2006) assessment. This despite written and oral communications to the EPA from the authors and sponsors. Rather the Draft Assessment acknowledges the MOA as defined by McGregor et al. (2006), as updated by Thompson et al., but without referencing those peer-reviewed publications (see quote below). Furthermore, the Draft Assessment dismisses the MOA, in favor of a mutagenic MOA, without making a quantitative assessment of the cytotoxicity with regenerative hyperplasia MOA as directed by the NAS (2011).”

“The Draft Assessment does not include a fair evaluation of the most widely accepted MOA for nasal tumor formation related to formaldehyde and does not follow EPA Cancer Guidelines [See also general comments on lack of documentation for MOA]... The exclusion of Thompson et al. (2020) from the Draft Assessment is noteworthy, as Dr. Thompson personally briefed the EPA IRIS staff on the peer-reviewed and published cytotoxicity MOA. This MOA for nasal tumors was recently adopted by the European Chemicals Agency (ECHA) and approved by the 32 countries in the European Union.” *[Please see docket for details and supporting information.]*

- **Public Comment (ToxStrategies):** “The following review articles relevant to the MOA of formaldehyde and thus the U.S. EPA risk assessment are not cited anywhere in the Toxicological Review, Assessment Overview, or Supplemental Information. It is unclear if these articles were not captured in U.S. EPA’s literature searches or whether they were captured but intentionally discarded by the U.S. EPA [Thompson et al. 2020; Gentry et al. 2020; Andersen et al. 2019; Thompson et al. 2018].

“U.S. EPA failed to integrate two highly relevant data streams [studies in ADH5 null mice and measurement of endogenous and exogenous formaldehyde-DNA adducts] into their MOA analysis.”

“Individually, the studies described above may not paint a coherent MOA for formaldehyde. In totality, however, they tell a remarkably cohesive picture of the likely MOA for formaldehyde-induced nasal tumors. Instead of integrating these data, the U.S. EPA documents are full of

speculative discussions of what might theoretically be capable of happening as evidenced by their refusal to develop either a linear or non-linear MOA and instead display networks of plausible mechanistic effects. If there is a study or studies that report that formaldehyde causes, for example, inflammation in the portal of entry and/or systemic tissues, then EPA accepts these effects at face value and further hypothesizes that they could play a meaningful role in an otherwise overly complex intractable MOA while ignoring high-quality molecular data that tell a remarkably coherent story. This is not how risk assessment should be conducted.”

“Assuming the term “in silico” on page 1-302 was an error and that U.S. EPA instead meant “in vivo” test systems have shown formaldehyde to induce genotoxic or mutagenic effects, this statement too is misleading. As discussed in Thompson et al. (2020), genotoxicity assays conducted in the nasal tissues of rats have reported negative findings for clastogenic and mutagenic markers. As already discussed, ADH5 null mice do not exhibit increases in markers of genotoxicity unless other genes involved in formaldehyde detoxification of DNA repair are also knocked out.”

“The nasal tumor MOA information provided in the Toxicological Overview is neither clear nor well-constructed. Section 4.2.3. Mode of action information, of the Toxicological Overview is vague and provides no graphical depiction of the hypothesized MOA. Table 34 lists “hypothesized mechanistic events” with no clear indication of the sequence of events. The first mechanistic event is “direct, or presumed direct, genotoxicity and mutagenicity”. The reviewer assumes that U.S. EPA has chosen the word ‘presumed direct’ because much of the evidence for this key event in animals is based on markers of exposure (e.g., DNA adducts and DNA-protein crosslinks) as opposed to markers of effect (e.g., evidence of clastogenic or mutagenic damage).”

“It is curious that the only real evidence for “direct” genotoxicity and mutagenicity comes from worker and student studies. The use of the term “direct” is highly misleading, as these are not controlled experiments and the workers and students were likely exposed to other agents (chemical or physical) that might increase micronuclei. Notable omissions from Table 35 are Speit et al. (2007) and Zeller et al. (2011).”

“Table A-22 “Summary of in vivo genotoxicity studies of formaldehyde inhalation exposure in experimental animals” is highly misleading regarding the evidence for in vivo genotoxicity in regions where tumors occur. A total of five studies is listed in the table section “Mutations.” Two of the 5 studies are based on the same underlying bioassay where rats were exposed for 2 years (Recio et al. 1992, Wolf et al. 1995). These studies looked for p53 mutations in tumor tissue, which is not a valid approach for assessing whether genotoxicity is an early initiating event in the MOA. Another study, Kitaeva et al. (1990) is in Russian and appears to have reported genotoxicity in bone marrow cells even though the highest exposure concentration was 1.5 mg/m³, which is not even carcinogenic to the nasal passages of rodents. The fourth positive study is Liu et al. (2009), where male mice were exposed to 0, 2, 20, or 200 mg/m³ formaldehyde for 2 hours and then mated six weeks later. Exposure to 200 mg/m³ was

reported to increase heritable mutations in offspring. It should be appreciated that the RD50 for mice is ~5 mg/m³ (see Table A-16 in the Supplemental Information). It is conceivable that exposure to 200 mg/m³ would have depressed respiration much further. Data presented in section A.3 discusses that this reflex bradypnea induces hypothermia in mice (decreasing body temperature by as much as 14 degrees). In addition, U.S. EPA states that reflex bradypnea “also results in decreased blood pO₂ and pCO₂ and increased blood pH.” It is well known that hypothermia can cause genotoxicity (Tweats et al. 2007). This has no relevance to humans because exposure to irritant gases does not result in extreme levels of heat loss due to the much lower surface area to volume ratio of large mammals compared to small mammals (Gordon et al. 2008). The fifth study in this section, Meng et al. (2010) did not find increases in mutant frequency in nasal tissue following exposure up to 18.5 mg/m³ for 13 weeks. This is the only relevant study in this section of the table.”

“Table A-22 section “Micronucleus” lists only one study reporting on micronuclei in tissue other than blood or bone marrow. Specifically, Neuss et al. (2010) is listed as negative for micronucleus formation in bronchioalveolar lavage cells. Missing from the table is Speit et al. (2011), which reported no increases in micronucleus formation in rat nasal tissue following 4 weeks of exposure up to ~18 mg/m³ formaldehyde. It is unclear why this study was not included in the table. The vast majority of studies listed in Table A-22 includes either genotoxicity effects beyond the portal of entry or DNA modifications in the nasal and other tissues. These modifications are not measures of heritable gene changes, and thus should not be considered genotoxicity. As already discussed, data in ADH5 null mice indicate that increased formaldehyde DNA adducts are not necessarily genotoxic.”

“In discussing the role of cell proliferation, U.S. EPA cites evidence for increased cell proliferation ≤2.5 mg/m³ as evidence for mitogenic proliferation that might increase cancer risk together with DNA damage. As already noted, these levels can result in squamous metaplasia, i.e., a transition from respiratory to squamous epithelium. As such, comparing the labelling index between treated and control animals is essentially comparing the proliferation rate in two different epithelial types.”

“On page 1-331, U.S. EPA states that glutaraldehyde cause many of the same effects as formaldehyde (squamous metaplasia, hyperplasia, and inflammation) yet does not increase nasal tumors. U.S. EPA uses this to conclude that increased cell proliferation alone is not sufficient to cause nasal tumors...To my knowledge, the MOA of glutaraldehyde has not been studied as extensively (if at all) as formaldehyde since 2006. As such, the database for glutaraldehyde is insufficient to inform the MOA of formaldehyde.”

“Table 35 lists ‘cellular mitogenesis in the absence of cytotoxic tissue pathology’ as another hypothesized mechanistic event. In the column titled ‘experimental evidence pertinent to mechanistic event’ there is a bullet stating that there is clear evidence for increased URT ‘cell proliferation under conditions also resulting in tissue pathology’ at ≥4 mg/m³. Unless this bullet

includes a typo, it is unclear how that bullet supports the hypothesized event. The next bullet states that there is 'suggestive evidence' for increased URT 'cell proliferation under conditions not clearly causing tissue pathology' $<4 \text{ mg/m}^3$. This bullet does support the hypothesized key event; however, note the overall weak terminology such as 'suggestive evidence' of cell proliferation 'not clearly causing' pathology. This language is weak and its purpose seems to be to establish cell proliferation at exposure concentrations more relevant to human exposure levels than the higher concentrations clearly associated with increasing cell proliferation and tumors in rodents."

"The U.S. EPA should revise its assessment such that the presence of DNA-protein crosslinks and formaldehyde-DNA adducts is not synonymous with genotoxicity."

"Much of the text on pages 1-315 to 1-320 includes discussion about the accumulation of DNA-protein crosslinks. This accumulation is discussed in the context of Leng et al. (2019) who reported that exposure to ≤ 0.3 ppm formaldehyde for 28 days did not result in the detection of exogenous formaldehyde-DNA adducts. U.S. EPA appears to be speculating that longer exposure durations to low concentrations might increase adducts. If accumulation were true, then one might expect that the constant biological production of endogenous formaldehyde would increase the levels of endogenous adducts over time. I am not aware of any articles that have demonstrated this. Considering that U.S. EPA believes formaldehyde adducts are pro-mutagenic but also acknowledge that nasal tumors in rodents are exceedingly rare, it would seem that endogenous adducts do not accumulate over time and nor would any exogenous adducts formed from exposure to ≤ 0.3 ppm formaldehyde accumulate over time. The U.S. EPA also fails to consider that evidence for accumulation of exogenous adducts over time might be due, in part, to remodeling to squamous epithelium where the superficial cell layers are dead yet likely accumulate exogenous adducts as exposure duration increases."

[Please see docket for details and supporting information.]

EPA Response (to both public comments above): The MOA analyses and decisions presented in the external review draft are retained in the revised assessment. The NASEM committee had no Tier 1 recommendations on this topic and EPA has addressed the panel findings and other comments on this topic. As noted previously, EPA conducts an independent analysis of the available articles, so the stance of EPA on the impact and utility of the papers to inform assessment conclusions can differ from the views and conclusions expressed by the study authors. Further, reviews and studies with no primary data are considered as supplemental materials and tracked in HERO.

The IRIS assessment carefully evaluated the temporal and dose-dependence of key events in the mutagenicity and cytotoxicity-induced regenerative cell proliferation MOAs. During this review, several lines of evidence were identified to support increases in genotoxicity markers that preceded other mechanistic changes, and which sometimes occurred at lower exposure levels than those

known to cause cytotoxicity (see Section 3.2.5). This included changes in exposed human workers as well as in several experimental animal species, all strongly supporting a role for direct DNA damage leading to mutagenicity in formaldehyde exposure-induced URT carcinogenesis.

The analyses also revealed large temporal and spatial (location in the nasal passages) variability in the available data on cell proliferation in experimental animals (see characterization of uncertainty and variability in cell replication rates in Appendix D.2.2, noting that direct evidence in humans is lacking). This variability complicates interpretations regarding a threshold dose for this mechanism. This uncertainty is further illustrated in the IRIS assessment review of the BBDR modeling in Appendix D.2.2. EPA estimated that the mutagenic component in the BBDR modeling by Conolly et al. (2003) contributes between 2% and 80% of the predicted probability of tumor incidence in the low-dose region, and between 58%–74% of the predicted probability of tumor incidence in the high-dose range of exposures where tumors were observed, depending on whether a J-shape or hockey-stick shape is assumed for the dose response for cell proliferation.

Overall, the available evidence does not support a conclusion that cytotoxicity-driven regenerative cell proliferation is the sole driver of formaldehyde-induced carcinogenicity, nor do the data support that a significant contribution of mutagenicity at lower doses is unlikely.

- **Public Comment (American Chemistry Council):** "The 2005 Guidelines provide that while a linear extrapolation would generally be the default for DNA reactive chemicals, a threshold approach would be allowed given sufficient evidence. The descriptor "Not likely to be carcinogenic to humans at concentrations that do not cause nasal tumors in rodents" should be used, as should a threshold approach for deriving a toxicity value."

"Animal evidence clearly demonstrate a threshold for induction of squamous cell carcinoma, which corresponds to concentrations above where cell proliferation is induced (Monticello et al. 1991; Connolly et al. 2002 as cited in BfR 2006; Swenberg et al. 1980; Kerns et al. 1983; Monticello et al. 1996)."

"EPA grossly misinterpreted the work of Dr. Marsh, which the 2022 Draft Assessment cites throughout...Of the Marsh et al. papers that were not cited in the 2022 Draft Assessment, EPA omitted the following important studies... [Marsh et al. 2019, Marsh et al. 2014, Marsh and Youk 2004]...The 2022 Draft Assessment also misinterprets and misrepresents several of the Marsh et al. papers that it cites. [Marsh et al. 1996, Marsh et al. 2007b, Marsh and Youk 2005, Marsh et al. 2007, Mart et al. 2016, Marsh et al. 2002]."

"EPA's Cancer Guidelines emphasize 'a critical analysis of all the available information that is relevant to assessing the carcinogenic risk,' rather than reliance on default options as the starting point. A nonlinear approach should be utilized when, as in the case of formaldehyde, 'there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses.' The Cancer Guidelines also stress the importance of relying upon, 'common sense

reasonable applications of assumptions and policy, and transparency...to avoid unrealistically high [risk] estimates.” *[Please see docket for details and supporting information.]*

EPA Response: EPA judged that the evidence is sufficient to conclude that a mutagenic MOA of formaldehyde is operative in formaldehyde-induced nasopharyngeal carcinogenicity, and this was supported by the NASEM peer review (e.g., “While there is uncertainty in the degree to which nonmutagenic processes may also contribute to the carcinogenic activity of formaldehyde inhalation at the point-of-entry tissues, there is sufficient evidence to support the assumption that a mutagenic MOA is involved in the carcinogenesis of formaldehyde in the upper aerodigestive tract in humans.”). The 2005 Cancer Guidelines ([U.S. EPA, 2005](#)) make it clear that when a mutagenic MOA is operative, linear extrapolation is used to derive the IUR and ADAFs are applied to develop risk estimates. EPA notes that reviews and studies with no primary data are considered as supplemental materials and tracked in HERO.

The IRIS assessment includes hundreds of pages evaluating and considering the mechanistic evidence in accordance with EPA’s cancer MOA framework. This includes extensive documentation of the evaluations necessary to scientifically support the nasal cancer MOA conclusions drawn, which were supported by the NASEM external peer review. The assertions that the nasal cancer MOA is limited to cytotoxicity/regenerative proliferation and that mutagenicity is not supported at lower doses are not sustained by the available scientific evidence. Specifically, evaluations of the mechanistic evidence relevant to the nasal cancer MOA are documented in Appendix B.3 for studies of inflammatory changes, tissue injury, and cellular proliferation, and in Appendix C.3 for genotoxicity studies. The extensive nasal cancer MOA information is synthesized together according to the EPA cancer guidelines in Section 3.2.5. In addition, Appendix C.1 describes the available toxicokinetic data and dosimetry modeling, and Appendix D.2 documents the strengths and uncertainties of those models for use in describing nasal cancer risk, with the summary application of those evaluations to deriving an inhalation unit risk described in Section 5.2.1.

A key consideration in applying the 2005 EPA cancer guidelines is the strong evidence supporting that formaldehyde is both a direct- as well as an indirect-acting mutagen. However, there is no clear demarcation in dose that separates these actions (see EPA response to the prior public comment above on the nasal cancer MOA), a conclusion supported by the extensive analyses in the IRIS assessment.

The IRIS assessment is in line with both the general and specific recommendation in the EPA cancer guidelines. As noted in the assessment and in the response to the prior MOA comment above, formaldehyde is shown to have direct mutagenic action. As recommended in Appendix A of the cancer guidelines, “... the linear approach is used ... for example, when a conclusion is made that an agent directly causes alterations in DNA, a kind of interaction that not only theoretically requires one reaction but also is likely to be additive to ongoing, spontaneous gene mutation.” In the case of

formaldehyde, the point in the cancer guidelines regarding additivity to background is particularly relevant because of the highly variable endogenous levels in humans.

- **Public Comment (University of North Carolina at Chapel Hill):** "The EPA IRIS Draft recognized the potentially important role of epigenetic regulation in MOA of nasal tumor, yet further data from animal and human studies are needed...I appreciate that the EPA IRIS Draft recognized the potential important role of epigenetic regulation in the MOA of formaldehyde by stating 'DNA methylation and histone modification can promote carcinogenesis through steric regulation of enhancer/promoter binding and transcription factor-DNA association, thereby affecting gene transcription' and 'suggesting that these epigenetic effects may play a causal role in human URT cancer formation.' However, no studies have exclusively examined histone PTM and DNA methylation in formaldehyde-exposed animals or human. Therefore, the data used in the IRIS Draft were largely limited to cultured cells or results based on N6-Formyllysine that is not a highly relevant biomarker of formaldehyde exposure and epigenetic regulation, which represents a significant data gap in understanding MOA of formaldehyde-induced nasal tumor." "Recommendation: Further studies (animal and human) are needed to improve understanding of the role of mutation and epigenetic factors, including histone modification and DNA methylation, contributing to the cytotoxicity with regenerative hyperplasia MOA."
[Please see docket for details and supporting information.]

EPA Response: EPA appreciates and agrees with this future recommendation that additional studies on the topic of epigenetic regulation would be informative.

- **Public Comment (Cardno ChemRisk [now Stantec]):** "Page 159, lines 3-11: Comment 1: Marsh et al. (1996) concluded that the NCI cohort formaldehyde exposure estimates may be inflated in Plant 1 plant of the NCI cohort study (Plant 1 in Wallingford, CT), where many of the NPC cases occurred, all of which were associated with the highest exposure categories. The 2022 draft interpretation suggests that all exposures in the NCI study, which includes 10 plants, would have been impacted by exposures in Plant 1, and thus that the true risk would have been overpredicted in the entire study. This is a serious misrepresentation of the Marsh et al. (1996) findings that were limited to Plant 1."
"Page 1-209, lines 1-11: Comment 2: This is the source of the serious misrepresentation noted in Comment 1 above which could potentially mislead the reader to believe that all risks were understated in the NCI cohort study."
"Page 1-209, lines 32-39, Page 210, lines 1-3: Comment 3: The 2022 IRIS draft fails to consider Table 6 in Marsh et al 2007b, which reported results based on the interaction terms used in the case-control study modeling. This showed that when comparing workers with formaldehyde exposure only to workers with neither formaldehyde nor silversmithing or other metal work

exposure there was no elevation in the risk of NPC. In contrast, when comparing workers with silversmithing or other metal work to workers with only formaldehyde exposure the odds ratio for NPC was 13.37. These findings suggest that co-exposures to silversmithing or other metal work, which include risk factors for NPC and other upper respiratory system cancers, played a major role on the NPC findings that were unique to Plant 1 in the NCI study. These findings were essentially disregarded by the 2022 IRIS draft.”

“Marsh et al. (2007b) also reported but did not show results of interaction models for the category all other pharyngeal cancers (AOPC) that generally produced more robust risk estimates due to the larger number of cases. For example, the conventional baseline model further adjusted for year of hire revealed a 4.32-fold increased risk for subjects with silversmithing or other metal work only, whereas subjects exposed only to formaldehyde had no elevated risk of AOPC. Here subjects with both exposure factors had a decreased risk for AOPC due to the heavy influence of subjects with formaldehyde exposure only (10 of 16 cases).”

“Pages 1-210; lines 3-16: Comment 4: EPA’s argument that the results for NPC in the NCI cohort consistently show an association between formaldehyde and NPC risk is not supported by the data in Table 2 of the Marsh and Youk (2005) paper. Specifically, Table 2 shows that the SMR based on local rates for NPC among Plant 1 workers was 7.39 and statistically significant based on 6 of the 10 total NPC deaths in the NCI cohort study. In stark contrast, the corresponding SMR for workers in the remaining nine plants in the NCI study was 0.98 -- essentially a null value. This finding highlights the inconsistency of the results in the NCI cohort study and shows clearly that Plant 1 was unique with respect to non-formaldehyde risk factors for NPC. In the Marsh et al. (2007) case-control study discussed in Comment 3, it was concluded that the unique and profound findings for NPC in Plant 1 were likely due to risk factors associated with exposures in previous or post-Plant 1 jobs in the silversmithing or other metalworking industry. The authors stated “the results of our nested case-control study suggest that the large NPC mortality excess in the Wallingford cohort (Plant 1) may not be due to formaldehyde exposure but rather reflects the influence of external employment in the ferrous and nonferrous metal industries of the local area that entailed possible exposures to several suspected risk factors for upper respiratory system cancer (e.g., sulfuric acid mists, mineral acid, metal dusts and heat)”. This association reported in the Marsh et al. (2007) publication was given no consideration in the 2022 IRIS draft.”

“Page 1-210, lines 34-37: Comment 5: While no specific Marsh citations were stated in this conclusion, it should be noted that this conclusion is not consistent with that reached by Marsh and colleagues in several papers, most recently Marsh et al. (2016). In this second reanalysis of the NCI cohort data Marsh et al. concluded “Our updated analysis provided little or no evidence to support NCI’s suggestion of a persistent association between formaldehyde exposure and mortality from NPC. NCI’s suggestion continues to be driven heavily by anomalous findings in one study plant (Plant 1).” This and earlier Marsh et al. reanalyses of the NCI formaldehyde

cohort demonstrated that the results of the NCI study for NPC mortality were driven heavily by the results in Plant 1 which ultimately included six of 11 NPC cases. As noted in the above comments, Marsh et al. (2007) found that the NPC excess unique to Plant 1 was likely due to external exposures in the ferrous and nonferrous metal industries of the local area.”

“Page 1-239, Table 1-33: Comment 6: The Marsh et al. (2007a) citation is incorrectly stated in the reference list. This actually should be what is stated as 2007b.”

“Page 1-254, Lines 1-8: Comment 7: The 2022 IRIS draft cites only SMRs based on US rates, whereas the Marsh et al. (2007b) article also provided the more valid SMRs based on local county population rates. In this case, the local rate based SMRs were lower than those cited by IRIS.”

“Page 1-254, lines 15-19: Comment 8: This is a confusing and incorrect statement because the Marsh et al. (2002) paper did not present results for oropharyngeal / hypopharyngeal cancer in relation to years of formaldehyde exposure. Results were presented for the category “all pharyngeal cancer” and the category “NPC only.” It appears that EPA re-calculated SMRs using data from the Marsh et al. (2002) paper. As noted below, there may be errors in EPA’s calculations of the confidence intervals.”

“Page 1-254, lines 29-34: Comment 9: Same response as comment 8.”

“Page 1-255, lines 3-5: Comment 10: First, this reference should be Marsh et al (2007b). Second, the results shown for hypopharyngeal and pharyngeal cancer unspecified are reversed. Third, no mention is made of the local rate-based SMRs for these categories, which in every case were lower than those based on US rates.”

“Page 1-255; lines 3-5: Comment 11: The SMRs and CIs for Marsh et al. (2002) ‘Exposure to formaldehyde >0.2ppm’ were “calculated using the Mid-P method” by EPA, but could not be replicated in calculations using the specifications in the table footnotes; specifically, the CIs calculated were different than I calculated using the stated methods. Per my calculations, the Level 1 SMR and 95% CI should be 1.51 (0.61-3.13) and the Level 2 SMR and 95% CI should be 2.01 (0.98-3.68). As a result, the Level 2 95% CI would no longer be considered statistically significant as our calculated lower bound is less than one. These errors in calculations thus could have affected how EPA interpreted Marsh et al. (2007b); these calculations should be corrected and the updated conclusions incorporated accordingly.”

EPA Response: EPA has reviewed the cancer evidence synthesis and dose-response sections (including the calculations) and incorporated several typographical corrections. Importantly, however, the revised assessment retains the strong conclusions regarding NPC cancer hazard and risk (Sections 3.2.5 and 5.2), as supported by the consensus peer review panel (see findings above). The NASEM committee did not have Tier 1 recommendations on this topic and EPA has addressed the other committee comments on this topic. In addition, the conclusions on cancer by other federal and international agencies are summarized in Appendix G. Specifically, the hazard conclusion for

human carcinogenicity in the IRIS assessment is consistent with those drawn by other expert review panels. Formaldehyde was classified as a known carcinogen by the (NTP, 2011a) and a Group 1 carcinogen by (IARC, 2006, 2012), both based on evidence for nasal cancers in humans and animals and myeloid leukemia in humans, with supporting data on mechanisms of carcinogenesis. In addition, an expert committee convened by the NAS confirmed the conclusions of the NTP 12th Report on Carcinogens (RoC) and conducted an independent review of the literature through 2013, concluding that formaldehyde is a known carcinogen.

Note that the peer review also supported the methods used to evaluate and synthesize the available cancer studies, including the studies by Marsh et al. A few specific points are included below for clarity. Marsh et al. (2002) stated “our estimates of median AIE to formaldehyde with and without co-exposure to particulates are more than ten times lower than corresponding values estimated for the same Wallingford workers in the NCI study (Blair et al., 1986).” In discussion the potential for information bias, EPA noted that systematic measurement error could bias results if exposures were overestimated by NCI. EPA stated that “If this were true, then the same amount of observed risk in Beane Freeman et al. (2013) would be apportioned to one-tenth the same exposure, which would yield an exposure-response 10-fold greater in magnitude. The claim by Marsh et al. (2007, 2002) suggests a one-sided uncertainty in the exposure-response reported by Beane Freeman et al. (2013), which may be 10 times more potent than reported.” EPA acknowledged that there could have been exposure measurement error as Marsh et al. suggested and EPA noted that overestimating exposure would have diminished the apparent exposure-response.

- **Public Comment (ScitoVation):** “I believe there is an issue with the interpretation of a manuscript that I was the senior author... the p53 point mutations observed in the Recio et al. (1992) study, which EPA cites as evidence of a formaldehyde-induced initiating event in their draft document, is not plausible for several reasons... There is no pathway for DPC to cause the point mutations observed exclusively at GC base pairs. Genotoxicity observed in respiratory tissues are not measures of mutagenic events, particularly point mutations: genotoxicity [does not equal] mutagen[i]city. There is no evidence to support a temporal nature of a mutagenic carcinogen as outlined by EPA. Finally, as determined by NTP, there is no data to support a role for p53 in formaldehyde-induced neoplasia. Although I recognize that p53 mutations are considered hallmarks of cancer, the concern I have is the misrepresentation in your review of the Recio et al., 1992 manuscript indicating that these late occurring mutations are caused by formaldehyde induced DNA lesions. With the lack of any data to support a mutagenic event prior to tumor outcome in the target tissues, I conclude that the p53 mutations observed in SCC from formaldehyde exposed rats are late occurring passenger mutations resulting from known genome instability that occurs in cancers, are not formaldehyde-induced point mutations and

do not support a mutagenic mode-of-action for tumor outcomes from formaldehyde exposed rats."

EPA Response: The MOA conclusion that mutagenicity contributes to formaldehyde inhalation-induced nasal cancers is retained in the revised assessment, consistent with the consensus peer review findings (above). EPA has addressed all panel comments on this topic. As noted previously, EPA conducts an independent analysis of the available articles, so the stance of EPA on the impact and utility of the papers to inform assessment conclusions can differ from the views and conclusions expressed by the study authors. As discussed in prior responses to related public comments, the available evidence does not support a conclusion that cytotoxicity-driven regenerative cell proliferation is the sole driver of formaldehyde-induced nasal carcinogenicity, nor do the data support that a significant contribution of mutagenicity at lower doses is unlikely.

- **Public Comment (Natural Resources Defense Council):** "NRDC supports the EPA conclusion that – with its highest rating of confidence - “evidence demonstrates” a causal link between formaldehyde exposure and three cancer endpoints, nasopharyngeal cancer (NPC), myeloid leukemia and sinonasal cancer (SNC). NRDC agrees that this is consistent with the scientific evidence."

"The 2010 IRIS report that “the associations between myeloid leukemia and formaldehyde exposure are positive and consistent” and that “human epidemiological evidence is sufficient to conclude a causal association between formaldehyde exposure and nasopharyngeal cancer, nasal and paranasal cancer ... [and] myeloid leukemia.” There are no post-2010 data that casts doubt upon that connection. The risk of this endpoint should be included in EPA’s overall cancer risk."

"NRDC supports EPA’s evaluation that the scientific evidence demonstrates that exposure to formaldehyde causes myeloid leukemia. Although EPA has “low” confidence in its risk estimate for myeloid leukemia (compared to “medium” confidence in its NPC-based risk figure) EPA could increase scientific confidence in the data by leveraging the meta-analysis of Zhang et al 2009 (discussed above) to support the risk estimate. Use of the meta-analysis can increase the precision of an effect estimate, by basing the estimate on a larger number of studies ,and can also be used to quantify effects across sufficiently homogeneous studies. Meta-analyses frequently underpin Health Impact Assessments (HIA) and cost-benefit analyses (CBA) of interventions, such as policies to reduce air pollution. Meta-analyses can provide important opportunities when synthesizing study results to strengthen hazard evaluations, and should be used by EPA with appropriate considerations of study quality etc."

"Zhang et al 2009 propose a biologically plausible mechanism for leukemia as follows: “formaldehyde may act on bone marrow directly or, alternatively, may cause leukemia by damaging the hematopoietic stem or early progenitor cells that are located in the circulating

blood or nasal passages, which then travel to the bone marrow and become leukemic stem cells." Where the EPA Draft states that, "No MOA has been established to explain how formaldehyde inhalation can cause myeloid leukemia without systemic distribution..." (p. 54) this statement - and sentiment- should be corrected to note the proposed mechanism of Zhang et al 2009."

EPA Response: The conclusion regarding lack of a MOA explaining formaldehyde inhalation-induced myeloid leukemia has been retained in the revised assessment (see Section 3.3.3). As noted previously, EPA conducts an independent analysis of the available articles, so the stance of EPA on the impact and utility of the papers to inform assessment conclusions can differ from the views and conclusions expressed by the study authors.

Evidence Syntheses and Synthesis Judgments for Lymphohematopoietic Cancers

Peer Review Findings and Comments

- **Finding (9):** With respect to cancer hazard identification for lymphohematopoietic cancer in humans exposed to formaldehyde, EPA was responsive to previous recommendations of the NRC (2011, 2014) and focused on the most specific diagnoses of myeloid leukemia, lymphatic leukemia, multiple myeloma, and Hodgkin lymphoma.
- **Finding (10):** With respect to cancer hazard identification for lymphohematopoietic cancers in humans exposed to formaldehyde, EPA used appropriate methods to synthesize the current state of the science and presents conclusions regarding the hazard identification analysis that accord with its framework and criteria, and are based on expert judgment with the support of the available scientific evidence.
- **Tier 2 Suggested Revision (Recommendation 5.3):** To add clarity, EPA should, in the captions of figures displaying the findings of epidemiological studies for the different cancers, provide references to the numbers of the tables that describe the confidence in each study (low, medium, or high) and "results" (high vs. medium confidence, as presented in Figure 1-38).

EPA Response to Tier 2 Suggested Revision 5.3: References to the associated tables of epidemiological results for each cancer have been added to the captions of the cancer-specific figures.

- **Finding (11):** With respect to cancer hazard identification for lymphohematopoietic cancers in animals exposed to formaldehyde, EPA used its state-of-practice methods to synthesize the current state of the science, and presents conclusions regarding the hazard identification analysis based on its expert judgment with the support of the available scientific evidence.

EPA Response to Finding 11: While no response is necessary, EPA is clarifying the following misstatement from the peer review report: "One chronic-duration inhalation study that reported detailed information on formaldehyde-induced leukemia or lymphoma in rodents (Battelle, 1982) was deemed *indeterminate* because its results remain unpublished." This study was not deemed *indeterminate*; it was actually deemed a *high* confidence study in the external draft assessment (now classified as a *medium* confidence study in the revised assessment, for the reasons described above). The animal evidence overall was deemed *indeterminate* due to the limitations in the available animal evidence that are discussed in the evidence synthesis. The revised presentation of the studies and conclusions in this synthesis section should help to eliminate such misperceptions.

- **Finding (12):** With respect to evidence on MOA for lymphohematopoietic cancers, EPA used its state-of-practice methods to synthesize the knowledge. The presentation of conclusions regarding the mechanistic evidence and how it supports hazard identification is appropriate.
- **Finding (13):** EPA presents an overall clear and succinct evidence integration summary for effects of formaldehyde inhalation on lymphohematopoietic cancers. EPA followed its state of practice for evidence integration at the time the 2022 Draft Assessment was being developed in evaluating studies of cancer outcomes in humans and animals, as well as mechanistic evidence, where available.

Related Public Comments

- **Public Comment (American Chemistry Council):** "The opaque and inconsistent process of evidence integration in the 2022 Draft Assessment is exemplified in EPA's treatment of the evidence for nasopharyngeal cancers (NPCs) relative to lymphohematopoietic malignancies. In both cases, EPA concluded that the 'evidence demonstrates' that formaldehyde inhalation causes NPCs and myeloid leukemia, 'given appropriate exposure circumstances.' However, the body of evidence for these two cancers are quite different and based on EPA's own criteria, should not have the same hazard determination...In the case of NPCs, nasal tumors occur in animals and there are plausible MOAs that have supporting evidence (primarily, cytotoxicity and regenerative proliferation) to support the epidemiological findings. In contrast, myeloid leukemia has no positive animal evidence nor biologically plausible MOA."

"EPA's 2005 Guidelines indicate that meta-analysis can follow systematic review and can be helpful when varying degrees of risk or conflicting results are present. A meta-analysis by Mundt et al. (2021) indicates the descriptor "Inadequate information to assess carcinogenic potential" should be used until a meta-analysis that separately analyzes AML (with control for tobacco smoking) and CML is conducted by EPA solely under its 2005 Guidelines."

“The experimental evidence in animals on formaldehyde inhalation was incorrectly assessed in the Draft Assessment, leading to an inaccurate determination that ‘the evidence available from animal studies is considered indeterminate for drawing conclusions as to whether or not formaldehyde exposure might cause leukemia or lymphoma.’ Formaldehyde is one of the most-studied chemicals in the world. Reflecting this, eleven cancer bioassays have been performed with formaldehyde (that have recently been reviewed by Thompson et al., 2020), via the inhalation route: seven in rats, two in mice, and two in hamsters. A complete animal experimental database in laboratory animals is often considered to consist of chronic bioassays in two species, which clearly formaldehyde exceeds. If the formaldehyde cancer bioassay database is considered indeterminate, then virtually no experimental database can meet the bar set in the Draft Assessment. We disagree with the low rating of the overall study quality of the database in the Draft Assessment. Notably the Draft Assessment rated one rat cancer study (Table 1-65) and one mouse cancer study as “high confidence” studies, clearly meeting the EPA definition of a complete database for assessing formaldehyde carcinogenicity. Thus, it is unclear why his database was considered “indeterminant” in the Draft Assessment. Neither of the two high confidence studies identified a treatment-related increase in leukemia; their exclusion from integration into the assessment of leukemogenic potential prevents the Draft’s conclusions from reflecting best available science.”

“In synthesizing the results of the epidemiology studies, EPA emphasized consistency, magnitude of effects, and dose-response gradients. For example, the EPA reported ‘consistent increases in risk across a set of high and medium confidence, independent studies with varied study designs and populations.’ The estimates of relative risk of myeloid leukemia, however, were not consistent within or between studies in relation to various formaldehyde exposure metrics...A conclusion of consistent associations between formaldehyde exposure and myeloid leukemia is not scientifically justifiable when the observed associations between formaldehyde measures and myeloid leukemia differ according to exposure metrics between studies (that is, consistency is not met when one study reports an association with peak exposure but not cumulative exposure, while another study reports an association with cumulative exposure but not peak exposure).”

“The scientific evidence does not support EPA’s conclusion that ‘evidence demonstrates’ formaldehyde causes myeloid leukemia. In the 2022 Draft assessment, EPA concluded that ‘the evidence demonstrates that formaldehyde inhalation causes myeloid leukemia in humans given appropriate exposure circumstances’⁹⁰ This conclusion mirrors the conclusion in the 2010 Draft Assessment. In its revised assessment of lymphohematopoietic (LHP) malignancies, EPA no longer categorizes LHPs as one large group, as recommended by NASEM (2011). EPA, however, continues to lump two distinct cancers -- acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), into myeloid leukemia.”

“The 2022 Draft Assessment concludes that the human evidence for myeloid leukemia is ‘robust’ based on ‘consistent increases in risk across a set of high and medium confidence... studies...strong associations...and...temporal relationship[s] consistent with causality.’⁵⁰ However, setting aside the inappropriate grouping of acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), the body of epidemiological evidence on myeloid leukemia does not provide ‘robust evidence’ of an association – it is neither consistent nor strong. Specifically, epidemiological studies of occupational groups historically highly exposed to formaldehyde generally do not demonstrate statistically significantly increased rates of myeloid leukemia or more specifically AML and CML:

1. No statistically significant excess risk above background rates of myeloid leukemia is reported in the three myeloid leukemia studies rated as having “effect estimates classified with high confidence” (Hauptmann et al., 2009; Beane Freeman et al., 2009; Meyers et al. 2013).
2. Results from studies with as high or higher quality observed no significant increases in AML, CML, or myeloid leukemia (Saber Hosnijeh et al. 2013, Talibov et al. 2014, Checkoway et al. 2015).
3. Results do not indicate consistent excesses across exposure measures in the studies rated “high confidence” (Hauptmann et al., 2009; Beane Freeman et al., 2009; Myers et al. 2013).

Overall, there is no ‘reasonable confidence that alternative explanations’ (i.e., chance) can be ruled out.”

“Given that EPA acknowledges in the 2022 Draft Assessment that there are no known MOAs for formaldehyde and lymphohematopoietic malignancies, EPA should have deprioritized these malignancies for assessment.”

“The Draft Assessment inappropriately combines myeloid and other/unspecified leukemias together into one class which it then refers to as “myeloid leukemia (p. 2-87) contrary to the NAS (2011) recommendation against combining incidence of different leukemia types.”

“NASEM has consistently recommended improvements to the evidence integration process, including its transparency. Evidence integration should focus on outcomes or endpoints with robust evidence and fully consider study quality. This should include consideration of MOA. As detailed further below, the 2022 draft does not fully meet this recommendation, particularly with respect to lymphohematopoietic malignancies. EPA concluded ‘evidence demonstrates’ that formaldehyde inhalation causes myeloid leukemia in humans, despite the weak human evidence, lack of evidence in animal studies, and lack of a biologically plausible MOA.”

“There is compelling evidence that inhaled formaldehyde is 1) not systemically distributed at ≤ 15 ppm, precluding a direct mutagenic MOA; and 2) not leukemogenic in animal models, including transgenic models designed to be sensitive for detecting leukemogenic effects. The Draft Assessment, however, sets aside the evidence that does not support a leukemogenic effect.

Rather, the Draft Assessment concludes that formaldehyde is a known human leukemogen, based solely on a non-statistically significant numerical association inconsistently observed in epidemiology studies. This is not reflective of data integration, causal determination based on weight of evidence, or sound science.”

“There is no established plausible mode of action for myeloid leukemias.

- EPA continues to rely on flawed studies purportedly demonstrating an MOA.
- EPA’s speculations on potential MOAs for myeloid leukemia lack evidence and therefore biological plausibility.
- EPA fails to consider Gentry et al. (2020) in assessing the biological plausibility of a causal association between inhalation of formaldehyde and myeloid leukemia”

“Failure to fully integrate evidence in this manner, along with numerous other limitations in EPA’s synthesis of the epidemiological evidence, are discussed in detail in the sections that follow. They can be broadly summarized as follows:

- EPA lumps together myeloid leukemias, failing to differentiate between AML and CML, which have very distinct genetics and risk factors.
- EPA fails to truly weigh and integrate the available evidence: it inappropriately downgrades and dismisses important re-analyses and studies, including Checkoway et al. (2015), which are highly informative regarding formaldehyde and LHP malignancies.
- EPA raises up flawed studies such as Hauptmann et al (2009).
- EPA mischaracterizes and overemphasizes risk estimates for purported peak exposure metrics relative to cumulative exposure metrics, which are consistently null.”

“Since the NAS (2011) review, more definitive data on the lack of systemic distribution of inhaled formaldehyde have become available (Lu et al., 2011; Moeller et al. 2011; Edrissi et al. 2013, 2017; Yu et al. 2015; Lai et al., 2016; Leng et al., 2019). It is noteworthy that the more recently published peer-reviewed literature provides further support for the conclusions reached by the NAS (2011) that there is a lack of causal evidence. Such evidence on causality, or the lack thereof, was recently assessed by Gentry et al. (2020) using the IPCS MOA framework. They concluded that LHPs are highly unlikely to be directly related to inhalation of formaldehyde.”

“The Draft Assessment maintains problematic conclusions on systemic genotoxicity/mutagenicity in circulating blood cells. The Draft Assessment continues to conclude that a mutagenic MOA is operable for systemic effects, despite conclusive evidence that formaldehyde is not systemically distributed after inhalation, at environmentally relevant concentrations. It is hard to understand why the EPA continues to conclude that a potential biomarker of a mutagenic effect on circulating blood cells, that is not dose-responsive, supports a linear low-dose leukemogenic response, when EPA has previously and repeatedly been advised this approach is problematic.”

“As discussed in these comments, EPA relies heavily on just a few flawed studies (Zhang et al. 2009, 2010; Lan et al. 2015) which ‘reported effects on myeloid progenitor cells cultured from peripheral blood of exposed workers compared to cells cultured from controls without occupational formaldehyde exposure.’⁵⁸ These studies have since been refuted in the literature (Mundt et al., 2017).”

“Furthermore, a direct genotoxic effect on the bone marrow, resulting in an impact on circulating cells, has been all but disproved by several studies published after Zhang et al.’s (2010) initial study including Lu et al. 2011, Moeller et al. 2011, Yu et al. 2015, and Lai et al. 2016. These studies show that due to the reactive nature of formaldehyde, exogenous formaldehyde is not able to move beyond “the portal of entry” (Mundt et al. 2017).”

“Furthermore, there is ample literature examining whether formaldehyde might cause mutations leading to cancer in blood progenitor cells. Drs. Albertini and Kaden note in their comment on the 2022 Draft Assessment that EPA failed to include two of their papers:

- Albertini RJ, Kaden DA. 2016. Do chromosome changes in blood cells implicate formaldehyde as a leukemogen? *Critical Reviews in Toxicology*, 47(2): 145-184.
- Albertini RJ, Kaden DA. 2020. Mutagenicity monitoring in humans: global versus specific origin of mutations. *Mutation Research/Reviews in Mutation Research*, 786: 108341.”

“EPA mistakenly assumes that biomarkers of exposure (or even effect) in circulating blood cells are the same as genotoxicity in target cells.”

“The Draft Assessment still considers indirect biomarkers of systemic genotoxicity as evidence supporting a genotoxic MOA. The Draft Assessment uses this mechanism to support the hypothesis of cytogenetic effects in circulating blood cells and calculate a linear IUR for leukemia in the absence of any dose-response observations for leukemia or demonstration of in vivo genotoxicity. The approach taken in the Draft Assessment clearly does not follow or adequately address the NAS comments above.”

“For example, in the highly influential National Cancer Institute (NCI) cohort study (Beane-Freeman et al. 2009), the strength and specificity of the exposure-response associations varied considerably over the period in which the cohort was followed. In addition, the reliance on the peak-exposure metric to determine causality in that study rather than the more conventional dose metric of cumulative exposure should be further justified, particularly in the absence of established modes of action.⁴⁴”

“... the draft concludes that that the strength of the human evidence for myeloid leukemia is “robust” based on “several” studies with consistent findings. EPA’s conclusion is based largely on the Beane Freeman analyses of the NCI cohort (pg. 1-542). EPA did not appropriately integrate apparently conflicting findings in the older epidemiological studies (Hauptman et al. (2009) funeral workers study, Beane Freeman et al. (2009) analysis of the NCI cohort) with the analyses published more recently, which demonstrate no excess in cancer risk (e.g., Checkoway et al. (2015) re-analysis of the NCI cohort). EPA should re-examine its evaluations of study

confidence (and risk of bias, especially including statistical analyses) and more fully integrate and interpret the earlier studies in the context of the broader body of more informative and updated studies." *[Please see docket for details and supporting information.]*

- **Public Comment (Louisiana Chemical Association):** "The classification of 'evidence demonstrates' that formaldehyde causes myeloid leukemia is not supported and should be reevaluated based on the growing amount of information which argues against a causal association. Based on EPA guidelines (Table 8 of EPA, 2022a), the classification of 'evidence suggests' is more appropriate given the conflicting and inconsistent findings in human epidemiological studies, the indications of chance, bias and confounders in key positive epidemiological studies, the consistent lack of statistically significant associations, negative animal studies, and the lack of mechanistic evidence supporting a mode of action." *[Please see docket for details and supporting information.]*

EPA Response (to both comments above): The revised assessment retains the conclusion of **evidence demonstrates** for myeloid leukemia. This conclusion was supported by the consensus peer review panel (finding 10) in their response to EPA's charge question on the topic: "With respect to cancer hazard identification for lymphohematopoietic cancers in humans exposed to formaldehyde, EPA used appropriate methods to synthesize the current state of the science and presents conclusions regarding the hazard identification analysis that accord with its framework and criteria, and are based on expert judgment with the support of the available scientific evidence." However, while the conclusion is unchanged, the assessment has been revised to add clarity and transparency regarding the factors most influential to the evidence synthesis and integration decisions for LHP cancers, including myeloid leukemia (see Section 3.3.3).

- **Public Comment (ToxStrategies):** "In support of leukemia and genotoxicity, the U.S. EPA acknowledges the lack of systemic delivery but then invokes non-specific local mechanisms causing systemic genotoxicity. These views are in direct contrast to other organizations. For example, the OECD test guideline 474 for in vivo micronucleus induction requires proof of systemic delivery of a test article when negative genotoxicity results are to be accepted for regulatory purposes. This implies that the genotoxicity experts who designed the test guidelines anticipate that genotoxicity in distal tissues requires systemic delivery of the test article. The doses in these guideline studies typically include a maximal tolerated dose that would presumably elicit local effects that would, if capable of inducing systemic genotoxicity, interfere with the interpretation of the test results. Since indirect genotoxicity mechanisms are not accounted for in guideline tests, it can be surmised that genotoxicity experts do not consider local effects a capable or meaningful contributor to systemic genotoxicity. Additionally, the purpose of genotoxicity testing guidelines and [a]s acknowledged by the NRC and U.S. EPA,

inhaled formaldehyde is not systemically distributed up to 15 ppm. As such, the likelihood that local effects are inducing systemic genotoxicity is low. Data from ADH5 null mice (see above) indicate that even 2-fold increases in serum formaldehyde levels do not cause genotoxicity. Given EPA's hypothesis that elevated formaldehyde at the portal of entry might cause inflammation or oxidative stress, one might assume that ADH5 null mice, having elevated formaldehyde in all tissues, would have higher levels of oxidative stress and DNA damage, yet this does not appear to be the case."

"Table 35 also lists as indirect support for genotoxicity 'strong and consistent evidence of mutagenicity (increased incidence of MN, CA, and chromosomal aneuploidies) in PBLs of human workers'. Elsewhere in the assessment, U.S. EPA acknowledges that inhaled formaldehyde does not distribute beyond the site of contact. As such, U.S. EPA must believe that these data indicate that there is strong and consistent evidence that low levels of inhaled formaldehyde are potent enough to induce systemic genetic damage through indirect mechanisms. Given that many scientists might find this difficult to believe, it is incumbent on the U.S. EPA to strengthen their position...However, U.S. EPA does little to bolster their conclusions. For example, there is no attempt to evaluate their conclusions vis-à-vis the controlled studies by Speit et al. (2007) and Zeller et al. (2011). U.S. EPA could bolster their conclusions by identifying other chemicals with strong experimental data demonstrating that inhalation exposure results in systemic genotoxicity without systemic delivery of the parent compound or metabolites; however, no such discussion was found..."

"In a subsection of 1.2.5 on page 1-303 it is stated that "long-term occupational exposure was associated with significantly increased MN in PBLs, and aneugenicity appears to be the predominant effect in peripheral tissues (see Section 1.3.3)." There are three problems in this one sentence. First, the U.S. EPA is referring to information supporting URT carcinogenicity that has not yet been discussed. This makes following their arguments unnecessarily difficult. Second, why would formaldehyde genotoxicity be associated with long-term exposure but not shorter-term exposure? Formaldehyde does not bioaccumulate and data indicate that formaldehyde-related adducts are labile. Similarly, if the damage were due to oxidative stress or inflammation, these too are transient effects that likely resolve. Skipping ahead to section 1.3.3 I found no explanation; moreover, if there is an explanation in the document it should be recapitulated whenever such data are used to support MOA. The third issue with the above quote is that U.S. EPA states that aneugenicity appears to be the predominant form of genotoxicity in peripheral blood lymphocytes. Considering that aneugenicity is broadly regarded as a threshold form of genotoxicity, it is curious why low-dose linear extrapolation was conducted. Again, I skipped ahead to section 1.3.3 but found no discussion on the implications of aneugenicity for low dose extrapolation. However, on page 1-524 U.S. EPA concludes that formaldehyde cause aneuploidy in PBLs and clastogenicity in nasal tissues. Notably, Speit et al. (2011) reported that formaldehyde induced clastogenicity but not

aneugenicity in cultured cells. Taken together, the reported associations between formaldehyde inhalation exposure and aneugenicity in worker PBLs suggests that such observations are due to something other than formaldehyde exposure."

- **Public Comment (Cardno ChemRisk [now Stantec]):** "The EPA largely disregarded the Mundt et al. (2017) fuller analysis of the Zhang et al. (2010) data...Mundt et al. (2017) requested the exposure data via an NCI Technology Transfer Agreement and was provided the average of the three exposure measures for each exposed worker, but not the individual measurements. Thus, the true range of exposure measurements could not be determined. However, they were able to evaluate the relationship, if any, between the average of the individual formaldehyde exposure measurements and all blood and genetic aneuploidy results (Mundt et al. 2017). These results demonstrated no correlation between formaldehyde exposure level (reportedly over at least a fourfold range) and any of the blood or aneuploidy results. Furthermore, the few assay results generated by tests that met the study protocol (e.g., numbers of cells counted, as pointed out by Gentry et al. (2013) appeared to be related to smoking (but not formaldehyde). "

"Overall, the 2022 Draft does not fully consider or integrate the findings of the fuller analyses that call into question Zhang et al. (2010)'s findings nor does it appropriately integrate evidence from cohort studies reporting ML (and specifically AML) results. The fact remains that there are no studies supporting a MOA for formaldehyde causing leukemias (including AML) and there are several key findings that detract from this hypothesis, e.g., 1) formaldehyde does not form either DNA: protein crosslinks or DNA adducts in bone marrow, 2) exogenous formaldehyde does not escape the portal of entry, 3) there remains no consistent statistically significant relationships between formaldehyde exposure and chromosome aberrations, sister chromatid exchanges or micronucleated cells in hematopoietic stem cells as conducted in animals, 4) studies in humans exposed to formaldehyde have failed to find a relationship between exposures and chromosomal changes in peripheral blood lymphocytes and 5) the available epidemiological evidence is consistent with no significant increase in LHM, specifically AML, among formaldehyde-exposed workers."

"The EPA 2022 Draft Report continues to rely on a cross-sectional study to support a possible MOA for an association between inhalation exposure to formaldehyde and LHMs, based on what they claim (even in the title of the paper) to be "leukemia-specific chromosome changes in cultured myeloid progenitor cells" (Zhang et al. 2010). Cross-sectional studies are incapable of demonstrating changes in clinical markers but might reflect underlying differences between compared groups."

"Additionally, the 2022 Draft does not integrate the fuller analyses of the Zhang et al. (2010) data with results of other studies reporting no exposure-dependent differences in blood parameters and genetic markers of formaldehyde-exposed relative to unexposed workers and other cytogenic studies (Casanova-Schmitz et al. 1984; Heck & Casanova, 2004; Lu et al.

2010) or studies examining chromosomal changes in peripheral blood lymphocytes (Bauchinger & Schmid, 1985; Cheotarev et al., 1986; Pala et al., 2008; Suruda et al., 1993; Thomson et al., 1984; Ying et al., 1999) or cytogenetic studies which show genotoxic effects, but fail to show effects on hematopoietic stem cells (He et al., 1998; Shaham et al., 2002; Yager et al., 1986; Ye et al., 2005). These studies largely align with the results of Mundt et al. (2017) and challenge the interpretation that exposure to formaldehyde may induce chromosome or genotoxic changes in blood or chromosomes." *[Please see docket for details and supporting information.]*

- **Public Comment (University of North Carolina at Chapel Hill):** "The IRIS Draft failed to provide adequate evidence to support proposed mechanisms for leukemia, given the fact that inhaled formaldehyde is not systemically distributed at concentrations ≤ 15 ppm...The study [Lu, Collins, et al. 2010b] demonstrated that exogenous formaldehyde resulted in DNA adducts in rat respiratory nasal mucosa, but did not form [13CD2]-adducts in distant organs remote to the portal of entry. Therefore, the finding provides strong evidence to support a genotoxic and/or cytotoxic MOA for the carcinogenesis of inhaled formaldehyde in respiratory nasal epithelium, but provides evidence against the biological plausibility of inhaled formaldehyde causing leukemia or other systemic effects...The EPA IRIS Draft mentioned several possible mechanisms leading to leukemia from formaldehyde exposure, such as 'Formaldehyde-induced DNA damage to peripheral blood leukocytes', 'formaldehyde-induced systemic oxidative stress', 'formaldehyde-induced changes in the bone marrow niche', etc. However, these hypothesized mechanistic events are not supported or severely confounded by other factors."

"Recommendation: The EPA IRIS Draft should provide direct evidence, rather than hypothesis in identifying the mechanisms responsible for leukemia. Further animal exposure studies are warranted to examine whether EPA hypothesized mechanistic events are supported by data, or not, before integration into a weight-of-evidence determination."

"The IRIS Draft did not demonstrate adequate understanding of the chemistry underlying formaldehyde reactions with DNA and protein...The EPA IRIS Draft recognized the formation of protein adducts by stating that 'Formaldehyde can interact with macromolecules...or protein adducts, such as N6-formyllysine.' or 'Formaldehyde has been shown to bind to histones and chromatin forming N6-formyllysine'. This may exemplify that the EPA IRIS Draft had no adequate understanding of chemistry underlying formaldehyde reactions with DNA and protein. N6-Formyllysine is by no means a major and highly relevant protein adduct induced by formaldehyde. I and others have demonstrated that Schiff base on lysine residue is the predominant protein adduct induced by formaldehyde...Protein binding has significant impact on the formation of DNA adducts induced by exogenous formaldehyde exposure. For example, we have demonstrated that protein binding can determine the available amount of exogenous formaldehyde to target DNA to form DNA adducts...The EPA IRIS Draft did not provide any perspective on how protein binding would affect the fate of exogenous formaldehyde across

tissue space... as my colleagues and I have demonstrated that inhaled formaldehyde at low doses (300 ppb and below) does not induce exogenous DNA adducts even in nasal epithelium. Then, how could exogenous formaldehyde further traverse the epithelium and enter deeper components of the tissues, such as NALT, to induce leukemia?" "Recommendation: EPA needs to revise the IRIS Draft to develop more thorough understanding of chemistry underlying formaldehyde reactions with DNA and protein and how formaldehyde-specific chemistry, such as protein binding, may affect the fate of formaldehyde across tissue space to better support evidence-based formaldehyde risk assessment. Further experiments would be warranted to examine protein binding across tissue space."

"The IRIS Draft misinterpreted the findings of our critical, highly human exposure relevant low dose 28-day study, that precludes a mutagenic MOA at concentrations ≤ 0.3 ppm...The EPA IRIS Draft commented that 'However, in a more recent study with a lower detection limit for adducts and testing lower formaldehyde exposure levels, Leng et al. (2019) did not observe an increase in exogenous hmDNA adducts or DPXs, including in nasal and respiratory tissues as well as at systemic sites (e.g., bone marrow), at formaldehyde levels of 0, 1, 30, or 300 ppb (up to 0.37 mg/m³) after exposure for 28 days.' Certainly, there is a difference between 'not observe an increase' in the EPA IRIS Draft and 'exogenous adducts were not detectable' as my colleagues and I reported...I appreciate that the EPA IRIS Draft acknowledged that our low dose study is helpful in improving understanding of DPX formation by stating 'The lack of detectable exogenous adducts in the URT at 0.3 ppm (0.37 mg/m³) helps to inform the evolving understanding of formaldehyde-induced DPX at lower concentrations, which would benefit from additional study.' However, EPA IRIS Draft did not explain what additional study would be needed...The EPA IRIS Draft failed to provide reasonable explanations or discuss why no exogenous formaldehyde induced DNA adduct or DPC was detected at low doses."

"Recommendation: Considerably more weight-of-evidence should be assigned to our critical, low dose study. The absence of exogenous DNA adducts/DPC in any tissue, against a background of endogenous formaldehyde over 3,000x higher than the detection limit, should be interpreted in the context of interactions between formaldehyde and protein/DNA, formaldehyde carcinogenicity and the potential for genotoxicity in the absence of DNA exposure. Further clarification on 'additional study' is warranted."

"The IRIS Draft showed misunderstanding of formaldehyde-induced DNA-protein crosslinks and its role in formaldehyde carcinogenicity...The EPA IRIS Draft stated that 'While some DPXs may undergo hydrolysis to form N²-hmdG adducts following exogenous formaldehyde exposure, other DPXs appear to be quite stable in vivo; it may be these latter DPXs that play a more important role in formaldehyde-mediated respiratory tract mutagenicity and carcinogenicity.' However, there is no data to support that Cys-CH₂-dG is more important than Lys-CH₂-dG in formaldehyde mutagenicity and carcinogenicity...Likewise, the EPA IRIS Draft states that 'the inability to detect ¹³C, d₂-N⁶-hmdA was surprising, since ¹³C, d₂-N²-hmdG is

reliably quantifiable following low levels of exposure, and increases in an exposure-dependent manner in both rodents, and nonhuman primates (Yu et al., 2015b; Swenberg et al., 2013); the reason for the apparent absence of 13C, d2-N6-hmdA adducts formed by reaction with exogenous formaldehyde remains unknown.' Statements like this again may reflect the lack of adequate understanding of the chemistry underlying formaldehyde reactions with DNA and protein." Recommendation: EPA develops adequate understanding of the formation and fate of formaldehyde-induced DPC and revises the IRIS Draft to address the inaccuracy or mischaracterization in the current EPA IRIS Draft."

[Please see docket for details and supporting information.]

- **Public Comment (Ramboll):** "We recently reviewed the US Environmental Protection Agency (USEPA) draft evaluation of noncancer endpoints in the Draft document "Toxicological Review of Formaldehyde—Inhalation" and were dismayed to find that two of our recent papers were not considered in your assessment.

The first paper, "Do chromosome changes in blood cells implicate formaldehyde as a leukemogen?" addresses an important topic with respect to the USEPA conclusion that formaldehyde can cause myeloid leukemia. It is a comprehensive review of genotoxicity studies of formaldehyde, seeking in vivo evidence in support of the biological plausibility of formaldehyde's role in the induction of leukemia. In this review, we conclude that although chromosome-level genetic effects, as manifest as chromosomal aberration, or micronuclei formation, in peripheral blood lymphocytes, may be positively associated with the subsequent development of cancer, even when these effects are induced in non-target tissues such as peripheral blood lymphocytes (PBLs). There is no implication of causation in these association studies. The association may simply be due to host susceptibility to developing chromosome aberrations, even those that occur spontaneously. This is consistent with mechanisms of cancer development, in which chromosomal-level mutational changes are important key events in cancer induction. Note, these chromosome-level mutational events in PBLs are independent markers of cancer risk, and not the cause of the subsequent cancer - which occur in the target organ (e.g. the bone marrow niche). Elevated frequencies of chromosomal aberrations or micronuclei in PBLs of workers exposed to formaldehyde therefore do not implicate formaldehyde as a leukemogen, and the use of these studies to suggest that they do is a misinterpretation of those studies.

The second paper, "Mutagenicity monitoring in humans: global versus specific origin of mutations," is more indirectly relevant to the USEPA draft report, but nevertheless important. It addresses what in vivo mutation monitoring tells us about the target organ of origin. Assays examining genotoxicity in circulating PBLs in adults do not necessarily reflect a bone marrow origin, and in all likelihood reflect changes occurring in the periphery e.g. circulating blood or peripheral tissues. In newborns and young infants or in rare cases of hematopoietic disorders

where the genetic change originated in the bone marrow, the frequency of such alterations in the circulating blood cells would be expected to be 100-fold or more over background, as seen in the few unusual cases discussed in our paper. This second paper extensively reviews the kinetics of blood cells, and illustrates why chromosome changes measured in PBLs in adults do not implicate the bone marrow in their genesis in normal adults. To equate chromosome mutations in rodents with those in humans is to conclude that the mechanism that induces leukemia in the former are also present in the latter. This conclusion is faulty because rodent experimental studies typically measure the aberrations in bone marrow while human observational studies do so in PBLs. By ignoring this critical difference, the EPA fails to recognize that current technology allows routine measurement of bone marrow chromosome mutations in humans by using the reticulocyte micronucleus assays. Results of such studies would allow a critical evaluation of the ability of formaldehyde to induce chromosome mutations in bone marrow cells - something that should be a requisite before concluding that it does.”

“Although the Zhang et al. (2010a) study has been widely interpreted to suggest the in vivo origins of aneuploidy or initiating genomic instability, the protocol used in this study as well as the underlying data do not support this interpretation. Instead, the data supports aneuploidy arising during in vitro expansion.”

“While this publication [McGregor et al. 2006] was included, a similar publication on which I am the primary author (Gentry et al. 2020; reference provided below), was not included. The Gentry et al. (2020) publication also applied the IPCS human framework for the analysis of the MOAS that have been postulated for the development of leukemia following formaldehyde inhalation. The integration of the available science for formaldehyde in this publication not only demonstrated the limited amount of data that support any hypothesized MOAS, it also demonstrated the significant amount of research supporting the null hypothesis that there is no causal association between formaldehyde inhalation exposure and leukemia. Further, these analyses increase confidence in the conclusion that there is a lack of biological plausibility for a causal association between formaldehyde inhalation exposure and leukemia... Based on the inclusion of the McGregor et al. (2006) study, I would have expected the consideration of the results of the Gentry et al. (2020) study in the current Draft IRIS Assessment. The science presented in this publication is critical in the determination of the lack of biological plausibility of a causal association between inhalation of formaldehyde and myeloid leukemia and should be considered in finalizing the Toxicological Review of Formaldehyde.”

EPA Response (to the four public comments above): The publications noted above are both review articles, not primary data papers. EPA conducted an independent analysis of the available primary data evidence on formaldehyde and the conclusions drawn may or may not agree with those of the study authors or other review authors. Review articles are tracked as supplemental

material and are not necessarily cited in the assessment. For the specific example raised by the commenter, the review by McGregor et al. (2006) is cited in the IRIS assessment three times to summarize information on glutaraldehyde, a URT irritant that doesn't appear to cause nasal tumors in rats, for comparison to formaldehyde, which does cause such tumors. It is not cited as any type of framework for the MOA analysis. The review by Gentry et al. (2020) does not introduce new evidence informative to EPA's independent evaluation of MOA. EPA's conclusions on the primary evidence pertinent to LHP cancer MOA were extensively discussed (Section 3.3.3) and supported by the NASEM panel. Two points of note are provided. One is that knowledge of MOA is not a pre-requisite for identifying a hazard in EPA guidelines or the IRIS Handbook. The second is that the EPA's cancer guidelines (2005) indicate that when a MOA is unknown, a low-dose linear extrapolation is to be applied. Importantly, however, a quantitative estimate for myeloid leukemia risk is not included in the IUR in the revised assessment, due to too much uncertainty in the underlying data. Accordingly, comments about low-dose extrapolation on LHP cancer risk are viewed as potential future considerations.

F.4.2. Dose-Response Analysis of Cancer Effects of Formaldehyde

[Please note that for this section, public comments are collated at the end of the section rather than for each subtopic (as was the organization for prior sections)].

Charge Question 7. Inhalation Unit Risk (IUR) for Cancer.

[Please also see Section "EPA's use of Toxicokinetic Data and Dosimetry Models" above for additional comments relevant to cancer dose-response modeling using toxicokinetic and dosimetric approaches.]

An IUR for cancer is derived on the basis of nasal cancers using data on nasopharyngeal cancers (NPCs) in a human study from the National Cancer Institute (NCI), specifically the results reported in (Beane Freeman et al., 2013). In addition, comparative estimates are provided on the basis of modeling of nasal tumors in exposed rodents. Finally, although not included in the draft IUR, an estimate for myeloid leukemia is presented. Please comment on the clarity and scientific justification for each specific decision in the draft cancer dose-response analyses outlined in (a) to (d), below, including study selection; POD estimates; and confidence in the calculated values. Part (e) includes a specific, additional question on myeloid leukemia.

- a. The NCI study results on NPCs were ultimately selected and used to develop the draft IUR estimate. A lifetable analysis was used to develop a POD and, given the mutagenic MOA for this cancer type, a linear extrapolation was applied. Age-dependent adjustment factors (ADAFs) were applied to this estimate, in accordance with EPA guidelines when a mutagenic MOA is supported. Confidence in the IUR is medium.
- b. As a comparison for the modeling of the human data, data from two chronic rat bioassays were used to develop an estimate of nasal cancer risk. Multiple models, including a biologically-based dose-response model, were evaluated and used to

extrapolate the results in rats to a POD in humans. A linear extrapolation was applied based on a mutagenic MOA for formaldehyde. Furthermore, a candidate RfC based on cell proliferation was also developed for comparison.

- c. For sinonasal cancer, the draft draws an evidence integration judgment of **evidence demonstrates**. Given the lack of quantifiable data to allow the use of dose-response information to identify a POD, the IUR does not incorporate potential contributions to risk for sinonasal cancer. Please comment on this decision and, if this is not supported, include a recommended method to account for this cancer risk.
- d. For myeloid leukemia, a unit risk estimate is presented using the NCI study results (Beane Freeman et al., 2009). The derivation of a unit risk estimate for myeloid leukemia from these data is not straightforward, and several quantitative risk assessment approaches were considered. The selected data set used to derive the myeloid leukemia unit risk estimate combined the results from myeloid leukemia with results for other/unspecified leukemias based on other NCI studies showing that one third to one half of other/unspecified leukemias on death certificates were diagnosed as myeloid leukemias in the hospital. ADAFs were not applied to this estimate, as the assessment concludes that the MOA is unknown.
- e. Although the draft concludes that the evidence demonstrates that formaldehyde inhalation causes myeloid leukemia, the only data available to develop a unit risk estimate for myeloid leukemia are uncertain. The draft Toxicological Review discusses the strengths and limitations of the myeloid leukemia estimate in detail. Please comment specifically on how the unit risk estimate for myeloid leukemia should inform the IUR for cancer, if at all.

Peer Review Comment Summary

- 0 Tier 1 Recommended Revisions
- 14 Tier 2 Suggested Revisions (Note: one was unnumbered in the NASEM study report)
- 1 Tier 3 Future Consideration
- 34 Consensus Panel Findings (numbered below)

General Scope and Approach

Peer Review Findings and Comments

- **Finding (1):** The overall approach and conduct of the cancer dose-response analysis is consistent with EPA's state-of-practice methods for deriving inhalation unit risk estimates. The 2022 Draft Assessment adequately and transparently evaluates the scientific evidence, and generally documents the dose-response analysis overall in a well-organized and transparent manner. The analyses generally follow the process outlined in the 2022 IRIS Handbook and are consistent with the 2005 Guidelines for Carcinogen Risk Assessment (EPA, 2005a, 2022). As

documented in Appendix D and in sections on cancer dose-response analyses, the decision points and analyses are also responsive to the recommendations of the 2011 NRC committee.

- **Finding (2):** The derivation of unit risk is documented in approximately 200 pages in total across the three documents in the 2022 Draft Assessment—the Assessment Overview, Main Assessment, and Appendices—and some redundancies are evident within and across the documents. The sections documenting the derivations based on epidemiological evidence are transparent, and overall are well written and understandable.

EPA Response to Finding 2: EPA notes that the Assessment Overview has not been retained in the revised assessment (again, noting that this document was not intended as a component of the principle analyses, but rather a distilled summary overview of the Toxicological Review and Appendices).

Inhalation Unit Risk for Nasopharyngeal Cancer from Epidemiological Data

Study Selection

Peer Review Findings and Comments

- **Finding (3):** EPA's selection of the Beane Freeman et al. (2013) occupational retrospective mortality study is appropriate and is consistent with the earlier recommendation of the 2011 NRC committee (NRC, 2011, p. 88) that EPA should update the unit risk value when this study became available. While the selection of this study is appropriate, and the major factors for its selection are described, the narrative discussion lacks clarity. The 2011 NRC committee called for EPA to "develop, state, and systematically apply a set of selection criteria for studies and cancer end points" (NRC, 2011, p. 145).
- **Tier 2 Suggested Revision (Recommendation 5.4):** While the criteria for selecting the Beane Freeman et al. (2013) study can reasonably be discerned from the 2022 Draft Assessment, EPA should provide clearer statements of the criteria and comparison of studies with such criteria, in tabular format, to improve transparency and clarity. EPA should add to such a table other studies that evaluated the same cancer outcome so it is apparent why the selected study was superior for the purposes of dose-response analysis.

EPA Response to Tier 2 Suggested Revision 5.4: EPA has added considerations for selecting studies to advance to dose-response analysis (i.e., are they more/most appropriate for use in estimating a POD) to the assessment methods (Section 2.7 in the revised Toxicological Review); these general considerations are applicable to both cancer and noncancer health effects. Note that decisions on which studies are advanced involves pros and cons of the available evidence rather

than strict criteria. A table documenting the application of the considerations to the available studies was added for each quantified cancer type in Section 5.2 of the revised assessment to clarify the justification for study selection in deriving cancer toxicity values.

Endpoint Selection

Peer Review Findings and Comments

- **Finding (4):** The selection of nasopharyngeal cancer mortality is appropriate given its causal link to formaldehyde exposure in humans. Nasopharyngeal cancer incidence would be the preferred endpoint over mortality, but could not be modeled because of the study design (mortality cohort). The decision not to develop a unit risk for the sinonasal cancer outcome can be justified on the basis of the small number of cases and the quality of the dose-response relationship in the study.

Dose Metric Selection

Peer Review Findings and Comments

- **Finding (5):** The selection of cumulative exposure as the dose metric is adequately justified. The use of cumulative exposure as the dose metric in dose-response modeling is consistent with the 2022 IRIS Handbook (EPA, 2022 p. 8-4): “Cumulative exposure (or a dose metric that can be converted to cumulative exposure) is generally the preferred exposure metric for cancer responses; exposure estimates can include a lag period, if warranted.” EPA’s language in Guidelines for Carcinogen Risk Assessment (EPA, 2005a, p. 3-26) is less definitive with regard to preferential use of the cumulative exposure metric: “Unless there is evidence to the contrary in a particular case, the cumulative dose received over a lifetime, expressed as average daily exposure prorated over a lifetime, is recommended as an appropriate measure of exposure to a carcinogen... This approach becomes more problematical as the exposures in question become more intense but less frequent, especially when there is evidence that the agent has shown dose-rate effects” (EPA, 1986a).
 - *Additional committee comments relevant to Finding 5:* “Although EPA stated that the peak exposure metric produced the strongest exposure-response relationships, it did not use this metric because it is not based on exposure concentration measurements. In addition, EPA expressed uncertainty about how to use peak exposure-based estimates in predicting risks for lifetime exposure to continuous low exposures. Because the average exposure metric did not account for duration and duration does not account for level of exposure, these metrics also were not used.”

Model Selection and Fit

Peer Review Findings and Comments

- **Finding (6):** EPA followed a process consistent with its state-of-practice methods in conducting the dose-response analysis on nasopharyngeal cancers. Log-linear Poisson regression is a standard, widely used, and acceptable approach for modeling large occupational data sets such as that reported in Beane Freeman et al. (2013) (25,610 workers; 998,239 person-years of follow-up). The selected lag period of 15 years was based on expert judgment with supporting evidence; the committee finds this to be appropriate for modeling solid tumors.
- **Finding (7):** There is uncertainty about the degree to which peak exposure produces the strongest exposure-response relationships. Inclusion of the zero-response group shows a slightly less significant trend for peak exposure ($p = 0.1$ for trend) compared with the use of cumulative exposure as a metric ($p = 0.07$).
- **Tier 2 Suggested Revision (Recommendation 5.5):** EPA should acknowledge the uncertainty involved in interpreting the analyses on the degree to which exposure-response relationships are stronger than cumulative exposure for determination of peak exposure and risk.

EPA Response to Tier 2 Suggested Revision 5.5: The assessment has been revised to acknowledge uncertainty that peak exposure produces the strongest exposure-response relationships (see Section 5.2).

Point of Departure and Inhalation Unit Risk for Nasopharyngeal Cancer Mortality

Peer Review Findings and Comments

- **Finding (8):** In evaluating the nasopharyngeal cancer outcome, EPA's approach of using a life-table procedure to account for competing causes of death in applying the regression coefficient to estimate extra-risk levels is a standard approach for dose-response assessments that are based on epidemiological evidence.
- **Finding (9):** EPA's choice of an extra-risk value of 0.05 percent for the POD is consistent with its Guidelines for Carcinogen Risk Assessment (EPA, 2005a, p. 3-16): "The POD is an estimated dose (expressed in human-equivalent terms) near the lower end of the observed range without significant extrapolation to lower doses." Because elevated relative risks in the Beane Freeman et al. (2013) study were associated with significant findings in the range of 2.94 to 11.54, the POD of 0.11 ppm, which is associated with a relative risk of 3.6, can be considered as being at the lower end of the observed range.

Supplemental Information—Formaldehyde (Inhalation)

- **Finding (10):** EPA's calculation of the unit risk for mortality is appropriate and consistent with its Guidelines for Carcinogen Risk Assessment (EPA, 2005a, p. 3-23).
- **Finding (11):** Insufficient information is given on the details of the dose-response analysis and the derivation of the unit risk values. For example, the 2022 Draft Assessment states, "An adjustment was also made for the 15-year lag period" (p. 2-50), without explaining how the adjustment was made. In addition, Appendix B, Table B-18 shows no exposure in infancy and childhood in the life-table example for the derivation of a unit risk for environmental exposures.
- **Tier 2 Suggested Revision (Recommendation 5.6):** EPA should state how the adjustment for the 15-year lag was made for nasopharyngeal cancer mortality, and explain the assignment of zero exposure in Table B-18.

EPA Response to Tier 2 Suggested Revision 5.6: The assessment text was revised to clarify how the adjustment for a 15-year exposure lag was made for NPC mortality and explain the zero exposures (see Appendix D.2.1 of the revised assessment).

Inhalation Unit Risk for Nasopharyngeal Cancer Incidence

Peer Review Findings and Comments

- **Finding (12):** EPA's derivation of the unit risk estimate for formaldehyde-associated nasopharyngeal cancer incidence was appropriately done, and the preference for that estimate over the mortality-based unit risk estimate is appropriate and consistent with the 2022 IRIS Handbook (EPA, 2022, p. 8-4) and EPA's Guidelines for Carcinogen Risk Assessment (EPA, 2022, p. 3-12).
- **Finding (13):** The prediction of the number of annual incident cases in the U.S. at upper ends of outdoor (5 ppb) and indoor (20 ppb) formaldehyde exposure levels as a "reality check" on the inhalation unit risk is a useful exercise that would be improved by acknowledging some of the possible environmental and other causative factors of nasopharyngeal cancer in the United States.

EPA Response: Factors that might contribute to NPC risk were evaluated for consideration during evaluation of potential confounding within individual health effect studies. However, as IRIS assessments do not include exposure assessment and perspective on risk due to different exposure scenarios are the purview of subsequent EPA (or other) risk assessments, the recommended discussion was not added in the revised IRIS assessment.

- **Tier 2 Suggested Revision (Recommendation 5.7):** In Appendix B, Table B-12, increasing the number of significant figures in columns P, H, and L to align with column I would add transparency by making it easier for readers to follow and understand the calculations for nasopharyngeal cancer incidence.

EPA Response to Tier 2 Suggested Revision 5.7: EPA has added significant figures to the table entries where such details were available.

Inhalation Unit Risk for Nasal Cancer from Animal Bioassay Data

Study Selection

Peer Review Findings and Comments

- **Finding (14):** EPA's selection of studies was appropriate. The studies were identified as having *high* confidence (Section 1.2.5), and each used large numbers of animals per dose group. However, Table 1-37 (p. 1-296) in Section 1.2.5 lays out five *high*-confidence studies with dose-response data. The reason for not selecting the three other high-quality studies is not discussed or referenced in the dose-response section "Animal Nasal Tumor Incidence."

EPA Response to Finding 14: EPA has added text to Section 5.2.1 that discusses issues with the studies on rats by [Woutersen et al. \(1989\)](#); [Sellakumar et al. \(1985\)](#); [Kamata et al. \(1997\)](#); [Holmstrom et al. \(1989b\)](#) and the merits of the [\(Kerns et al., 1983\)](#) and [\(Monticello et al., 1996\)](#) studies for conducting dose-response analyses. The section also indicates why the studies on mice were not suited for dose-response.

- **Finding (15):** Combining similarly designed studies is an acceptable approach when data harmonization is feasible. While EPA notes that "the incidences are similar in the two studies," it does not fully discuss the homogeneity or heterogeneity of the two studies and did not adopt statistical approaches to address study heterogeneity. Further, EPA does not clearly state that the data relied upon in the analysis are documented in a memorandum by Elizabeth Gross Bermudez from the Chemical Industry Institute of Toxicology (CIIT) correcting the incidence data and dose levels used in the two studies. Both studies were conducted by CIIT. Kerns and colleagues (1983) report that they used the concentrations 0, 2.0, 5.6, or 14.3 ppm with 6 hours per day and 5 days per week exposure for up to 24-months, followed by a nonexposure period of 6 months and with interim sacrifices at 6, 12, 18, 24, 27, and 30 months. Monticello and colleagues (1996) report that they used concentrations of 0, 0.7, 2.0, 6.0, 10, or 15 ppm with 6 hours per day and 5 days per week exposure for up to 24 months and interim sacrifices at 3, 6, 12, 18, and 24 months. EPA reports concentration levels of 0, 0.7, 2.0, 6.01, 9.93, or 14.96 ppm for the two studies combined, as provided in the Bermudez memorandum and in Table 2-20 of

the 2022 Draft Assessment. Listing Bermudez as one of the references for the animal incidence data in Table 2-20 on p. 2-54 without further explanation is insufficient and confusing.

- **Tier 2 Suggested Revision (Recommendation 5.8):** EPA should describe more clearly the procedure and justification for pooling the data from two animal studies into one analysis, and clarify that combined and corrected incidence data are contained in the Bermudez memorandum, which is not readily accessible to the public. The individual animal data for time-to-tumor occurrence used in the model should be provided in an appendix.

•
EPA Response to Tier 2 Suggested Revision 5.8: EPA has added text related to the F344 rat nasal cancer incidence data that addresses the following (with supporting analyses) in Appendix D.2.2 of the revised assessment:

- Dose-response data pooled from the ([Monticello et al., 1996](#); [Kerns et al., 1983](#)) studies were found to be statistically homogenous, and supporting combining the two studies for increased power in the dose-response analysis.
 - In 2004, CIIT issued a memo to EPA that corrected errors in the original report for the ([Kerns et al., 1983](#)) study; the memo ([Bermudez, 2004](#)) is reproduced in the Appendix in its entirety.
 - The individual animal data obtained from CIIT are reproduced.
- **Finding (16):** Another advantage of the two CIIT studies is that they provide time-dependent SCC incidence data, and also have companion mechanistic investigations pertaining to the nasal carcinogenicity of formaldehyde. The mechanistic data generated for the CIIT studies include site-specific DNA–protein crosslinks (DPC) as a marker of tissue dose, as well as site-specific changes in cell labeling as a measure of cell division rate.

Cancer Endpoint Selection

Peer Review Findings and Comments

- **Finding (17):** EPA's selection of SCC as the cancer endpoint is appropriate and consistent with its state-of-practice methods.

Dose-Response Modeling

Peer Review Findings and Comments

- **Finding (18):** EPA's reanalysis largely reproduced the results of Conolly et al. (2003) (Figure 2-4). EPA conducted a thorough uncertainty analysis of the BBDR model, investigating the structure of the model, uncertainties of the model parameters, and model sensitivities to these parameters (Appendix B, Section B.2.2). EPA provides an in-depth discussion of uncertainties from four sources that have potentially larger impact: PBPK model for DPC, use of historical

controls, cell replication rates from the labeling data, and model specification for initiated cell kinetics.

- **Finding (19):** Together, these modeling efforts were highly responsive to the recommendations of the 2011 NRC committee regarding the use of CFD and BBDR models and modeling of time-to-tumor and time-to-death data.
- **Finding (20):** EPA does not present details of model specification or results of the MSW model and the Weibull incidence model in either the Main Assessment or Appendix B. The form of the Weibull model is not presented. The use of these models came with many restrictions on the parameters that affected the model-fitting results. Regarding the MSW model, it is unclear how EPA treated the lapsed-time parameter t_0 —a crucial parameter—although EPA does describe the approach to coding animals with lethal versus incidental tumors. It is also unclear why EPA set the first 5 coefficients to 0 and estimated only the coefficient for the term of fifth power in the multistage polynomial. Although the reader is referred to Appendix B, Section B.2.2 for details, the information on MSW modeling could not be located. The relationships between the administered concentration and the DPC and flux metrics for the rat and the human are not provided. Dose-response fits for the MSW for administered concentration using time-to-tumor data would be informative and useful for comparison given the described uncertainties in the estimates of the dose metrics.
- **Tier 2 Suggested Revision (Recommendation 5.9):** To enhance transparency, EPA should provide additional detail on the modeling, including constraints imposed on model parameters, the results of model fitting (goodness-of-fit test), and the approach used to define lag parameters. The relationship between administered dose and the DNA– protein crosslinks and flux dose metrics should also be provided. Given the uncertainties in the dose surrogates, a dose-response analysis and benchmark concentration calculations using administered concentrations should be provided as a point of comparison.

EPA Response to Tier 2 Suggested Revision 5.9:

- Details regarding the Multistage Weibull time-to-tumor modeling (parameters and model fits) of the ([Monticello et al., 1996](#); [Kerns et al., 1983](#)) studies had been accidentally left out of the document submitted for peer-review and EPA thanks the reviewer for pointing this out. A section in Appendix D.2.2 (referenced in Section 5.2.1 of the main text under *Time-to-tumor modeling without using mechanistic data*) now provides details of the dose-response analyses, estimation of parameters, and plots of model fits for fatal and incidental tumors. The value for the lag parameter t_0 was zero. The first 5 coefficients were estimated in the course of the model fit to be zero (they were not fixed at this value).

- A goodness-of-fit test could not be carried out as this is not available in the software, therefore the model fit was assessed by visual comparison against Kaplan-Meier survival estimates.
- A Table providing the flux levels has been added in the revised Appendix.
- A Table providing flux levels against average DPX levels has also been added in the revised Appendix. The DNA protein cross link estimates used for the dose-metric in the ([Schlosser et al., 2003](#)) study were generated using a physiologically based pharmacokinetic model that used CFD-model generated values of formaldehyde flux to the tissue as input. This is now clearly stated in the text.
- EPA agrees with the recommendation to provide results based on administered exposure concentration of formaldehyde as the dose-metric. These are now added in Appendix D.2.2.

Benchmark Dose Modeling

Peer Review Findings and Comments

- **Finding (21):** The estimates of BMC and BMCL at benchmark response (BMR) levels of 0.01, 0.05, and 0.10 are comparable across the MSW and Weibull (with threshold) models and the BBDR models. EPA also shows (in Table 2-22) that the BMC and BMCL estimates at the BMR = 0.005 level are similar in the two versions of BBDR. Human equivalent concentration (HEC) estimates are also similar. As expected, the Weibull threshold model produced slightly higher estimates. EPA's exercise showed that, assuming the models fit the data well, the model-related uncertainty is limited.

Uncertainty and Variability

- **Finding (22):** EPA's discussion of the variability and uncertainty in the BBDR models is extensive. The 2022 Draft Assessment correctly states that the statistical uncertainties outside of the data range for the model can be higher. However, the example provided for the MSW model (p. 2-69, line 7-10) is difficult to follow, in part because the full results of the model are not given. The MSW model is quoted identically twice (p. 2-69, line 7- 10; p. 2-79, line 7-9) for dose-response estimation and low-dose risk without extrapolation, respectively.
- **Tier 2 Suggested Revision (Recommendation 5.10):** EPA should organize the discussion of uncertainties and variabilities in a manner that is easier to follow, such as by models or by process (models, benchmark concentration estimation, lower dose extrapolation, or extrapolation from animal data to humans).

EPA Response to Tier 2 Suggested Revision 5.10: The uncertainties and variabilities discussion has been revised, as described above. However, given that the uncertainties overlap and are interwoven, the discussion was not re-organized as suggested.

- **Tier 2 Suggested Revision (Recommendation 5.11):** The results from different models and different databases are remarkably similar, supporting each other and suggesting a good degree of robustness. EPA should highlight this robustness to a greater degree while not losing sight of uncertainties within individual studies, endpoints, and models.

EPA Response to Tier 2 Suggested Revision 5.11: The similarities in the estimates, which increase confidence in the selected estimate, are highlighted and emphasized in the revised draft (Section 5.2.1).

Selection of a Unit Risk Estimate for Nasal Cancer

Peer Review Findings and Comments

- **Finding (23):** EPA's selection of the inhalation unit risk based on human nasopharyngeal cancer is appropriate and acceptable. The committee concurs with EPA's choice of cumulative exposure as the metric.

Uncertainties and Confidence in the Preferred Unit Risk Estimate for Nasal Cancers

Peer Review Findings and Comments

- **Finding (24):** EPA discusses the uncertainties for the human inhalation unit risk estimate after discussing inhalation unit risk estimates for animal SCC data and the selection of a preferred unit risk estimate. With only 10 cases of nasopharyngeal cancer-specific deaths in the NCI cohort, the use of nasopharyngeal cancer for deriving an inhalation unit risk carries uncertainties, although the monotonic trend (in rate ratio based on the Poisson regression model) somewhat strengthens the dose-response trend ($p = 0.07$). The strength of dose-response between formaldehyde exposure and nasopharyngeal cancer-related deaths can also be affected by other factors, including the confounding of age and misclassification of exposure, which create additional and important uncertainties for discussion.
 - *Additional committee comments related to Finding 24:* "...a very rare cancer. This study followed more than 25,000 workers for over 40 years and observed a statistically significant increase in RR associated with the highest category of average exposure intensity, however, only 10 cases occurred. The small number of deaths creates uncertainties for the dose-response modeling (borderline significant trend test for cumulative exposure including exposed and unexposed person-years, $p = 0.07$).
- **Tier 2 Suggested Revision (Recommendation 5.12):** EPA should discuss the extent to which the inhalation unit risk estimates based on animal squamous cell carcinoma data and mechanistic data provide supporting evidence for the inhalation unit risk based on the human nasopharyngeal carcinoma data.

EPA Response to Tier 2 Suggested Revision 5.12: Section 5.2.1 (e.g., Table 5-42) shows that the unit risks computed from animal and human data are comparable to a surprising degree, and the text surrounding the Table makes this point.

- **Finding (25):** EPA mischaracterizes the p-value for trend as a p-value for goodness of fit (e.g., Table 2-28; p. 86, lines 8–9; p. 91, lines 13–14).
- **Tier 2 Suggested Revision (Recommendation 5.13):** EPA should address technical errors, such as mischaracterization of a trend p-value, with a thorough and technical edit and proofreading.

EPA Response to Tier 2 Suggested Revision 5.13: EPA has addressed these technical errors in the revised assessment.

Inhalation Unit Risk for Myeloid Leukemia from Epidemiological Data

Study Selection

Peer Review Findings and Comments

- **Finding (26):** The Beane Freeman et al. (2009) study was appropriately selected, and the rationale for its selection over Hauptmann et al. (2009) is adequate.

Inhalation Unit Risk Value for Myeloid Leukemia

Peer Review Findings and Comments

- **Finding (27):** EPA's use of the same general approach for myeloid leukemia as for nasopharyngeal cancer in deriving unit risk estimate was appropriate. The grouping of myeloid leukemia with other/unspecified leukemia to address possible underreporting of myeloid leukemia was a practical approach, but as recognized in the 2022 Draft Assessment, likely created overcounting of myeloid leukemia since half to two-thirds of the other/unspecified category could be nonmyeloid leukemia. Both under- and overreporting of myeloid leukemia can alter the true shape of the dose-response by impacting the cancer deaths across exposure levels and statistical power in an uncertain fashion.
- **Finding (28):** EPA states that the p-value of 0.44 for trend is indicative of a poor model fit. This conclusion is erroneous, and this error occurs throughout this section (e.g., p. 2-86, line 8-9; p. 2-91, line 14; Table 2-36, last bullet point). Rather, this p-value is indicative of the lack of statistical significance for the association between myeloid leukemia death risk and cumulative formaldehyde exposure, which results largely from the nonmonotonic relative risk trend seen

in the reported data (0.82 and 1.02 in the second and third exposure categories, respectively; Table 2-29). In contrast, a monotonic trend is observed under the metric of peak exposure ($p = 0.07$; Table 2-29).

- **Tier 2 Suggested Revision (Recommendation 5.14):** EPA should discuss the implications and interpretation of nonmonotonic dose-response relationships observed with the cumulative exposure metric (e.g., p. 2-92, lines 2–4).

EPA Response to Tier 2 Suggested Revision 5.14: The uncertainties in the dose-response assessment of myeloid leukemia are outlined in Section 5.2.2 of the revised assessment, with additional discussion in Appendix D.2.3. The revision makes clear that the unit risks that can be calculated are too uncertain for use in the IUR.

Uncertainty in the Myeloid Leukemia Inhalation Unit Risk

Peer Review Findings and Comments

- **Finding (29):** The degree of monotonicity of the dose-response in the data dominates the statistical significance of the exposure-response trend. Therefore, combining other/unspecified leukemia with myeloid leukemia does not necessarily reduce the uncertainty due to underreporting of myeloid leukemia.
- **Finding (30):** EPA estimated inhalation unit risks for all leukemias, myeloid leukemia and other/unspecified leukemia combined, and myeloid leukemia separately, and concluded that these estimates were similar (Tables, 2-34 and 2-35). In part because of the weak dose-response relationship for cumulative exposure and myeloid leukemia data in the NCI cohort, the likely significant underreporting of myeloid leukemia, and the uncertainty in the optimal exposure metric, EPA determined that the inhalation unit risk estimate for myeloid leukemia is of low confidence. The committee concurs with the decision not to carry the myeloid leukemia risk estimate forward into the overall inhalation unit risk estimate for formaldehyde.
- **Tier 2 Suggested Revision (Recommendation 5.15):** In the discussion of uncertainties and confidence in the inhalation unit risk for myeloid leukemia, EPA should include the unknown dose rate-response relationship, the choice of statistical model and method, and the lack of understanding of mechanism. The three estimates in Table 2-35 should be presented as alternative, low-confidence inhalation unit risk estimates for myeloid leukemia without selection of a preferred estimate. EPA should not characterize the combining of other/unspecified leukemia with myeloid leukemia as “the best approach.”

EPA Response to Tier 2 Suggested Revision 5.15: The uncertainties in the dose-response assessment of myeloid leukemia are outlined in Section 5.2.2 of the revised assessment, with additional discussion in Appendix D.2.3. The revision classifies the unit risk that can be calculated is too uncertain for use in the IUR. Discussion of a “preferred” unit risk have been removed throughout. EPA has clarified that the unit risk estimate combining other/unspecified leukemia is “the best available approach” rather than “the best approach,” making it clear that while EPA does still consider it to be the best estimate that is currently derived in the assessment (as it leverages the most available information), it remains too uncertain for use in the IUR.

Human-Based Unit Risk Estimates for Potential Increased Early-Life Susceptibility

Decision to Apply Adjustment for Increased Early-Life Susceptibility

Peer Review Findings and Comments

- **Finding (31):** EPA’s application of ADAFs is consistent with its Supplemental Guidance for Assessing Susceptibility from Early-life Exposure to Carcinogens (EPA, 2005b), which states (p. 36): “ADAFs are only to be used for agents with a mutagenic mode of action for carcinogenesis when chemical-specific data are absent.”
 - *Additional committee comments related to Finding 31:* While there is uncertainty in the degree to which nonmutagenic processes may also contribute to the carcinogenic activity of formaldehyde inhalation at the point-of-entry tissues, there is sufficient evidence to support the assumption that a mutagenic MOA is involved in the carcinogenesis of formaldehyde in the upper aerodigestive tract in humans. Furthermore, there are no formaldehyde-specific data to inform adjustments for early-life exposure, which further supports use of the default procedure as discussed in the *Supplemental Guidance for Assessing Susceptibility from Early-life Exposure to Carcinogens* (EPA, 2005b). Finally, this [EPA’s] approach is concordant with the *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a) which states (p. 3-22): “If there are multiple modes of action at a single tumor site, one linear and another nonlinear, then both approaches are used to decouple and consider the respective contributions of each mode of action in different dose ranges. For example, an agent can act predominantly through cytotoxicity at high doses and through mutagenicity at lower doses where cytotoxicity does not occur.”

Approach to Adjustment

Peer Review Findings and Comments

- **Finding (32):** The use of multiple terms for the same item is confusing, and the “preferred inhalation unit risk” conflicts with the definition of “inhalation unit risk” in EPA’s online IRIS glossary: “Inhalation Unit Risk Definition: The upper-bound excess lifetime cancer risk

estimated to result from continuous exposure to an agent at a concentration of 1 µg/m³ in air. The “ADAF-adjusted unit risk estimate” meets this definition, while the “preferred” estimate does not. EPA intends the “preferred” estimate to be used in applications for which ADAFs are used to address less-than-lifetime exposures, particularly early in life, or risks when exposure varies with age.

- *Additional committee comments related to Finding 32:* "Calculations were then made using this “adult-based” inhalation unit risk and ADAFs to derive a total lifetime inhalation unit risk estimate assuming exposure from birth to 70 years of age. The resulting estimate is termed a “lifetime unit risk estimate” (p. 2-99). The “adult based” unit risk estimate is identified as the “preferred inhalation unit risk estimate,” and the “lifetime unit risk estimate” is termed the “ADAF-adjusted unit risk estimate”. (p. 2-102)."

EPA Response to Finding 32: The revised Toxicological Review no longer uses the term “preferred” in this manner.

- **Tier 3 Future Consideration (Recommendation 5.16):** Terminology for inhalation unit risk estimates and for potency values used in applying ADAFs should be consistent across the IRIS Program, including with terms in the IRIS glossary.

EPA Response to Tier 3 Future Consideration 5.16: This future consideration encouraging consistency in future assessments is noted.

- **Tier 2 Suggested Revision (Recommendation 5.17):** For clarity, EPA should include the lifetable calculations for the adult-only unit risk estimate in Appendix B.

EPA Response to Tier 2 Suggested Revision 5.17: An illustration of the lifetable analysis is provided in Appendix D.2.1 of the revised assessment.

Confidence in the Unit Risk Estimate

Peer Review Findings and Comments

- **Finding (33):** The sources of uncertainty are well described, and the committee finds the assignment of medium confidence appropriate and consistent with EPA’s state-of-practice methods. The committee agrees that there is substantial uncertainty regarding extrapolation to lower doses, but notes that the degree of extrapolation is less than typical in environmental health risk assessment because the POD was already at the risk level of 0.05 percent excess risk and well within the range of observation. The committee also agrees that the failure to incorporate in the unit risk estimate cancer activity for cancers other than nasopharyngeal that

are causally related to formaldehyde exposure (myeloid leukemia and sinonasal cancer) could raise the possibility of a bias toward underestimation of risk.

Appropriate Exposure Circumstance

Peer Review Findings and Comments

- **Finding (34):** The dose-response analysis in Section 2 does not explicitly address the meaning of “appropriate exposure circumstances” in the context of the cancer hazard identifications in Section 1, and in fact nowhere in Section 2 is that term found. Consequently, readers are left uninformed as to how EPA is qualifying its cancer hazard conclusions.
- **Tier 2 Suggested Revision (Recommendation “5.18”; [Note: revision not numbered in NASEM report]):** EPA should clearly articulate what is meant by “appropriate exposure circumstances” in Section 2 or abandon the use of the term.

EPA Response to Tier 2 Suggested Revision 5.18: For consistency with the IRIS Handbook and current approaches, this phrasing was replaced with “sufficient exposure conditions” throughout. In addition, footnotes and explanations have been added to clarify that this phrasing is used to highlight in the hazard identification-specific sections that the dose-response analyses defining the conditions of exposure that might be necessary for the effects to manifest are evaluated in the separate dose-response section (for those health effects with sufficient evidence of a hazard). Specifically, “For each credible hazard identified (in this assessment, judgments of evidence demonstrates or evidence indicates [likely]; see Section 2.6 for details on drawing these judgments), the “sufficient exposure conditions” (e.g., exposure level, duration, timing, frequency) alluded to during hazard identification in Section 3 are more fully evaluated and defined through dose-response analysis in Section 5, including, depending on the evidence available, the derivation of toxicity values.” It is noted that this language is not used in association with the carcinogenicity conclusions; the revised assessment was reviewed to remove any such inferences.

Public Comments on Dose-Response Analysis of Cancer Effects of Formaldehyde

- **Public Comment (Louisiana Chemical Association):** “The inhalation unit risk (IUR) for BPC was developed using the NCI cohort study (Beane Freeman et al., 2013) as “it is the only one with sufficient individual exposure data for dose response modeling” (EPA, 2022a). The weak and inconsistent associations, methodological issues, and external confounders of the study are mentioned above and are discussed in detail in the cited references.

The IUR for NPC is based on a mutagenic mode of action (MOA) which assumes a linear non-threshold (LNT) dose response (EPA, 2005). The available science does not support the use of a LNT assumption to estimate toxicity criteria for formaldehyde, a chemical which EPA

acknowledges has a mixed MOA and evidence of non-linear dose response relationships (EPA, 2022a & b)."

"EPA applied age-dependent adjustment factors (ADAFs) to develop the UR for NPC which is typically done for chemicals with a mutagenic MOA. However, EPA acknowledges that the carcinogenicity of formaldehyde can be attributed only in part to a mutagenic MOA. The scientific defensibility of applying ADAFs to the toxicity criteria for a chemical known to have a multi-faceted MOA, non-linear dose-response relationships, and an effect threshold is questionable."

"The 2022 NPC IUR is 1.1×10^{-5} per $\mu\text{g}/\text{m}^3$ (1.1 excess cancer cases are expected to develop per 100,000 people if exposed daily for a lifetime to $1 \mu\text{g}/\text{m}^3$). The overall confidence level of EPA in the NPC IUR is medium based largely on 'The small number of cases that contributed to the statistical analysis and resulting imprecision in modeling the shape of the dose-response curve' (EPA, 2022a). The 2022 NPC IUR is similar to the 1991 IUR of 1.3×10^{-5} based on nasal squamous cell carcinomas in F344 rats (EPA, 2022a)."

[Please see docket for details and supporting information.]

- **Public Comment (American Chemistry Council):** "With regard to nasal tumors, there is substantive evidence indicating these tumors are the result of cytotoxicity and regenerative proliferation. In its derivation of IURs based on animal data, EPA presents an analysis of potential threshold-like effects (i.e., an RfC based on cellular proliferation). Ultimately, however, EPA concludes that because the formaldehyde-induced tumors could not solely be attributed to cell proliferation and that the evidence "at least in part" supported a mutagenic MOA, a linear no-threshold approach was supported. However, the existence of multiple MOAs does not preclude EPA from deriving toxicity values based on threshold-like responses. In the case of formaldehyde, if genotoxicity were to occur, it is expected only above those exposures associated with regenerative cell proliferation, as noted by the ECHA RAC Committee (2020) quoted above. Given the robust scientific evidence that the non-genotoxic MOA predominates and would be protective of any other MOA for carcinogenicity, EPA should consider assessing formaldehyde nasal cancer potency using a threshold approach, rather than a linear, no-threshold IUR."

"The Draft Assessment concludes that a mutagenic MOA is operative for nasal tumors and, therefore, low dose linear extrapolation is required and fails to integrate data demonstrating that both the tumorigenic response and exposure to the molecular target (i.e., DNA) are highly non-linear."

"The draft Formaldehyde Review does not mention the MOA evaluation by Thompson et al. (2020). This analysis of the dose-response indicates there is a threshold for genotoxicity and that it is higher than the threshold for nasal tumors. As such, a threshold approach based on nasal tumors in rats would be protective for any tumors resulting from genotoxicity. The MOA

information, together with the robust toxicity database for formaldehyde, provides ample evidence to "justify a conclusion that mutagenicity is not operative at low doses and focus on a non-linear approach," in accordance with the 2005 Guidelines. In addition to the updated MOA by Thompson et al. (2020), authoritative sources have concluded there is a threshold for both nasal tumors and genotoxicity: i. ECHA Committee for Risk Assessment (RAC). 13 March 2020. Opinion on an Annex XV Dossier Proposing Restrictions on Formaldehyde and Formaldehyde Releasers...ii. WHO. 2010. WHO Guidelines for Indoor Air Quality: Selected Pollutants. World Health Organization, Regional Office for Europe."

"Concordance of effects and other information that supports a threshold MOA, supported by dosimetry was excluded from the assessment. Understanding that the nasal tumors form only at exposure levels at which normal homeostasis has been upset is a critical point in apprehending why a threshold is observed in tumor response. These data [Thompson et al. 2020; Andersen et al. 2010] argue strongly against the use of low dose linear extrapolation into the zone of homeostasis (below background levels of endogenously produced formaldehyde)."

"EPA's carcinogenicity risk-assessment guidelines recommend considering alternative models, especially when biologically based dose-response models are unavailable (EPA 2005). That exercise is especially important when a single study with uncertainties associated with selected cancers and inconsistency with exposure metrics is used. Despite this guidance the Draft Assessment concludes that Formaldehyde is a Human Nasal Carcinogen that acts via a genotoxic mechanism, with a linear dose response, and excludes consideration of the cytotoxic and regenerative hyperplasia threshold MOA recently updated by Thompson et al. (2020), as an alternative MOA."

"... EPA's linear unit risk estimate for NPC is essentially unchanged from the value derived over 30 years ago, despite the prodigious growth in the scientific database on formaldehyde indicating that formaldehyde is a threshold carcinogen."

"Toxicodynamics and toxicokinetics considerations, demonstrating highly non-linear dosimetry, were not taken into consideration when deriving quantitative estimates of risk."

"EPA's formaldehyde cancer slope factor is not based in reality. It projects that background formaldehyde levels of 5 and 20 ppb will result in 8 and 20% of the annual NPC US incidence in non-smokers, or of 16 and 40% of the population when smokers are included. Moreover, using an EPA cited background NPC incidence of 0.6 per 100,000 folks, EPA's cancer slope factor for formaldehyde, and average indoor formaldehyde concentrations, projects incidences at 20 or 51% of the annual NPC US incidence rate. These values clearly do not match the available literature, which shows minimal, if any, background NPC due to formaldehyde, nor is it consistent with highly reliable biomarker of exposure/molecular dosimetry data that indicates little to any exogenous formaldehyde entering cells at average indoor air concentrations, against a high background of endogenous exposure of the DNA. The molecular dosimetry data demonstrates that inhalation exposures could not have added risk of more than 1/3,000th of

the endogenous risk. With an average incidence rate of ~2,000 per year in the US, based molecular dosimetry, at maximum formaldehyde could be causing <1 case per year of nasal cancer in a population of 300,000,000 US residents. Clearly, formaldehyde does not present an unreasonable risk of nasal cancer in the US, at average indoor air concentrations.”

[Please see docket for details and supporting information.]

- **Public Comment (University of North Carolina at Chapel Hill):** "The IRIS Draft did not appreciate or misunderstood the key molecular dosimetry data using DNA adducts as the formaldehyde-specific biomarker. The EPA IRIS Draft simply used a sentence to state that 'an increase in exogenous formaldehyde adducts has been observed in rat nasal tissue at 0.7–15 ppm', which clearly did not appreciate or understand the significance of the critical molecular dosimetry data set." "I appreciate that EPA agreed with formaldehyde mutagenic MOA, however, how could the IRIS Draft still perform a linear low-dose extrapolation given our clear evidence that formaldehyde interacted with DNA in a highly non-linear fashion?" "Recommendation: The EPA IRIS Draft should discuss and consider the critical molecular dosimetry data and the non-linear dose response for DNA adducts in the risk estimates for inhaled formaldehyde"

[Please see docket for details and supporting information.]

- **Public Comment (ToxStrategies):** "More importantly, if U.S. EPA believes that these are the two primary drivers of URT carcinogenicity and there is broad scientific consensus that there are exposures that do not increase proliferation and exposures that do not increase exogenous adducts, then it is perplexing that one of these apparently threshold mechanisms could not serve as a basis for an RfC protective against URT carcinogenicity. The application of uncertainty factors could account for any remaining uncertainty U.S. EPA has about where these thresholds occur in humans. Instead, U.S. EPA appears to accept every publication equally to the point of decision paralysis about whether there is any lower bound on exposures that do not result in proliferation or mutation-inducing adducts and therefore U.S. EPA defaults to linear no threshold cancer slope factor development."

EPA Response (to the four public comments above): Please see prior EPA responses relating to the conclusion of an operant mutagenic MOA for formaldehyde-induced nasal cancers that is retained in the revised assessment (as supported by the peer review).

Specifically, based on the MOA analysis in Section 3.2.5 conducted according to EPA's cancer MOA framework and as summarized during the derivation of unit risk estimates from the animal and human data in Section 5.2.1, it was concluded that both mutagenicity and cytotoxicity-induced regenerative proliferation are key events in nasal cancers caused by formaldehyde inhalation exposure. It was further concluded that there is "strong and consistent evidence for both MOAs" and that "multiple well-conducted studies demonstrate effects associated with mutagenicity (e.g.,

chromosomal changes) at exposure levels well below those causing cellular cytotoxicity, which is incompatible with a theory of carcinogenesis based solely upon exceedance of a cytotoxicity-based threshold.” Additionally, as stated in section 3.2.5, “...formaldehyde may stimulate nasal epithelial cell proliferation to some extent, even in the absence of frank tissue cytotoxicity. Instead of considering independent, sequential series of key events for each of these mechanistic considerations, evidence for genotoxicity and mutagenicity, cellular proliferation (independent from tissue pathology), and cytotoxicity-induced regenerative tissue proliferation is evaluated in an integrated manner, whereby hypothesized mutagenesis and increased cellular turnover initiate and then augment URT carcinogenesis as a function of exposure duration, periodicity, and tissue dose.”

This is finally summarized in the section on “Summary and integration of mechanistic pathways” as follows: “The evidence ... was assembled into a putative URT cancer MOA network highlighting the potential contributions of genotoxicity and cytotoxicity-induced regenerative proliferation (see Figure 3 24), as well as incorporating the influences of increased cell turnover independent of tissue pathology, underlying chronic inflammation and epigenetic activity”

The extensive EPA analyses of the nasal cancer MOA evidence resulted in the selection of a linear low-dose extrapolation in calculations of nasal cancer unit risk estimates (Section 5.2).

- **Public Comment (Natural Resources Defense Council):** “NRDC is concerned that the risk of SNC is not given appropriate weight; it should be included, quantitatively, in the overall cancer risk calculation. As NIEHS notes, “EPA has concluded that (1) formaldehyde is genotoxic in humans (based on studies using buccal, blood, and nasal tissues from exposed humans) and in experimental systems, (2) a genotoxic mode of action plays a role in the development of nasal tumors (SNC) and NPC, and (3) there is moderate confidence for a causal association between formaldehyde exposure and SNC risk from human studies,” (NIEHS p. 12). The hazard for SNC from formaldehyde is established by the existing science; failure to include it in an overall risk estimate may result in an underestimate of risk.”

“NRDC agrees with EPA’s use of an ADAF-adjusted Inhalation Unit Risk (IUR) for NPC of 1.1×10^{-5} per ug/m³ (0.013 per ppm) (Table 2-40, p. 721). This value represents the upper-bound estimate of the increased lifetime risk of cancer from inhaling 1 ug/m³ of formaldehyde over a lifetime. The ADAF adjustments are to address exposure over a 70-year lifetime, which includes vulnerable periods of childhood, and are required by EPA’s Cancer Guidelines because the NPC cancer is due, at least in part, to a mutagenic mode of action. (p. 58).”

“NRDC is concerned that the failure to include SNC in its overall risk finding (the inhalation unit risk, IUR), which is instead based only on the risk of NPC, will result in an underestimate of risk. Excluding both SNC and myeloid leukemia risk from the calculated IUR is pretending the risks don’t exist when in fact they are scientifically established hazards. NRDC suggests that EPA could instead present a range of overall cancer risk, from NPC-only to NPC and myeloid leukemia, and including SNC risks. This is more consistent with EPA policy to add cancer risks,

and would provide both a scientific and policy honesty to EPA's evaluation. Providing this range of overall risk values will inform decisions by regulatory agencies at local and state levels, other countries, industry, unions, communities, consumers, and others."

EPA Response: The revised assessment emphasizes the uncertainty associated with the inability to incorporate estimates for SNC (or myeloid leukemia) risk into the IUR, and it acknowledges that cancer risk is thus expected to be underestimated by the IUR. However, there is no appropriate quantitative adjustment that can be applied for the lack of data on SNC and thus the revised assessment does not incorporate any such adjustment.

- **Public Comment (Louisiana Chemical Association):** "EPA was compelled to develop a IUR estimate because of its judgement that the available 'evidence demonstrates' that formaldehyde causes myeloid leukemia. As discussed above, LCA and other groups and scientists disagree with this designation of causality due to weak or absent causal associations, inconsistent findings, no established mode of action, etc. The methodology used by EPA to develop the IUR for myeloid leukemia further demonstrates why 'evidence demonstrates' is inappropriate."

"Because of the insufficient human and animal data base, EPA used exposure data for myeloid leukemia plus other/unspecified leukemias (which may include cases of myeloid leukemia but does include cases of non-myeloid leukemias) to develop the IUR. Due to the lack of data showing causal associations, EPA used exposure data that did not show an association with formaldehyde exposure. Because mechanistic data are insufficient to determine a MOA for formaldehyde induction of myeloid leukemia if it occurs, EPA used a default linear low-dose extrapolation, the shortcomings of which were presented under the discussion of the IUR for NPC. EPA (2002a) states that it used 'an innovative approach' i.e., one not yet subject to review and approval by the scientific community, to derive and present potential unit risk estimates for myeloid leukemia...EPA has developed an IUR for myeloid leukemia which seems scientifically indefensible and in which the Agency admits it has low confidence."

[Please see docket for details and supporting information.]

- **Public Comment (American Chemistry Council):** "For leukemia dose response assessment, the Draft Assessment uses several methods, both for deriving the inhalation unit risk (IUR) factor for myeloid leukemia and for different combinations of myeloid leukemia and other types of leukemia. Each of the derived IURs, presented in Tables 2-31 through 2-25 fall within the range of 0.03 to 0.48 (per mg/m³). No mention of relative dosimetry related to naturally occurring background concentrations of formaldehyde is included in the assessment of uncertainties in Table 2-36 or included in the section (2.2.5) of the draft assessment that was titled 'Cancer Risk Based on Background Cancer Incidence and Internal Dose of Endogenous and Exogenous Formaldehyde.'"

"It seems that an alternative approach would be to recognize that there are considerable uncertainties in the Draft Assessment cancer risk estimates, that there is direct evidence against an association between leukemia and formaldehyde at environmentally relevant concentrations, and that the associations between formaldehyde and myeloid leukemia were not statistically significant in the study used to calculate the IUR."

"The 2022 draft Formaldehyde Review indicates that epidemiological data are preferred for dose-response analysis and derivation of toxicity values. In many cases, the epidemiological evidence is not suitable for use in quantitative dose-response due to insufficient exposure-response information or other issues. For formaldehyde, only animal data are adequate to describe the dose-response (i.e., threshold or 'hockey-stick') relationship between formaldehyde and cancer, and these are limited to nasal cancer. Despite acknowledging the evidence for LHPs is of 'low confidence,' EPA derives an IUR nonetheless. For specific leukemias (or other specific LHM), neither the animal toxicology nor the epidemiological evidence demonstrates a clear causal relationship and therefore deriving a slope factor or unit risk for leukemia is inappropriate."

"The Draft Assessment does not integrate the evidence developing IURs for nasal cancer and leukemia and fails to integrate of animal studies, toxicodynamics, toxicokinetics, or dosimetry into the modeling."

[Please see docket for details and supporting information.]

- **Public Comment (University of California San Francisco):** "EPA did not include all cancer endpoints in the estimated inhalation unit risk. EPA has appropriately determined that the evidence demonstrates that formaldehyde inhalation causes an increased risk of myeloid leukemia in humans. This determination is consistent with previous findings by International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP), with the NAS affirming the NTP conclusion. EPA, however, does not incorporate the quantitative risk of myeloid leukemia into its estimated inhalation unit risk for formaldehyde, saying that this exclusion is due to uncertainty in the interpretation of the modeling results. It is not appropriate to exclude myeloid leukemia from the quantification of formaldehyde cancer risk, regardless of the uncertainty. EPA's National Air Toxics Assessment (NATA) has identified formaldehyde as the largest nation-wide contributor to cancer risk from air toxics. Exclusion of myeloid leukemia from calculation of the IRIS inhalation unit risk will result in consistent underestimation of cancer risk and will adversely affect risk management decisions, particularly in over-burdened communities that experience poor health status in part because of exposure to formaldehyde and other air toxics. EPA should use the results of its best modeling efforts for myeloid leukemia risk from formaldehyde inhalation and incorporate those results into its overall estimate of the inhalation unit risk for formaldehyde. Based on the

current draft, that means use of the model results using data from the National Cancer Institute (NCI) study.”

- **Public Comment (Environmental Defense Fund):** “EDF recommends that the agency consider using the unit risk for myeloid leukemia to inform the overall cancer IUR. As stated previously, EPA did conclude that the available scientific evidence “demonstrates” that formaldehyde inhalation causes myeloid leukemia. Furthermore, as EPA stated in the Draft Assessment, the public health burden should be considered; nasopharyngeal cancer is rare and, in comparison, myeloid leukemia is more prevalent (pg. 712). EDF does not have specific recommendations for how to include the unit risk of myeloid leukemia to that of the overall cancer IUR. Rather, we ask the agency to consider the following points as it reviews comments on how to potentially update the cancer IUR. When deciding if and how the unit risk for myeloid leukemia should inform the cancer IUR, EPA should consider the existing weakness associated with the current IUR. Our specific points to consider are as follows:
 - Uncertainty with the exposure metric
 - Potential bias associated with heterogeneity among the industrial plants included in the NCI studies

The National Academies also flagged these similar concerns about the Hauptmann et al., 2004 study, which was used to derive the IUR for nasopharyngeal cancer in the 2011 draft formaldehyde assessment in its review of the 2011 document.⁴ We highlight these areas of uncertainty again as we believe they would be important to consider as EPA evaluates the benefit of considering myeloid leukemia in the cancer IUR.”

[Please see docket for details and supporting information.]

- **Public Comment (Natural Resources Defense Council):** “EPA acknowledges some uncertainty in the low dose-response due to the potential for endogenous formaldehyde levels to reduce the uptake of inhaled formaldehyde. Importantly, as EPA concludes, this limitation is negligent given robust scientific evidence demonstrating that formaldehyde has genotoxic, mutagenic and cytogenic mode of toxicity, thus supporting a linear low-dose extrapolation (p. 158, 171).”

“NRDC urges EPA to consider applying ADAFs to the myeloid leukemia risk estimate. EPA did not apply ADAFs to this endpoint. NRDC supports the use of ADAF lifetime adjustments. NRDC joins the broad scientific community in advocating that this adjustment should be applied to all carcinogens, not just those that are mutagenic. This is done by Cal-EPA OEHHA, for example.”

“NRDC is concerned that the proposed IUR will strip away much-needed health protections, as it is roughly 10-fold less protective than EPA’s 2010 draft risk estimate of 1.1×10^{-4} per ug/m³ (0.13 per ppm) based on myeloid leukemia. In fact, it sets IRIS back about a quarter-

century, to the 1991 cancer risk estimate of 1.3×10^{-5} per ug/m³ that remains in the IRIS database."

"NRDC disagrees with excluding the risk estimates for myeloid leukemia in EPA's overall risk estimate (the inhalation unit risk, IUR), which is instead based only on the risk of NPC. This is an especially significant oversight given that myeloid leukemia is a much more common disease than NPC, which is a very rare cancer. EPA explains its omission of the myeloid leukemia risk as due to some uncertainty and therefore a lower confidence in the exposure-response modelling results (Tox Draft p. 57). Nonetheless, the IRIS draft calculates a risk estimate for myeloid leukemia risk, of 3.4×10^{-5} per ug/m³ (0.042 per ppm) (Table 2-35, p. 710)."

EPA Response (to the five public comments above): Please see previous EPA responses related to the myeloid leukemia hazard decision (a judgment of *evidence demonstrates* is retained in the revised assessment) and quantitative estimates (a unit risk estimate for myeloid leukemia is not incorporated into the selected IUR). Both of these decisions are consistent with the advice of the consensus peer review panel, as outlined above. Note that although the results in the only study usable for quantification of myeloid leukemia were ultimately concluded to be too uncertain to support a unit risk estimate, the selected principal epidemiology study is robust for use in quantitative analysis of nasopharyngeal cancer. The assessment recognizes that, primarily due to the inability to address myeloid leukemia risk, the IUR is expected to be an underestimate (of an unknown magnitude) of the actual cancer risk.

- **Public Comment (Toxicology Excellence in Risk Assessment):** "EPA's choice of dosimeter is wrong. EPA's own text indicates that that the choice of dosimeter for the formation of tumors, either nasal pharyngeal or leukemia, is related more to the peak concentration rather than the cumulative exposure. Thus, EPA needs to develop a low dose response extrapolation on the basis of peak exposure, despite the apparent difficulty in doing so. Otherwise, EPA's projected lifetime cancer risks are not credible."

"EPA should also consider the recent findings of the Alliance for Risk Assessment (ARA) Beyond Science and Decisions workshop XIII, where formaldehyde was used as an example of a new approach to dosimetry that may offer some insights.¹³ Simply put, this case study suggests that formaldehyde cannot penetrate the cell nucleus at low concentrations, thus supporting the threshold approach suggested by Thompson et al. (2020). In such cases, EPA (2005) cancer guidelines dictate the use of RfDs or RfCs as the basis of the low dose extrapolation rather than a linear low dose modeling." *[Please see docket for details and supporting information.]*

EPA Response: The revised assessment includes an IUR based on NPC risk. EPA disagrees that peak exposure is the appropriate exposure metric for developing lifetime cancer risks for a number

of reasons enumerated in the assessment (e.g., cumulative exposure is preferred; NPC risk is increased in relation to a variety of different exposure metrics; the definition of peak exposure varies across studies and not directly translatable to the chronic, lifetime exposure conditions addressed by the IUR).

F.5. GENERAL PUBLIC COMMENTS AND PUBLIC COMMENTS NOT ADDRESSED UNDER THE TOPIC AREAS ABOVE

[Note: EPA has provided responses in Appendix F.1 to F.4 on topic areas addressed by the IRIS assessment. The comments below are either general comments not needing an EPA response or they are not relevant to the scientific analyses in the IRIS formaldehyde assessment.⁴⁶]

- **Public Comment (Natural Resources Defense Council):** "Overall, we appreciate the evaluation. It is a thorough and well-reasoned scientific evaluation of the inhalation risks of formaldehyde, including both cancer and non-cancer health endpoints."
- **Public Comment (Environmental Defense Fund):** "EDF applauds EPA's Office of Research and Development's IRIS program for a thorough, clear, well documented robust assessment that clearly demonstrates that the IRIS program heeded the advice of the National Academies of Sciences, Engineering, and Medicine (NASEM) in their 2014 and 2018 reports on the IRIS program."
- **Public Comment (University of California San Francisco):** "The EPA has not allowed for adequate stakeholder engagement during the comment period. Formaldehyde is an environmental justice and affordable housing concern. Lower-income communities are disproportionately at risk of exposure to formaldehyde and resulting health effects from pressed wood products in homes built with less costly building materials. Given this, EPA should have better incorporated into the 60-day comment period opportunities for affected communities as well as academics to engage with EPA on the Formaldehyde Toxicological Review. We have stated our concerns about effectively engaging communities in the public comment process in previous comments submitted to EPA."
- **Public Comment (U.S. Poultry and Egg Association):** "We wish to underscore that the formaldehyde draft assessment should be informed by the best available science and developed

⁴⁶ IRIS assessments are not risk assessments and they do not address exposure assessment or risk management considerations. These are the purview of other EPA program and regional offices, and thus responses are not provided to these comments. Responses are also not provided to comments related to EPA, IRIS, or NASEM processes or policies. EPA draws independent scientific conclusions based on the evidence, which can differ from conclusions by others (see Appendix G for a summary). IRIS assessments do not include evaluations of such potential differences, and thus, responses to comments of this nature are not provided.

through a transparent and unbiased process. We are concerned that EPA has neither achieved this objective nor meaningfully conferred with USDA and FDA on pertinent issues related to animal agriculture and food safety."

- **Public Comment (American Veterinary Medical Association):** "We note several federal agencies have commented on the Draft IRIS Toxicological Review of Formaldehyde (Inhalation), but we did not see comment from FDA or USDA, both of which have familiarity with the animal health, public health, and food safety uses of formaldehyde."
- **Public Comment (United States Chamber of Commerce):** "Moreover, because EPA failed to incorporate fundamental concerns around key issues during the intra-agency and interagency review process, the agency therefore should coordinate with Office of Management and Budget (OMB) to conduct a formal interagency review of the draft formaldehyde IRIS assessment that facilitates review and comment from experts at agencies familiar with use of formaldehyde across the economy (such as FDA and USDA, for example)."
- **Public Comment (American Chemistry Council):** "At Step 3 of its process, EPA solicits input from other federal agencies. In this case, EPA provided other federal agencies only four weeks to respond to its voluminous materials. While this time would be short under normal circumstances, EPA's 30 days included the period from Christmas through New Year's, a period when many in the federal government are out of the office, further reducing the actual time available for other agencies to review. Perhaps as a result of this, several federal agencies from whom responses should have been expected, did not respond. Given the lack of a clear record on the review, however, we are unable to discern why these agencies did not respond. For this reason, and others discussed below, EPA should reengage with other federal agencies to ensure it has obtained their considered opinions."

"EPA has not sought meaningful comment on draft charge questions and peer review committee task, and EPA and NASEM sequencing of public comment, 2022 Draft Assessment revisions, and selection of reviewers undermines the rigor of the peer review."

"EPA's draft peer review charge questions¹⁹¹ unduly narrow the scope of the review, raising key scientific, legal, and policy issues on the quality and independence of the peer review process. For example, there are no questions regarding the 2011 NASEM recommendations on the 2010 Draft Assessment."

"With regard to the lack of transparency, EPA has not docketed its responses to the interagency comments on the 2022 Draft Assessment. The failure to document any changes to the 2022 Draft Assessment (including its appendices) based on interagency comment further hampers the public's ability to comment on this complex and lengthy document."

“The interagency review process conducted on the 2022 Draft Assessment demonstrates why a more structured interagency review process is appropriate, especially for assessments, such as the 2022 Draft Assessment, which have the potential to cause major regulatory and other impacts if they are adopted and used in subsequent regulatory actions. Submitting draft assessments for review under E.O. 12866 as “significant guidance documents” would result in a process that is more transparent, responsive, accountable, deliberate, and advantageous for several reasons.”

“Consistent with the prior NAS review of this assessment and the valuable NAS feedback to improve the IRIS process as well as EPA’s legal and scientific requirements, ACC and the Panel recommend the following steps:

1. Request the Office of Management and Budget to conduct a formal interagency review for the draft formaldehyde IRIS assessment and charge questions prior to their release for public comment.
2. Publish and respond to interagency comments on draft charge questions prior to commencing the public comment period or peer review process.
3. Consistent with EPA⁵ and White House policies,⁶ take public comment on draft charge questions before initiating the peer review. In light of these clear directives on sequencing, EPA and NAS should evaluate those comments prior to selection of the panel “so that appropriate expertise is included to address all charge questions,” and, if appropriate, re-open the nomination process.
4. EPA should incorporate the recommended charge questions (Appendix 1) and those received during the public comment period and update the committee task.
5. EPA should not restrict the committee’s task to “responding only to the materials provided by the EPA.”

[Please see docket for details and supporting information.]

- **Public Comment (Anonymous):** “This comment seeks to highlight for EPA as well as reviewers from the National Academy of Sciences, Engineering, and Medicine the content of relevant feedback in EPA’s general IRIS docket (<http://www.regulations.gov/docket/EPA-HQ-ORD-2014-0211>), for which the Agency has directed comments on the IRIS process in recent years (<https://iris.epa.gov/Dockets/>). Attached are examples of submissions which may be relevant to the review of the draft formaldehyde assessment.” *[Please see docket for details and supporting information.]*
- **Public Comment (Anonymous):** “Also attached are several economic, legal, public policy, and editorial publications highly relevant to this assessment and the IRIS process. [Graham and Liu 2014; Williams 2019; Graham 2006; Logomasini 2020; Logomasini 2019; Dourson 2018].” *[Please see docket for details and supporting information.]*

- **Public Comment (American Chemistry Council):** EPA has already taken steps that at a minimum raise questions regarding the appearance of a lack of impartiality. While NASEM has not released a provisional list of panel members who will review the 2022 Draft Assessment, the assessment cites multiple publications coauthored by the Staff Officer with well-documented involvement with the IRIS program, this assessment, the response to the previous NASEM recommendations, and EPA advocacy on this matter. Even if not explicitly prohibited by the above cited NASEM policy, participation of this Staff Officer raises significant issues, particularly as the 2022 Draft Assessment relies on the Staff Officer's publications violates EPA policies on independence and reviewing one's own work. In addition, the solution provided by NASEM's policies for a conflicted or biased panelist who nonetheless is the only source of needed expertise is to "balance" that individual's perspective on the panel, a remedy that is not possible in the case of a conflicted Staff Officer."

"The OMB Peer Review Guidance cautions agencies to "avoid repeated use of the same reviewer on multiple assessments unless his or her participation is essential and cannot be obtained elsewhere." EPA Peer Review Handbook uses similar language: "The principle is to avoid the repeated use of the same reviewer on multiple assessments unless his/her participation is essential and the expertise cannot be obtained elsewhere."¹⁸⁰ Despite this clear guidance, the responsible Staff Officer has stated unequivocally that the EPA sponsored NASEM committee will include members from the previous 2011 NASEM committee that reviewed the 2010 draft IRIS formaldehyde assessment."

- **Public Comment (Roger O. McClellan):** "I request that EPA finalize the IRIS Formaldehyde document without the involvement of a committee formed by the National Academies of Science, Engineering and Medicine... The proposed involvement of a NASEM committee in bringing the IRIS document to closure is inappropriate. The NASEM modus operandi with the majority of committees deliberations conducted in closed sessions, out of view of the public, without detailed transcripts is not consistent with EPA's requirements that all EPA Advisory Committee activities be open to the public with the opportunity for public input at each meeting with all proceedings documented and transcripts available to interested parties. The Task Order agreement executed between EPA and the NASEM in the Fall of 2021 should be canceled by mutual agreement of both parties... If you, as EPA Administrator, determine that finalization of the IRIS Document will benefit from input from a Scientific Advisory Committee of qualified experts, you have the legal authority to appoint such a Committee to function with oversight by a qualified EPA Federal Government Employee...In my opinion, the EPA does not need any further advice from the NASEM on this matter. I submit that with each NASEM review there is a potential for Committee members to offer more detailed prescriptive advice for EPA to follow to reach the regulatory conclusions the NASEM Committee members would like from EPA."

Supplemental Information—Formaldehyde (Inhalation)

[Please see docket for details and supporting information.]

- **Public Comment (Formacare):** “We are concerned about the draft IRIS Risk Assessment as it is not consistent with the scientific and regulatory conclusions on formaldehyde in the European Union.”

“In the IRIS Risk Assessment, calculated unit risk estimates were in the range of 6.4×10^{-6} and 3.4×10^{-5} per $\mu\text{g}/\text{m}^3$ for the respective cancer types and reference concentrations (RfCs) for non-cancer health effects were derived in the order of 0.001-0.01 mg/m^3 . It is worth noting that, as given in the WHO indoor guidance documentation from 2010 and updated in 2017, the formaldehyde concentration in exhaled air from humans is just in the same range: “Human exhaled air contains formaldehyde in concentration in the order of 0.001–0.01 mg/m^3 , with an average value of about 0.005 mg/m^3 ”. Formacare would recommend putting the unit risk estimates and RfCs for the respective diseases in perspective with the respective prevalence considering actual exposures. This could avoid that the assessment misses capturing the possible risk from formaldehyde exposure in indoor environments and at the workplace.”
- **Public Comment (United States Chamber of Commerce):** “Additionally, the exposure limits and dose response criteria (i.e., Reference Concentrations and Inhalation Unit Risk Factors) proposed are 100 to 1000 times more stringent than the exposure limits/dose response criteria recently established or under consideration in the European Union. These ‘protective’ concentration limits are similar to concentrations in human breath and are at or below the background formaldehyde concentrations in rural air.”
- **Public Comment (Independent Lubricant Manufacturers Association):** “While ILMA members understand EPA’s interest in protecting consumers and workers, their experience is that the use of formaldehyde-release biocides have resulted in few issues over decades of use. In part, this is due to an evolving industrial landscape, including increased ventilation, PPE, and safety protocols to reduce well-known and studied hazards, like those caused by the proliferation of microbiological toxins. MWF companies are concerned that, by EPA’s ill-supported Draft Assessment, one of the most powerful tools for worker protection will be effectively compromised.”
- **Public Comment (U.S. Poultry and Egg Association):** “From our initial review of EPA’s formaldehyde assessment, we note that there is no descriptive reference or acknowledgement of the presence, use, benefit or possible hazards associated with formaldehyde applications in the animal agriculture sector.”

- **Public Comment (American Chemistry Council):** On p. 3-29 ‘An overall RfC for formaldehyde of 0.007 mg/m³ was selected. This value is within the narrow range (0.006-0.009 mg/m³) of the group of respiratory system-related RfCs, which together are interpreted with High confidence (sensory irritation, pulmonary function, allergy-related conditions, and current asthma prevalence or degree of control.’ EPA guidelines allow a considerable degree of freedom in identifying LOAELs, NOAELs, modeling dose-response, and selecting uncertainty factors. The statement above, however, gives the appearance of conclusions being targeted to a pre-determined number.”

“Inappropriately the Draft Assessment does not justify the large quantitative differences in EPA’s hazard and dose-response assessments when compared to recently published exposure guidelines and limits by both WHO and the European Commission, respectively. Each of these authoritative bodies derived safe exposure levels up to three orders of magnitude higher than the safe exposure limits (RfCs and IURs) presented in the Draft Assessment.”

“As such, the Draft IRIS Assessment should be benchmarked against competent authority evaluations [WHO, SCOEL, ECHA] performed under similar assessment guidelines. Additionally, any substantial deviations from other competent authorities conclusions must be documented and discussed in the Draft IRIS Assessment. Any further revisions to the Draft Assessment, must address the major differences between the derived no-effect levels (DNELs) and Occupational exposure limits (OELs) when compared to the RfCs and IURs proposed.”

“The Draft Assessment reaffirms the oral RfD developed in 1990 and is currently on IRIS. This exposure limit raises public concern unduly with little likelihood of any recognizable health benefits...A simple ground truthing demonstrates the inappropriate degree of conservatism in the medium confidence RfD. It is well known that formaldehyde is an endogenously produced natural product of metabolism. In addition, there are many studies of the naturally occurring concentrations of formaldehyde in food. For example, in their evaluation of the literature regarding background concentrations of formaldehyde in food, the European Food Safety Authority (EFSA, 2014) calculated the mean concentration of formaldehyde in foods to be 100 mg/kg. Assuming an average human body weight of 70 kg and the oral RfD of 0.2 mg/kg/day, the safe oral dose is 14 mg/person/day. Assuming consumption of 1 kg of food/day/70 kg human, the average human ingests 100 mg formaldehyde/day. As such, our normal diet exceeds the safe exposure level by 7 times the allowable limit. Clearly, this does not reflect actual risk, is not derived from the best available science, is overly conservative, and unnecessarily alarmist.”

“In addition, EPA has signaled that, in the formaldehyde risk evaluation under the amended Toxic Substances Control Act (TSCA), it “plans to include information developed from the draft IRIS hazard and dose response assessment.”⁸ The push to use draft IRIS information in a regulatory setting without finalizing the assessment or addressing comments on the underlying work product runs contrary to EPA policies and ACC comments on the scoping document. It

Supplemental Information—Formaldehyde (Inhalation)

also suggests that the Agency' rush to deploy the draft could short-circuit a rigorous peer review process."

"U.S. EPA's use of draft assessment violates statutory requirements for "Best Available Science"...Perhaps most concerning, U.S. EPA has signaled that, in its forthcoming draft and final formaldehyde risk evaluation under TSCA, it "plans to include information in a regulatory setting prior to finalizing the assessment, by incorporating both public and peer review comments, runs contrary to EPA policies and would render superfluous both public comment and a rigorous peer review process."

"EPA's failure to proactively discourage use of draft assessment violates agency policies. Failure to proactively prevent use of the draft IRIS assessment by those inside and outside the Agency would contradict long-standing EPA policies on peer review, information quality, and intergovernmental collaboration." *[Please see docket for details and supporting information.]*

APPENDIX G. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

Table G-1. Hazard conclusions and toxicity values developed by other national and international health agencies

Organization	Conclusions and toxicity values
Agency for Toxic Substances and Disease Registry (ATSDR, 1999)	Chronic inhalation minimal risk levels (MRL) = 0.008 ppm using a composite uncertainty factor (UF) of 30, based on clinical symptoms of irritation of eyes and upper respiratory tract and mild damage to the nasal epithelium in chronically exposed workers (Holmstrom et al., 1989c); Intermediate MRL = 0.03 ppm using composite UF of 30 based on nasopharyngeal irritation in Cynomolgus monkeys (Rusch et al., 1983); Acute MRL = 0.04 ppm using UF = 9 based on nasal and eye irritation in human volunteers (Pazdrak et al., 1993).
Interim Acute Exposure Guideline Levels (AEGs) for Formaldehyde, National Advisory Committee for AEGs for Hazardous Substances (NAC/AEGL, 2008)	AEGL-1 (non disabling)—0.90 ppm (1.1 mg/m ³) for exposures ranging from 10 min to 8 hr to protect against mild irritation, based on mild irritation in human subjects. AEGL-2 (disabling)—14 ppm (17 mg/m ³) for exposures ranging from 10 min to 8 hr to protect against mild lacrimation with adaptation in humans. AEGL-3 (lethal)—100 ppm (123 mg/m ³) for a 10-min exposure to 35 ppm (43 mg/m ³) for an 8-hr exposure, the highest nonlethal values in the rat.
National Toxicology Program (NTP, 2011b)	Known to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in humans (consistent findings for nasopharyngeal, sinonasal, and myeloid leukemia) and supporting data on mechanisms of carcinogenesis (NTP, 2011b).
National Institute of Occupational Safety and Health (NIOSH, 2011, https://www.cdc.gov/niosh/idlh/50000.html)	Potential occupational carcinogen. Recommended exposure limit (REL)—0.016 ppm (0.04 mg/m ³) TWA for up to a 10-hr workday and a 40-hr work wk.
Occupational Safety and Health Standard 1910.1048	Permissible exposure limit (PEL) for general industry—0.75 ppm (0.92 mg/m ³) TWA for an 8-hr workday; Short-term exposure limit: 2 ppm (2.5 mg/m ³), 15-min duration.
International Agency for Research on Cancer, Monograph Vol. 88 (IARC, 2006); Monograph Vol. 100F (IARC, 2012)	Sufficient evidence in humans for the carcinogenicity of formaldehyde based on nasopharyngeal cancer and leukemia (Group 1). Sufficient evidence in experimental animals for the carcinogenicity of formaldehyde.
European Union, European Commission, Scientific Committee on Occupational Exposure Limits (SCOEL, 2017)	Carcinogen group C: genotoxic carcinogen with a mode-of-action-based threshold. Occupational exposure limit (OEL)—8h-TWA of 0.3 ppm (0.369 mg/m ³); STEL 15 min of 0.6 ppm (0.738 mg/m ³) based on cytotoxic irritation in studies of human volunteers.
Health Canada (2006, https://www.canada.ca/en/health-canada/services/publications/healthy)	Short-term exposure: 123 µg/m ³ (1-hr average) based on eye, nose, and throat irritation (Kulle, 1993); long-term exposure: 50 µg/m ³ (8-hr average) based on respiratory symptoms in children with asthma (Rumchev et al., 2002).

Supplemental Information—Formaldehyde (Inhalation)

Organization	Conclusions and toxicity values
-living/residential-indoor-air-quality-guideline-formaldehyde.html Residential Indoor Air Quality Guideline	
(Health Canada, 2001) Priority Substances List Assessment Report	The inhalation of formaldehyde under conditions that induce cytotoxicity and sustained regenerative proliferation is considered to present a carcinogenic hazard to humans.

REFERENCES

- [Abrams, WR; Kallen, RG.](#) (1976). Equilibria and kinetics of N-hydroxymethylamine formation from aromatic exocyclic amines and formaldehyde. Effects of nucleophilicity and catalyst strength upon mechanisms of catalysis of carbinolamine formation¹. *J Am Chem Soc* 98: 7777-7789. <http://dx.doi.org/10.1021/ja00440a052>.
- [Abramson, MJ; Perret, JL; Dharmage, SC; McDonald, VM; McDonald, CF.](#) (2014). Distinguishing adult-onset asthma from COPD: a review and a new approach [Review]. *Int J Chron Obstruct Pulmon Dis* 9: 945-962. <http://dx.doi.org/10.2147/COPD.S46761>.
- [Abreu, M, d; Neto, AC; Carvalho, G; Casquillo, NV; Carvalho, N; Okuro, R; Ribeiro, GC; Machado, M; Cardozo, A; Silva, AS; Barboza, T; Vasconcellos, LR; Rodrigues, DA; Camilo, L; Carneiro, L; Jandre, F; Pino, AV; Giannella-Neto, A; Zin, WA; Corrêa, LH; Souza, MN; Carvalho, AR.](#) (2016). Does acute exposure to aldehydes impair pulmonary function and structure? *Respir Physiol Neurobiol* 229: 34-42. <http://dx.doi.org/10.1016/j.resp.2016.04.002>.
- [ACGIH](#) (American Conference of Governmental Industrial Hygienists). (2001). Acetaldehyde [TLV/BEI]. In *Documentation of the threshold limit values and biological exposure indices* (7th ed., pp. A1-A5). Cincinnati, OH.
- [Acheson, ED; Barnes, HR; Gardner, MJ; Osmond, C; Pannett, B; Taylor, CP.](#) (1984a). Cohort study of formaldehyde process workers [Letter]. *Lancet* 2: 403. [http://dx.doi.org/10.1016/s0140-6736\(84\)90568-3](http://dx.doi.org/10.1016/s0140-6736(84)90568-3).
- [Acheson, ED; Gardner, MJ; Pannett, B; Barnes, HR; Osmond, C; Taylor, CP.](#) (1984b). Formaldehyde in the British chemical industry: An occupational cohort study. *Lancet* 323: 611-616. [http://dx.doi.org/10.1016/S0140-6736\(84\)91007-9](http://dx.doi.org/10.1016/S0140-6736(84)91007-9).
- [Adams, DO; Hamilton, TA; Lauer, LD; Dean, JH.](#) (1987). The effect of formaldehyde exposure upon the mononuclear phagocyte system of mice. *Toxicol Appl Pharmacol* 88: 165-174. [http://dx.doi.org/10.1016/0041-008x\(87\)90002-0](http://dx.doi.org/10.1016/0041-008x(87)90002-0).
- [Aglan, MA; Mansour, GN.](#) (2018). Hair straightening products and the risk of occupational formaldehyde exposure in hairstylists. *Drug Chem Toxicol* 43: 1-8. <http://dx.doi.org/10.1080/01480545.2018.1508215>.
- [Ahmed, S; Tsukahara, S; Tin-Tin-Win-Shwe; Yamamoto, S; Kunugita, N; Arashidani, K; Fujimaki, H.](#) (2007). Effects of low-level formaldehyde exposure on synaptic plasticity-related gene expression in the hippocampus of immunized mice. *J Neuroimmunol* 186: 104-111. <http://dx.doi.org/10.1016/j.jneuroim.2007.03.010>.
- [Ahn, KH; Kim, SK; Lee, JM; Jeon, HJ; Lee, DH; Kim, DK.](#) (2010). Proteomic analysis of bronchoalveolar lavage fluid obtained from rats exposed to formaldehyde. *J Health Sci* 56: 287-295. <http://dx.doi.org/10.1248/jhs.56.287>.
- [Akbar-Khanzadeh, F; Mlynek, JS.](#) (1997). Changes in respiratory function after one and three hours of exposure to formaldehyde in non-smoking subjects. *Occup Environ Med* 54: 296-300. <http://dx.doi.org/10.1136/oem.54.5.296>.
- [Akbar-Khanzadeh, F; Vaquerano, MU; Akbar-Khanzadeh, M; Bisesi, MS.](#) (1994). Formaldehyde exposure, acute pulmonary response, and exposure control options in a gross anatomy laboratory. *Am J Ind Med* 26: 61-75. <http://dx.doi.org/10.1002/ajim.4700260106>.
- [Al-Saraj, AA.](#) (2009). Teratogenic effect of formaldehyde in rabbits. *Iraqi Journal of Veterinary Sciences* 23: 1-4.

Supplemental Information—Formaldehyde (Inhalation)

- [Alarie, Y.](#) (1981). Toxicological evaluation of airborne chemical irritants and allergens using respiratory reflex reactions. In BKJ Leong (Ed.), *Inhalation toxicology and technology* (pp. 207-231). Ann Arbor, MI: Ann Arbor Science Publishers, Inc.
- [Albert, M; Garcia, BC; Kuhnert, C; Peschla, R; Maurer, G.](#) (2000). Vapor-liquid equilibrium of aqueous solutions of formaldehyde and methanol. *AIChE J* 46: 1676-1687.
<http://dx.doi.org/10.1002/aic.690460818>.
- [Albert, RE; Sellakumar, AR; Laskin, S; Kuschner, M; Nelson, N; Snyder, CA.](#) (1982). Gaseous formaldehyde and hydrogen chloride induction of nasal cancer in the rat. *J Natl Cancer Inst* 68: 597-603.
- [Alderson, T.](#) (1967). Induction of genetically recombinant chromosomes in the absence of induced mutation. *Nature* 215: 1281-1283.
- [Alexandersson, R.](#) (1988). Decreased lung function and exposure to formaldehyde in the wood working industry. A five-year follow-up. *Arh Hig Rada Toksikol* 39: 421-424.
- [Alexandersson, R; Hedenstierna, G.](#) (1988). Respiratory hazards associated with exposure to formaldehyde and solvents in acid-curing paints. *Arch Environ Health* 43: 222-227.
<http://dx.doi.org/10.1080/00039896.1988.9934937>.
- [Alexandersson, R; Hedenstierna, G.](#) (1989). Pulmonary function in wood workers exposed to formaldehyde: A prospective study. *Arch Environ Health* 44: 5-11.
<http://dx.doi.org/10.1080/00039896.1989.9935865>.
- [Alexandersson, R; Hedenstierna, G; Kolmodin-Hedman, B.](#) (1982). Exposure to formaldehyde: effects on pulmonary function. *Arch Environ Health* 37: 279-284.
<http://dx.doi.org/10.1080/00039896.1982.10667579>.
- [Amdur, MO.](#) (1960). The response of guinea pigs to inhalation of formaldehyde and formic acid alone and with a sodium chloride aerosol. *Int J Environ Pollut* 3: 201-220.
- [Amiri, A; Turner-Henson, A.](#) (2017). The roles of formaldehyde exposure and oxidative stress in fetal growth in the second trimester. *J Obstet Gynecol Neonatal Nurs* 46: 51-62.
<http://dx.doi.org/10.1016/j.jogn.2016.08.007>.
- [An, J; Li, F; Qin, Y; Zhang, H; Ding, S.](#) (2019). Low concentrations of FA exhibits the Hormesis effect by affecting cell division and the Warburg effect. *Ecotoxicol Environ Saf* 183: 109576.
<http://dx.doi.org/10.1016/j.ecoenv.2019.109576>.
- [Anandarajan, V; Noguchi, C; Oleksak, J; Grothusen, G; Terlecky, D; Noguchi, E.](#) (2020). Genetic investigation of formaldehyde-induced DNA damage response in *Schizosaccharomyces pombe*. *Curr Genet* 66: 593-605. <http://dx.doi.org/10.1007/s00294-020-01057-z>.
- [Andersen, I.](#) (1979). Formaldehyde in the indoor environment - health implications and the setting of standards. In PO Fanger; O Valbjorn (Eds.), *Indoor climate: Effects on human comfort, performance, and health in residential, commercial, and light-industry buildings* (pp. 65-87). Copenhagen, Denmark: Danish Building Research Institute.
- [Andersen, I; Molhave, L.](#) (1983). Controlled human studies with formaldehyde. In JE Gibson (Ed.), *Formaldehyde toxicity* (pp. 154-165). Washington, DC: Hemisphere Publishing.
- [Andersen, ME; Clewell, HJ; Bermudez, E; Dodd, DE; Willson, GA; Campbell, JL; Thomas, RS.](#) (2010). Formaldehyde: integrating dosimetry, cytotoxicity, and genomics to understand dose-dependent transitions for an endogenous compound. *Toxicol Sci* 118: 716-731.
<http://dx.doi.org/10.1093/toxsci/kfq303>.
- [Andersen, ME; Clewell, HJ, III; Bermudez, E; Willson, GA; Thomas, RS.](#) (2008). Genomic signatures and dose-dependent transitions in nasal epithelial responses to inhaled formaldehyde in the rat. *Toxicol Sci* 105: 368-383. <http://dx.doi.org/10.1093/toxsci/kfn097>.
- [Anderson, D; Yu, TW; Phillips, BJ; Schmezer, P.](#) (1994). The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the COMET assay. *Mutat Res* 307: 261-271. [http://dx.doi.org/10.1016/0027-5107\(94\)90300-x](http://dx.doi.org/10.1016/0027-5107(94)90300-x).

- [Andersson, B; Eriksson, B; Falsen, E; Fogh, A; Hanson, LA; Nylén, O; Peterson, H; Svanborg Edén, C.](#) (1981). Adhesion of *Streptococcus pneumoniae* to human pharyngeal epithelial cells in vitro: differences in adhesive capacity among strains isolated from subjects with otitis media, septicemia, or meningitis or from healthy carriers. *Infect Immun* 32: 311-317.
- [Andersson, DA; Gentry, C; Moss, S; Bevan, S.](#) (2008). Transient Receptor Potential A1 Is a Sensory Receptor for Multiple Products of Oxidative Stress. *J Neurosci* 28: 2485-2494. <http://dx.doi.org/10.1523/JNEUROSCI.5369-07.2008>.
- [Andersson, M; Agurell, E; Vaghef, H; Bolcsfoldi, G; Hellman, B.](#) (2003). Extended-term cultures of human T-lymphocytes and the comet assay: a useful combination when testing for genotoxicity in vitro? *Mutat Res* 540: 43-55. [http://dx.doi.org/10.1016/S1383-5718\(03\)00169-4](http://dx.doi.org/10.1016/S1383-5718(03)00169-4).
- [Andjelkovich, DA; Janszen, DB; Brown, MH; Richardson, RB; Miller, FJ.](#) (1995). Mortality of iron foundry workers: IV. Analysis of a subcohort exposed to formaldehyde. *J Occup Environ Med* 37: 826-837. <http://dx.doi.org/10.1097/00043764-199507000-00012>.
- [Ankley, GT; Bennett, RS; Erickson, RJ; Hoff, DJ; Hornung, MW; Johnson, RD; Mount, DR; Nichols, JW; Russom, CL; Schmieder, PK; Serrano, JA; Tietge, JE; Villeneuve, DL.](#) (2010). Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment [Review]. *Environ Toxicol Chem* 29: 730-741. <http://dx.doi.org/10.1002/etc.34>.
- [Annesi-Maesano, I; Hulin, M; Lavaud, F; Raherison, C; Kopferschmitt, C; de Blay, F; Charpin, DA; Denis, C.](#) (2012). Poor air quality in classrooms related to asthma and rhinitis in primary schoolchildren of the French 6 Cities Study. *Thorax* 67: 682-688. <http://dx.doi.org/10.1136/thoraxjnl-2011-200391>.
- [Apfelbach, R; Weiler, E.](#) (1991). Sensitivity to odors in wistar rats is reduced after low-level formaldehyde-gas exposure. *Naturwissenschaften* 78: 221-223. <http://dx.doi.org/10.1007/bf01136085>.
- [Appelman, LM; Woutersen, RA; Zwart, A; Falke, HE; Feron, VJ.](#) (1988). One-year inhalation toxicity study of formaldehyde in male rats with a damaged or undamaged nasal mucosa. *J Appl Toxicol* 8: 85-90. <http://dx.doi.org/10.1002/jat.2550080204>.
- [Arican, RY; Sahin, Z; Ustunel, I; Sarikcioglu, L; Ozdem, S; Oguz, N.](#) (2009). Effects of formaldehyde inhalation on the junctional proteins of nasal respiratory mucosa of rats. *Exp Toxicol Pathol* 61: 297-305. <http://dx.doi.org/10.1016/j.etp.2008.09.005>.
- [Armstrong, RW; Imrey, PB; Lye, MS; Armstrong, MJ; Yu, MC; Sani, S.](#) (2000). Nasopharyngeal carcinoma in Malaysian Chinese: occupational exposures to particles, formaldehyde and heat. *Int J Epidemiol* 29: 991-998. <http://dx.doi.org/10.1093/ije/29.6.991>.
- [Arslan-Acaroz, D; Bayşu-Sozbilir, N.](#) (2020). Ameliorative effect of boric acid against formaldehyde-induced oxidative stress in A549 cell lines. *Environ Sci Pollut Res Int* 27: 4067-4074. <http://dx.doi.org/10.1007/s11356-019-06986-y>.
- [Asgedom, AA; Bratveit, M; Moen, BE.](#) (2019). High Prevalence of Respiratory Symptoms among Particleboard Workers in Ethiopia: A Cross-Sectional Study. *Int J Environ Res Public Health* 16. <http://dx.doi.org/10.3390/ijerph16122158>.
- [Askar, EM; Halloull, NM.](#) (2018). Formaldehyde-induced neurotoxicity in rat cerebellar cortex and possible protective effects of fatty acids from omega 3 and wheat germ oil supplement: a histopathological and biochemical study. *J Histotechnol* 41: 79-87. <http://dx.doi.org/10.1080/01478885.2018.1458176>.
- [Aslan, H; Songur, A; Tunc, AT; Ozen, OA; Bas, O; Yagmurca, M; Turgut, M; Sarsilmaz, M; Kaplan, S.](#) (2006). Effects of formaldehyde exposure on granule cell number and volume of dentate gyrus: a histopathological and stereological study. *Brain Res* 1122: 191-200. <http://dx.doi.org/10.1016/j.brainres.2006.09.005>.

Supplemental Information—Formaldehyde (Inhalation)

- [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (1999). Toxicological profile for formaldehyde [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. <http://www.atsdr.cdc.gov/toxprofiles/tp111.pdf>.
- [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (2007). Health consultation: Formaldehyde sampling at FEMA temporary housing units, Baton Rouge, Louisiana. Atlanta, GA. http://www.atsdr.cdc.gov/HAC/PHA/fema_housing_formaldehyde/formaldehyde_report_0507.pdf.
- [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (2008). Draft for toxicological profile for formaldehyde [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
- [Attia, D; Mansour, N; Taha, F; El Dein, AS](#). (2014). Assessment of lipid peroxidation and p53 as a biomarker of carcinogenesis among workers exposed to formaldehyde in cosmetic industry. *Toxicol Ind Health* 32: 1097-1105. <http://dx.doi.org/10.1177/0748233714547152>.
- [Auerbach, C; Moser, H](#). (1953a). An analysis of the mutagenic action of formaldehyde-food. I. Sensitivity of *Drosophila* germ cells. *MGG Mol gen genet* 85: 479-504. <http://dx.doi.org/10.1007/BF00308298>.
- [Auerbach, C; Moser, H](#). (1953b). Analysis of the mutagenic action of formaldehyde food. II. The mutagenic potentialities of the treatment. *MGG Mol gen genet* 85: 547-563. <http://dx.doi.org/10.1007/BF00308300>.
- [Auerbach, C; Moutschen-Dahmen, M; Moutschen, J](#). (1977). Genetic and cytogenetical effects of formaldehyde and related compounds [Review]. *DNA Repair* 39: 317-361. [http://dx.doi.org/10.1016/0165-1110\(77\)90011-2](http://dx.doi.org/10.1016/0165-1110(77)90011-2).
- [Augenreich, A; Stickford, J; Stute, N; Koontz, L; Cope, J; Bennett, C; Ratchford, SM](#). (2020). Vascular dysfunction and oxidative stress caused by acute formaldehyde exposure in female adults. *Am J Physiol Heart Circ Physiol* 319: H1369-H1379. <http://dx.doi.org/10.1152/ajpheart.00605.2020>.
- [Aung, W; Sakamoto, H; Sato, A; Yi, E; Thein, Z; Nwe, M; Shein, N; Linn, H; Uchiyama, S; Kunugita, N; Win-Shwe, T; Mar, O, hn](#). (2021). Indoor Formaldehyde Concentration, Personal Formaldehyde Exposure and Clinical Symptoms during Anatomy Dissection Sessions, University of Medicine 1, Yangon. *Int J Environ Res Public Health* 18. <http://dx.doi.org/10.3390/ijerph18020712>.
- [Axelsson, G; Lütz, C; Rylander, R](#). (1984). Exposure to solvents and outcome of pregnancy in university laboratory employees. *Br J Ind Med* 41: 305-312.
- [Aydemir, S; Akgun, SG; Beceren, A; Yuksel, M; Kumas, M; Erdogan, N; Sardas, S; Omurtag, GZ](#). (2017). Melatonin ameliorates oxidative DNA damage and protects against formaldehyde-induced oxidative stress in rats. *International Journal of Clinical and Experimental Medicine* 10: 6250-6261.
- [Aydin, S; Canpinar, H; Undeğer, U; Güç, D; Colakoğlu, M; Kars, A; Başaran, N](#). (2013). Assessment of immunotoxicity and genotoxicity in workers exposed to low concentrations of formaldehyde. *Arch Toxicol* 87: 145-153. <http://dx.doi.org/10.1007/s00204-012-0961-9>.
- [Aydin, S; Ogeturk, M; Kuloglu, T; Kavakli, A; Aydin, S](#). (2014). Effect of carnosine supplementation on apoptosis and irisin, total oxidant and antioxidants levels in the serum, liver and lung tissues in rats exposed to formaldehyde inhalation. *Peptides* 64C: 14-23. <http://dx.doi.org/10.1016/j.peptides.2014.11.008>.
- [Babiuk, C; Steinhagen, WH; Barrow, CS](#). (1985). Sensory irritation response to inhaled aldehydes after formaldehyde pretreatment. *Toxicol Appl Pharmacol* 79: 143-149. [http://dx.doi.org/10.1016/0041-008x\(85\)90376-x](http://dx.doi.org/10.1016/0041-008x(85)90376-x).
- [Bach, B; Pedersen, OF; Mølhave, L](#). (1990). Human performance during experimental formaldehyde exposure. *Environ Int* 16: 105-113. [http://dx.doi.org/10.1016/0160-4120\(90\)90150-5](http://dx.doi.org/10.1016/0160-4120(90)90150-5).

Supplemental Information—Formaldehyde (Inhalation)

- [Bakar, E; Ulucam, E; Cerkezkayabekir, A. \(2015\). Protective effects of proanthocyanidin and vitamin E against toxic effects of formaldehyde in kidney tissue. Biotech Histochem 90: 69-78. <http://dx.doi.org/10.3109/10520295.2014.954620>.](#)
- [Ballarin, C; Sarto, F; Giacomelli, L; Bartolucci, GB; Clonfero, E. \(1992\). Micronucleated cells in nasal mucosa of formaldehyde-exposed workers. Mutat Res Genet Toxicol 280: 1-7. \[http://dx.doi.org/10.1016/0165-1218\\(92\\)90012-O\]\(http://dx.doi.org/10.1016/0165-1218\(92\)90012-O\).](#)
- [Baluk, P; Bertrand, C; Geppetti, P; McDonald, DM; Nadel, JA. \(1995\). NK1 RECEPTORS MEDIATE LEUKOCYTE ADHESION IN NEUROGENIC INFLAMMATION IN THE RAT TRACHEA. Am J Physiol Lung Cell Mol Physiol 268: L263-L269.](#)
- [Band, PR; Le, ND; Fang, R; Threlfall, WJ; Astrakianakis, G; Anderson, JT; Keefe, A; Krewski, D. \(1997\). Cohort mortality study of pulp and paper mill workers in British Columbia, Canada. Am J Epidemiol 146: 186-194. <http://dx.doi.org/10.1093/oxfordjournals.aje.a009250>.](#)
- [Bansal, N; Uppal, V; Pathak, D. \(2011\). Toxic effect of formaldehyde on the respiratory organs of rabbits: a light and electron microscopic study. Toxicol Ind Health 27: 563-569. <http://dx.doi.org/10.1177/0748233710393398>.](#)
- [Barbosa, E; Dos Santos, ALA; Peteffi, GP; Schneider, A; Müller, D; Rovaris, D; Bau, CHD; Linden, R; Antunes, MV; Charão, MF. \(2019\). Increase of global DNA methylation patterns in beauty salon workers exposed to low levels of formaldehyde. Environ Sci Pollut Res Int 26: 1304-1314. <http://dx.doi.org/10.1007/s11356-018-3674-7>.](#)
- [Bardet, G; Achard, S; Loret, T; Desauziers, V; Momas, I; Seta, N. \(2014\). A model of human nasal epithelial cells adapted for direct and repeated exposure to airborne pollutants. Toxicol Lett 229: 144-149. <http://dx.doi.org/10.1016/j.toxlet.2014.05.023>.](#)
- [Barker, S; Weinfeld, M; Murray, D. \(2005\). DNA-protein crosslinks: their induction, repair, and biological consequences \[Review\]. Mutat Res 589: 111-135. <http://dx.doi.org/10.1016/j.mrrev.2004.11.003>.](#)
- [Barnes, PJ. \(1992\). Neurogenic inflammation and asthma \[Review\]. J Asthma 29: 165-180. <http://dx.doi.org/10.3109/02770909209099025>.](#)
- [Barnes, PJ. \(1998\). Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin Sci \(Lond\) 94: 557-572.](#)
- [Barnes, PJ; Baraniuk, JN; Belvisi, MG. \(1991a\). Neuropeptides in the respiratory tract. Part I \[Review\]. Am Rev Respir Dis 144: 1187-1198. <http://dx.doi.org/10.1164/ajrccm/144.5.1187>.](#)
- [Barnes, PJ; Baraniuk, JN; Belvisi, MG. \(1991b\). Neuropeptides in the respiratory tract. Part II \[Review\]. Am Rev Respir Dis 144: 1391-1399. <http://dx.doi.org/10.1164/ajrccm/144.6.1391>.](#)
- [Barrow, CS. \(1983\). Respiratory and metabolic response of rats and mice to formalin vapor \[Letter\]. Toxicology 28: 357-359. \[http://dx.doi.org/10.1016/0300-483X\\(83\\)90009-4\]\(http://dx.doi.org/10.1016/0300-483X\(83\)90009-4\).](#)
- [Barrow, CS; Steinhagen, WH; Chang, JCF. \(1983\). Formaldehyde sensory irritation. In JE Gibson \(Ed.\), Formaldehyde toxicity \(pp. 16-25\). Washington, DC: Hemisphere Publishing.](#)
- [Basler, A; Wvd, H; Scheutwinkel-Reich, M. \(1985\). Formaldehyde-induced sister chromatid exchanges in vitro and the influence of the exogenous metabolizing systems S9 mix and primary rat hepatocytes. Arch Toxicol 58: 10-13. <http://dx.doi.org/10.1007/bf00292609>.](#)
- [Bassig, BA; Zhang, L; Vermeulen, R; Tang, X; Li, G; Hu, W, ei; Guo, W; Purdue, MP; Yin, S; Rappaport, SM; Shen, M, in; Ji, Z; Qiu, C; Ge, Y; Hosgood, HD; Reiss, B; Wu, B; Xie, Y; Li, L; Yue, F, ei; Freeman, LEB; Blair, A; Hayes, RB; Huang, H; Smith, MT; Rothman, N; Lan, Q. \(2016\). Comparison of hematological alterations and markers of B-cell activation in workers exposed to benzene, formaldehyde and trichloroethylene. Carcinogenesis 37: 692-700. <http://dx.doi.org/10.1093/carcin/bgw053>.](#)

- [Batalha, JRF; Guimaraes, ET; Lobo, DJA; Lichtenfels, AJF, C; Deur, T; Carvalho, HA; Alves, ES; Domingos, M; Rodrigues, GS; Saldiva, PHN.](#) (1999). Exploring the clastogenic effects of air pollutants in Sao Paulo (Brazil) using the Tradescantia micronuclei assay. *DNA Repair* 426: 229-232.
- [Battelle](#) (Battelle Columbus Laboratories). (1981). Final report on a chronic inhalation toxicology study in rats and mice exposed to formaldehyde to Chemical Industry Institute of Toxicology: Volume 1. Research Triangle Park, NC: Chemical Industry Institute of Toxicology.
- [Battelle](#) (Battelle Columbus Laboratories). (1982). A chronic inhalation toxicology study in rats and mice exposed to formaldehyde. Research Triangle Park, NC: Chemical Industry Institute of Toxicology. http://al-pdf://CIIT_1982_FA1340_Vol1A_OCR-2178554371/CIIT_1982_FA1340_Vol1A_OCR.pdf.
- [Bauchinger, M; Schmid, E.](#) (1985). Cytogenetic effects in lymphocytes of formaldehyde workers of a paper factory. *Mutat Res Genet Toxicol* 158: 195-199. [http://dx.doi.org/10.1016/0165-1218\(85\)90085-0](http://dx.doi.org/10.1016/0165-1218(85)90085-0).
- [Bautista, DM; Jordt, SE; Nikai, T; Tsuruda, PR; Read, AJ; Poblete, J; Yamoah, EN; Basbaum, AI; Julius, D.](#) (2006). TRPA1 Mediates the Inflammatory Actions of Environmental Irritants and Proalgesic Agents. *Cell* 124: 1269-1282. <http://dx.doi.org/10.1016/j.cell.2006.02.023>.
- [Beane Freeman, LE; Blair, A; Lubin, JH; Stewart, PA; Hayes, RB; Hoover, RN; Hauptmann, M.](#) (2013). Mortality from solid tumors among workers in formaldehyde industries: an update of the NCI cohort. *Am J Ind Med* 56: 1015-1026. <http://dx.doi.org/10.1002/ajim.22214>.
- [Beane Freeman, LE; Blair, A; Lubin, JH; Stewart, PA; Hayes, RB; Hoover, RN; M, H.](#) (2009). Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries: The National Cancer Institute Cohort. *J Natl Cancer Inst* 101: 751-761. <http://dx.doi.org/10.1093/jnci/djp096>.
- [Beisswenger, C; Kandler, K; Hess, C; Garn, H; Felgentreff, K; Wegmann, M; Renz, H; Vogelmeier, C; Bals, R.](#) (2006). Allergic airway inflammation inhibits pulmonary antibacterial host defense. *J Immunol* 177: 1833-1837. <http://dx.doi.org/10.4049/jimmunol.177.3.1833>.
- [Beland, FA; Fullerton, NF; Heflich, RH.](#) (1984). Rapid isolation, hydrolysis and chromatography of formaldehyde-modified DNA. *J Chromatogr A* 308: 121-131. [http://dx.doi.org/10.1016/0378-4347\(84\)80202-9](http://dx.doi.org/10.1016/0378-4347(84)80202-9).
- [Bellavia, A; Dickerson, AS; Rotem, RS; Hansen, J; Gredal, O; Weisskopf, MG.](#) (2021). Joint and interactive effects between health comorbidities and environmental exposures in predicting amyotrophic lateral sclerosis. *Int J Hyg Environ Health* 231: 113655. <http://dx.doi.org/10.1016/j.ijheh.2020.113655>.
- [Bellisario, V; Mengozzi, G; Grignani, E; Bugiani, M; Sapino, A; Bussolati, G; Bono, R.](#) (2016). Towards a formalin-free hospital. Levels of 15-F2t-isoprostane and malondialdehyde to monitor exposure to formaldehyde in nurses from operating theatres. *Toxicology Research* 5: 1122-1129. <http://dx.doi.org/10.1039/c6tx00068a>.
- [Bender, JR; Mullin, LS; Grapel, GJ; Wilson, WE.](#) (1983). Eye irritation response of humans to formaldehyde. *Am Ind Hyg Assoc J* 44: 463-465. <http://dx.doi.org/10.1080/15298668391405139>.
- [Bentayeb, M; Norback, D; Bednarek, M; Bernard, A; Cai, G; Cerrai, S; Eleftheriou, KK; Gratziou, C; Holst, GJ; Lavaud, F; Nasilowski, J; Sestini, P; Sarno, G; Sigsgaard, T; Wieslander, G; Zielinski, J; Viegi, G; Annesi-Maesano, I; Study, G.](#) (2015). Indoor air quality, ventilation and respiratory health in elderly residents living in nursing homes in Europe. *Eur Respir J* 45: 1228-1238. <http://dx.doi.org/10.1183/09031936.00082414>.
- [Bergeron, C; Boulet, LP.](#) (2006). Structural changes in airway diseases: characteristics, mechanisms, consequences, and pharmacologic modulation [Review]. *Chest* 129: 1068-1087. <http://dx.doi.org/10.1378/chest.129.4.1068>.

Supplemental Information—Formaldehyde (Inhalation)

- [Berglund, B; Höglund, A; Esfandabad, HS.](#) (2012). A bisensory method for odor and irritation detection of formaldehyde and pyridine. *Chemosensory Perception* 5: 146-157. <http://dx.doi.org/10.1007/s12078-011-9101-9>.
- [Berglund, B; Nordin, S.](#) (1992). Detectability and perceived intensity for formaldehyde in smokers and non-smokers. *Chem Senses* 17: 291-306. <http://dx.doi.org/10.1093/chemse/17.3.291>.
- [Berk, JV; Hollowell, CD; Pepper, JH; Young, RA.](#) (1980). The impact of reduced ventilation on indoor air quality in residential buildings. In *Proceedings of the 73rd annual meeting of the Air Pollution Control Association*. Lexington, KY: Air Pollution Control Association. <https://www.osti.gov/biblio/5505445>.
- [Berke, JH.](#) (1987). Cytologic examination of the nasal mucosa in formaldehyde-exposed workers. *J Occup Environ Med* 29: 681-684.
- [Bermudez, E; Chen, Z; Gross, EA; Walker, CL; Recio, L; Pluta, L; Morgan, KT.](#) (1994). Characterization of cell lines derived from formaldehyde-induced nasal tumors in rats. *Mol Carcinog* 9: 193-199. <http://dx.doi.org/10.1002/mc.2940090403>.
- [Bermudez, EG.](#) (2004). HCHO studies - Tumor incidence [Memorandum]. Available online at (accessed [Bernardini, L; Barbosa, E; Charão, MF; Goethel, G; Muller, D; Bau, C; Steffens, NA; Stein, CS; Moresco, RN; Garcia, SC; Vencato, MS; Brucker, N.](#) (2020). Oxidative damage, inflammation, genotoxic effect, and global DNA methylation caused by inhalation of formaldehyde and the purpose of melatonin. *Toxicology Research* 9: 778-789. <http://dx.doi.org/10.1093/toxres/tfaa079>.
- [Berrino, F; Richiardi, L; Boffetta, P; Estève, J; Belletti, I; Raymond, L; Troschel, L; Pisani, P; Zubiri, L; Asuncion, N; Gubéran, E; Tuyns, A; Terracini, B; Merletti, F; Group, MJW.](#) (2003). Occupation and larynx and hypopharynx cancer: A job-exposure matrix approach in an international case-control study in France, Italy, Spain and Switzerland. *Cancer Causes Control* 14: 213-223. <http://dx.doi.org/10.1023/a:1023661206177>.
- [Bertazzi, PA; Pesatori, A; Guercilena, S; Consonni, D; Zocchetti, C.](#) (1989). Cancer risk among workers producing formaldehyde-based resins: Extension of follow-up. 80: 111-122.
- [Bertazzi, PA; Pesatori, AC; Radice, L; Zocchetti, C.](#) (1986). Exposure to formaldehyde and cancer mortality in a cohort of workers producing resins. *Scand J Work Environ Health* 12: 461-468. <http://dx.doi.org/doi:10.5271/sjweh.2111>.
- [Bessac, BF; Jordt, SE.](#) (2008). Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control [Review]. *Physiology (Bethesda)* 23: 360-370. <http://dx.doi.org/10.1152/physiol.00026.2008>.
- [Bhalla, DK; Mahavni, V; Nguyen, T; McClure, T.](#) (1991). Effects of acute exposure to formaldehyde on surface morphology of nasal epithelia in rats. *J Toxicol Environ Health* 33: 171-188. <http://dx.doi.org/10.1080/15287399109531516>.
- [Biagini, RE; Moorman, WJ; Knecht, EA; Clark, JC; Bernstein, IL.](#) (1989). Acute airway narrowing in monkeys from challenge with 2.5 ppm formaldehyde generated from formalin. *Arch Environ Health* 44: 12-17. <http://dx.doi.org/10.1080/00039896.1989.9935866>.
- [Bian, RX; Han, JY; Kim, JK; Choi, IS; Lee, SG; Park, JS; Jung, YD.](#) (2012). The effect of chronic formaldehyde exposure on the hippocampus in chronic cerebral hypoperfusion rat model. *Toxicol Environ Chem* 94: 1211-1224. <http://dx.doi.org/10.1080/02772248.2012.691505>.
- [Bickis, M; Krewski, D.](#) (1989). Statistical issues in the analysis of the long-term carcinogenicity bioassay in small rodents: an empirical evaluation of statistical decision rules. *Toxicol Sci* 12: 202-221.
- [Bienenstock, J; Mcdermott, MR.](#) (2005). Bronchus- and nasal-associated lymphoid tissues [Review]. *Immunol Rev* 206: 22-31. <http://dx.doi.org/10.1111/j.0105-2896.2005.00299.x>.
- [Billionnet, C; Gay, E; Kirchner, S; Leynaert, B; Annesi-Maesano, I.](#) (2011). Quantitative assessments of indoor air pollution and respiratory health in a population-based sample of French dwellings. *Environ Res* 111: 425-434. <http://dx.doi.org/10.1016/j.envres.2011.02.008>.

Supplemental Information—Formaldehyde (Inhalation)

- [Binawara, BK; Ranjnee, CS; Mathur, KC; Sharma, H; Goyal, K.](#) (2010). Acute effect of formalin on pulmonary function tests in medical students. *Pak J Physiol* 6: 8-10.
- [Binzak, BA; Vockley, JG; Jenkins, RB; Vockley, J.](#) (2000). Structure and analysis of the human dimethylglycine dehydrogenase gene. *Mol Genet Metab* 69: 181-187.
<http://dx.doi.org/10.1006/mgme.2000.2980>.
- [Blackburn, GR; Dooley, JFI; Schreiner, CA; Mackerer, C.](#) (1991). Specific identification of formaldehyde-mediated mutagenicity using the mouse lymphoma L5178Y TK +/- assay supplemented with formaldehyde dehydrogenase. *In Vitro Toxicol* 4: 121-132.
- [Blair, A; Linos, A; Stewart, PA; Burmeister, LF; Gibson, R; Everett, G; Schuman, L; Cantor, KP.](#) (1993). Evaluation of risks for non-Hodgkin's lymphoma by occupation and industry exposures from a case-control study. *Am J Ind Med* 23: 301-312. <http://dx.doi.org/10.1002/ajim.4700230207>.
- [Blair, A; Stewart, P; O'Berg, M; Gaffey, W; Walrath, J; Ward, J; Bales, R; Kaplan, S; Cubit, D.](#) (1986). Mortality among industrial workers exposed to formaldehyde. *J Natl Cancer Inst* 76: 1071-1084.
- [Blair, A; Stewart, PA; Hoover, RN.](#) (1990). Mortality from lung cancer among workers employed in formaldehyde industries. *Am J Ind Med* 17: 683-699.
<http://dx.doi.org/10.1002/ajim.4700170604>.
- [Blair, A; Stewart, PA; Hoover, RN; Fraumeni, JF, Jr; Walrath, J; O'Berg, M; Gaffey, W.](#) (1987). Cancers of the nasopharynx and oropharynx and formaldehyde exposure [Letter]. *J Natl Cancer Inst* 78: 191-193. <http://dx.doi.org/10.1093/jnci/78.1.191>.
- [Blair, A; Zheng, T; Linos, A; Stewart, PA; Zhang, YW; Cantor, KP.](#) (2001). Occupation and leukemia: A population-based case-control study in Iowa and Minnesota. *Am J Ind Med* 40: 3-14.
<http://dx.doi.org/10.1002/ajim.1066>.
- [Blasiak, J; Trzeciak, A; Malecka-Panas, E; Drzewoski, J; Wojewódzka, M.](#) (2000). In vitro genotoxicity of ethanol and acetaldehyde in human lymphocytes and the gastrointestinal tract mucosa cells. *Toxicol In Vitro* 14: 287-295. [http://dx.doi.org/10.1016/S0887-2333\(00\)00022-9](http://dx.doi.org/10.1016/S0887-2333(00)00022-9).
- [Boffetta, P; Stellman, SD; Garfinkel, L.](#) (1989). A case-control study of multiple myeloma nested in the American Cancer Society prospective study. *Int J Cancer* 43: 554-559.
<http://dx.doi.org/10.1002/ijc.2910430404>.
- [Bogaert, P; Tournoy, KG; Naessens, T; Grooten, J.](#) (2009). Where Asthma and Hypersensitivity Pneumonitis Meet and Differ Noneosinophilic Severe Asthma. *Am J Pathol* 174: 3-13.
<http://dx.doi.org/10.2353/ajpath.2009.071151>.
- [Bogdanffy, MS; Morgan, PH; Starr, TB; Morgan, KT.](#) (1987). Binding of formaldehyde to human and rat nasal mucus and bovine serum albumin. *Toxicol Lett* 38: 145-154.
[http://dx.doi.org/10.1016/0378-4274\(87\)90122-6](http://dx.doi.org/10.1016/0378-4274(87)90122-6).
- [Bogdanffy, MS; Plowchalk, DR; Sarangapani, R; Starr, TB; ME, A.](#) (2001). Mode-of-action-based dosimeters for interspecies extrapolation of vinyl acetate inhalation risk [Review]. *Inhal Toxicol* 13: 377-396. <http://dx.doi.org/10.1080/08958370119592>.
- [Bogdanffy, MS; Randall, HW; Morgan, KT.](#) (1986). Histochemical localization of aldehyde dehydrogenase in the respiratory tract of the Fischer-344 rat. *Toxicol Appl Pharmacol* 82: 560-567.
[http://dx.doi.org/10.1016/0041-008X\(86\)90291-7](http://dx.doi.org/10.1016/0041-008X(86)90291-7).
- [Bogdanffy, MS; Sarangapani, R; Plowchalk, DR; Jarabek, AM; Andersen, ME.](#) (1999). A biologically based risk assessment for vinyl acetate-induced cancer and noncancer inhalation toxicity. *Toxicol Sci* 51: 19-35. <http://dx.doi.org/10.1093/toxsci/51.1.19>.
- [Boja, JW; Nielsen, JA; Foldvary, E; Truitt, EB, Jr.](#) (1985). Acute low-level formaldehyde behavioural and neurochemical toxicity in the rat. *Prog Neuropsychopharmacol Biol Psychiatry* 9: 671-674.
[http://dx.doi.org/10.1016/0278-5846\(85\)90038-7](http://dx.doi.org/10.1016/0278-5846(85)90038-7).

- [Bokina, AI; Eksler, ND; Semenenko, AD; Merkur'yeva, RV.](#) (1976). Investigation of the mechanism of action of atmospheric pollutants on the central nervous system and comparative evaluation of methods of study. *Environ Health Perspect* 13: 37-42. <http://dx.doi.org/10.2307/3428235>.
- [Bolm-Audorff, U; Vogel, C; Woitowitz, H.](#) (1990). Occupation and smoking as risk factors of nasal and nasopharyngeal cancer. In RR Monson (Ed.), *Occupational epidemiology* (2nd ed., pp. 71-74). Boca Raton, FL: CRC Press.
- [Bonassi, S; El-Zein, R; Bolognesi, C; Fenech, M.](#) (2011). Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies [Review]. *Mutagenesis* 26: 93-100. <http://dx.doi.org/10.1093/mutage/geq075>.
- [Bonassi, S; Lando, C; Ceppi, M; Landi, S; Rossi, AM; Barale, R.](#) (2004a). No association between increased levels of high-frequency sister chromatid exchange cells (HFCs) and the risk of cancer in healthy individuals. *Environ Mol Mutagen* 43: 134-136. <http://dx.doi.org/10.1002/em.20006>.
- [Bonassi, S; Norppa, H; Ceppi, M; Stromberg, U; Vermeulen, R; Znaor, A; Cebulski-Wasilewska, A; Fabianova, E; Fucic, A; Gundy, S; Hansteen, IL; Knudsen, LE; Lazutka, J; Rossner, P; Sram, RJ; Boffetta, P.](#) (2008). Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries. *Carcinogenesis* 29: 1178-1183. <http://dx.doi.org/10.1093/carcin/bgn075>.
- [Bonassi, S; Znaor, A; Ceppi, M; Lando, C; Chang, WP; Holland, N; Kirsch-Volders, M; Zeiger, E; Ban, S; Barale, R; Bigatti, MP; Bolognesi, C; Cebulski-Wasilewska, A; Fabianova, E; Fucic, A; Hagmar, L; Joksic, G; Martelli, A; Migliore, L; Mirkova, E; Scarfi, MR; Zijno, A; Norppa, H; Fenech, M.](#) (2007). An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis* 28: 625-631. <http://dx.doi.org/10.1093/carcin/bgl177>.
- [Bonassi, S; Znaor, A; Norppa, H; Hagmar, L.](#) (2004b). Chromosomal aberrations and risk of cancer in humans: an epidemiologic perspective [Review]. *Cytogenet Genome Res* 104: 376-382. <http://dx.doi.org/10.1159/000077519>.
- [Boncler, M; Lukasiak, M; Dastych, J; Golanski, J; Watala, C.](#) (2019). Differentiated mitochondrial function in mouse 3T3 fibroblasts and human epithelial or endothelial cells in response to chemical exposure. *Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online* 124: 199-210. <http://dx.doi.org/10.1111/bcpt.13117>.
- [Bond, GG; Flores, GH; Shellenberger, RJ; Cartmill, JB; Fishbeck, WA; Cook, RR.](#) (1986). Nested case-control study of lung cancer among chemical workers. *Am J Epidemiol* 124: 53-66.
- [Bono, R; Munnia, A; Romanazzi, V; Bellisario, V; Cellai, F; Peluso, MEM.](#) (2016). Formaldehyde-induced toxicity in the nasal epithelia of workers of a plastic laminate plant. *Toxicology Research* 5: 752-760. <http://dx.doi.org/10.1039/c5tx00478k>.
- [Bono, R; Romanazzi, V; Munnia, A; Piro, S; Allione, A; Ricceri, F; Guarrera, S; Pignata, C; Matullo, G; Wang, P; Giese, RW; Peluso, M.](#) (2010). Malondialdehyde-deoxyguanosine adduct formation in workers of pathology wards: the role of air formaldehyde exposure. *Chem Res Toxicol* 23: 1342-1348. <http://dx.doi.org/10.1021/tx100083x>.
- [Bono, R; Romanazzi, V; Pirro, V; Degan, R; Pignata, C; Suppo, E; Pazzi, M; Vincenti, M.](#) (2012). Formaldehyde and tobacco smoke as alkylating agents: The formation of N-methylenvaline in pathologists and in plastic laminate workers. *Sci Total Environ* 414: 701-707. <http://dx.doi.org/10.1016/j.scitotenv.2011.10.047>.
- [Bono, R; Vincenti, M; Schiliro, T; Scursatone, E; Pignata, C; Gilli, G.](#) (2006). N-Methylenvaline in a group of subjects occupationally exposed to formaldehyde. *Toxicol Lett* 161: 10-17. <http://dx.doi.org/10.1016/j.toxlet.2005.07.016>.
- [Boreiko, CJ; Ragan, DL.](#) (1983). Formaldehyde effects in the C3H/10T½ cell transformation assay. In JE Gibson (Ed.), *Formaldehyde toxicity* (pp. 63-71). Washington, DC: Hemisphere Publishing Corporation.

- [Bos, PMJ; Busschers, M; Arts, JHE.](#) (2002). Evaluation of the sensory irritation test (Alarie test) for the assessment of respiratory tract irritation. *J Occup Environ Med* 44: 968-976.
<http://dx.doi.org/10.1097/01.jom.0000034342.94005.cc>.
- [Bosworth, D; Crofton-Sleigh, C; Venitt, S.](#) (1987). A forward mutation assay using ampicillin-resistance in *Escherichia coli* designed for investigating the mutagenicity of biological samples. *Mutagenesis* 2: 455-467.
- [Bouraoui, S; Mougou, S; Brahem, A; Tabka, F; Ben Khelifa, H; Harrabi, I; Mrizek, N; Elghezal, H; Saad, A.](#) (2013). A combination of micronucleus assay and fluorescence in situ hybridization analysis to evaluate the genotoxicity of formaldehyde. *Arch Environ Contam Toxicol* 64: 337-344.
<http://dx.doi.org/10.1007/s00244-012-9828-6>.
- [Boysen, M; Solberg, LA.](#) (1982). Changes in the nasal mucosa of furniture workers. A pilot study. *Scand J Work Environ Health* 8: 273-282. <http://dx.doi.org/10.5271/sjweh.2466>.
- [Boysen, M; Zadig, E; Digernes, V; Abeler, V; Reith, A.](#) (1990). Nasal mucosa in workers exposed to formaldehyde: a pilot study. *Occup Environ Med* 47: 116-121.
<http://dx.doi.org/10.1136/oem.47.2.116>.
- [Bracken, MJ; Leasa, DJ; Morgan, WKC.](#) (1985). Exposure to formaldehyde: Relationship to respiratory symptoms and function. *Can J Public Health* 76: 312-316.
- [Branco, P; Alvim-Ferraz, M; Martins, FG; Sousa, S.](#) (2019). Quantifying indoor air quality determinants in urban and rural nursery and primary schools. *Environ Res* 176: 108534.
<http://dx.doi.org/10.1016/j.envres.2019.108534>.
- [Branco, PT; Nunes, RA; Alvim-Ferraz, MC; Martins, FG; Sousa, SI.](#) (2015). Children's exposure to indoor air in urban nurseries - Part II: Gaseous pollutants' assessment. *Environ Res* 142: 662-670.
<http://dx.doi.org/10.1016/j.envres.2015.08.026>.
- [Branco, PTB; Alvim-Ferraz, MCM; Martins, FG; Ferraz, C; Vaz, LG; Sousa, SIV.](#) (2020a). Impact of indoor air pollution in nursery and primary schools on childhood asthma. *Sci Total Environ* 745: 140982.
<http://dx.doi.org/10.1016/j.scitotenv.2020.140982>.
- [Branco, PTB; Alvim-Ferraz, MCM; Martins, FG; Ferraz, C; Vaz, LG; Sousa, SIV.](#) (2020b). Impact of indoor air pollution in nursery and primary schools on childhood asthma: Supplemental Data [Supplemental Data]. *Sci Total Environ* 745.
- [Brand, G; Jacquot, L.](#) (2002). Sensitization and desensitization to allyl isothiocyanate (mustard oil) in the nasal cavity. *Chem Senses* 27: 593-598.
- [Breysse, PA.](#) (1984). Formaldehyde levels and accompanying symptoms associated with individuals residing in over 1000 conventional and mobile homes in the state of Washington. In B Berglund; T Lindvall; J Sundell (Eds.), *Indoor air: Proceedings of the 3rd International Conference on Indoor Air Quality and Climate Volume 3: Sensory and hyperreactivity reactions to sick buildings* (pp. 403-408). Stockholm, Sweden: Swedish Council for Building Research.
<https://search.proquest.com/docview/14197938?accountid=171501>.
- [Brinton, LA; Blot, WJ; Becker, JA; Winn, DM; Browder, JP; Farmer, JC, Jr; Fraumeni, JF, Jr.](#) (1984). A case-control study of cancers of the nasal cavity and paranasal sinuses. *Am J Epidemiol* 119: 896-906.
<http://dx.doi.org/10.1093/oxfordjournals.aje.a113812>.
- [Brinton, LA; Blot, WJ; Fraumeni, JF, Jr.](#) (1985). Nasal cancer in the textile and clothing industries. *Occup Environ Med* 42: 469-474.
- [Broder, I; Corey, P; Brasher, P; Lipa, M; Cole, P.](#) (1988a). Comparison of health of occupants and characteristics of houses among control homes and homes insulated with urea formaldehyde foam: III. Health and house variables following remedial work. *Environ Res* 45: 179-203.
[http://dx.doi.org/10.1016/S0013-9351\(88\)80046-X](http://dx.doi.org/10.1016/S0013-9351(88)80046-X).
- [Broder, I; Corey, P; Cole, P; Lipa, M; Mintz, S; Nethercott, JR.](#) (1988b). Comparison of health of occupants and characteristics of houses among control homes and homes insulated with urea

- formaldehyde foam: I Methodology. *Environ Res* 45: 141-155. [http://dx.doi.org/10.1016/S0013-9351\(88\)80044-6](http://dx.doi.org/10.1016/S0013-9351(88)80044-6).
- [Broder, I; Corey, P; Cole, P; Lipa, M; Mintz, S; Nethercott, JR.](#) (1988c). Comparison of health of occupants and characteristics of houses among control homes and homes insulated with urea formaldehyde foam: II initial health and house variables and exposure-response relationships. *Environ Res* 45: 156-178. [http://dx.doi.org/10.1016/S0013-9351\(88\)80045-8](http://dx.doi.org/10.1016/S0013-9351(88)80045-8).
- [Brondeau, MT; Bonnet, P; Guenier, JP; Simon, P; de Ceurritz, J.](#) (1990). Adrenal-dependent leucopenia after short-term exposure to various airborne irritants in rats. *J Appl Toxicol* 10: 83-86. <http://dx.doi.org/10.1002/jat.2550100204>.
- [Bruno, E; Somma, G; Russo, C; Porozaj, D; Pietroiusti, A; Alessandrini, M; Magrini, A.](#) (2018). Nasal cytology as a screening tool in formaldehyde-exposed workers. *Occup Med (Lond)* 68: 307-313. <http://dx.doi.org/10.1093/occmed/kqy052>.
- [Brusick, DJ.](#) (1983). Genetic and transforming activity of formaldehyde. In JE Gibson (Ed.), *Formaldehyde toxicity* (pp. 72-84). Washington, DC: Hemisphere Publishing.
- [Buckley, LA; Jiang, XZ; James, RA; Morgan, KT; Barrow, CS.](#) (1984). Respiratory tract lesions induced by sensory irritants at the RD50 concentration. *Toxicol Appl Pharmacol* 74: 417-429. [http://dx.doi.org/10.1016/0041-008X\(84\)90295-3](http://dx.doi.org/10.1016/0041-008X(84)90295-3).
- [Buckton, KE; Evans, HJ.](#) (1973). *Methods for the Analysis of Human Chromosome Aberrations*. Geneva: WHO.
- [Bull, RJ.](#) (2000). Mode of action of liver tumor induction by trichloroethylene and its metabolites, trichloroacetate and dichloroacetate [Review]. *Environ Health Perspect* 108: 241-259. <http://dx.doi.org/10.1289/ehp.00108s2241>.
- [Burgaz, S; Cakmak, G; Erdem, O; Yilmaz, M; Karakaya, AE.](#) (2001). Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. *Neoplasma* 48: 144-147.
- [Burgaz, S; Erdem, O; Cakmak, G; Erdem, N; Karakaya, A; Karakaya, AE.](#) (2002). Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to n-hexane, toluene, methyl ethyl ketone and formaldehyde. *Biomarkers* 7: 151-161. <http://dx.doi.org/10.1080/13547500110113242>.
- [Burgos-Barragan, G; Wit, N; Meiser, J; Dingler, FA; Pietzke, M; Mulderrig, L; Pontel, LB; Rosado, IV; Brewer, TF; Cordell, RL; Monks, PS; Chang, CJ; Vazquez, A; Patel, KJ.](#) (2017). Mammals divert endogenous genotoxic formaldehyde into one-carbon metabolism. *Nature* 548: 549-554. <http://dx.doi.org/10.1038/nature23481>.
- [Burns-Naas, LA; Hastings, KL; Ladics, GS; Makris, SL; Parker, GA; Holsapple, MP.](#) (2008). What's so special about the developing immune system? [Review]. *Int J Toxicol* 27: 223-254. <http://dx.doi.org/10.1080/10915810801978110>.
- [Callas, PW; Pastides, H; Hosmer, DW.](#) (1998). Empirical comparisons of proportional hazards, poisson, and logistic regression modeling of occupational cohort data. *Am J Ind Med* 33: 33-47. [http://dx.doi.org/10.1002/\(sici\)1097-0274\(199801\)33:1<33::aid-ajim5>3.0.co;2-x](http://dx.doi.org/10.1002/(sici)1097-0274(199801)33:1<33::aid-ajim5>3.0.co;2-x).
- [Campbell Jr, JL; Gentry, PR; Clewell III, HJ; Andersen, ME.](#) (2020). A kinetic analysis of DNA-deoxy guanine adducts in the nasal epithelium produced by inhaled formaldehyde in rats-assessing contributions to adduct production from both endogenous and exogenous sources of formaldehyde. *Toxicol Sci* 177: 325-333. <http://dx.doi.org/10.1093/toxsci/kfaa122>.
- [Cao, FH; Cai, J; Liu, ZM; Li, H; You, HH; Mei, YF; Yang, X; Ding, SM.](#) (2015). [Toxic effect of formaldehyde on mouse different brain regions]. *Sheng Li Xue Bao* 67: 497-504.
- [Cap, P; Dryahina, K; Pehal, F; Spanel, P.](#) (2008). Selected ion flow tube mass spectrometry of exhaled breath condensate headspace. *Rapid Commun Mass Spectrom* 22: 2844-2850. <http://dx.doi.org/10.1002/rcm.3685>.

- [Caria, H; Chaveca, T; Laires, A; Rueff, J.](#) (1995). Genotoxicity of quercetin in the micronucleus assay in mouse bone marrow erythrocytes, human lymphocytes, V79 cell line and identification of kinetochore-containing (CREST staining) micronuclei in human lymphocytes. *Mutat Res* 343: 85-94.
- [Carr, MJ; Undem, BJ.](#) (2001). Inflammation-induced plasticity of the afferent innervation of the airways. *Environ Health Perspect* 4: 567-571.
- [Casanova-Schmitz, M; David, RM; Heck, H.](#) (1984a). Oxidation of formaldehyde and acetaldehyde by NAD⁺-dependent dehydrogenases in rat nasal mucosal homogenates. *Biochem Pharmacol* 33: 1137-1142. [http://dx.doi.org/10.1016/0006-2952\(84\)90526-4](http://dx.doi.org/10.1016/0006-2952(84)90526-4).
- [Casanova-Schmitz, M; Heck, H.](#) (1983). Effects of formaldehyde exposure on the extractability of DNA from proteins in the rat nasal mucosa. *Toxicol Appl Pharmacol* 70: 121-132. [http://dx.doi.org/10.1016/0041-008x\(83\)90185-0](http://dx.doi.org/10.1016/0041-008x(83)90185-0).
- [Casanova-Schmitz, M; Starr, TB; Heck, HD.](#) (1984b). Differentiation between metabolic incorporation and covalent binding in the labeling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [14C]- and [3H]formaldehyde. *Toxicol Appl Pharmacol* 76: 26-44. [http://dx.doi.org/10.1016/0041-008x\(84\)90026-7](http://dx.doi.org/10.1016/0041-008x(84)90026-7).
- [Casanova, M; Bell, DA; Heck, H.](#) (1997). Dichloromethane metabolism to formaldehyde and reaction of formaldehyde with nucleic acids in hepatocytes of rodents and humans with and without glutathione S-transferase T1 and M1 genes. *Fundam Appl Toxicol* 37: 168-180. <http://dx.doi.org/10.1093/toxsci/37.2.168>.
- [Casanova, M; Deyo, DF; Heck, H.](#) (1989). Covalent binding of inhaled formaldehyde to DNA in the nasal mucosa of Fischer 344 rats: Analysis of formaldehyde and DNA by high-performance liquid chromatography and provisional pharmacokinetic interpretation. *Fundam Appl Toxicol* 12: 397-417. [http://dx.doi.org/10.1016/0272-0590\(89\)90015-8](http://dx.doi.org/10.1016/0272-0590(89)90015-8).
- [Casanova, M; Heck, H.](#) (1987). Further studies of the metabolic incorporation and covalent binding of inhaled [3H]- and [14C]formaldehyde in Fischer-344 rats: Effects of glutathione depletion. *Toxicol Appl Pharmacol* 89: 105-121. [http://dx.doi.org/10.1016/0041-008X\(87\)90181-5](http://dx.doi.org/10.1016/0041-008X(87)90181-5).
- [Casanova, M; Heck, H.](#) (1997). Lack of evidence for the involvement of formaldehyde in the hepatocarcinogenicity of methyl tertiary-butyl ether in CD-1 mice. *Chem Biol Interact* 105: 131-143.
- [Casanova, M; Heck, H; Everitt, JI; Harrington, WW, Jr; Popp, JA.](#) (1988). Formaldehyde concentrations in the blood of rhesus monkeys after inhalation exposure. *Food Chem Toxicol* 26: 715-716. [http://dx.doi.org/10.1016/0278-6915\(88\)90071-3](http://dx.doi.org/10.1016/0278-6915(88)90071-3).
- [Casanova, M; Morgan, KT; Gross, EA; Moss, OR; Heck, H.](#) (1994). DNA-protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde. *Fundam Appl Toxicol* 23: 525-536. <http://dx.doi.org/10.1006/faat.1994.1137>.
- [Casanova, M; Morgan, KT; Steinhagen, WH; Everitt, JI; Popp, JA; Heck, H.](#) (1991). Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. *Toxicol Sci* 17: 409-428. [http://dx.doi.org/10.1016/0272-0590\(91\)90230-2](http://dx.doi.org/10.1016/0272-0590(91)90230-2).
- [Casanovaschmitz, M; Starr, TB; Heck, HD.](#) (1984). DIFFERENTIATION BETWEEN METABOLIC INCORPORATION AND COVALENT BINDING IN THE LABELING OF MACROMOLECULES IN THE RAT NASAL-MUCOSA AND BONE-MARROW BY INHALED [C-14] FORMALDEHYDE AND [H-3] FORMALDEHYDE. *Toxicol Appl Pharmacol* 76: 26-44. [http://dx.doi.org/10.1016/0041-008X\(84\)90026-7](http://dx.doi.org/10.1016/0041-008X(84)90026-7).
- [Cassee, FR; Arts, JHE; Groten, JP; Feron, VJ.](#) (1996a). Sensory irritation to mixtures of formaldehyde, acrolein, and acetaldehyde in rats. *Arch Toxicol* 70: 329-337. <http://dx.doi.org/10.1007/s002040050282>.

Supplemental Information—Formaldehyde (Inhalation)

- [Cassee, FR; Feron, VJ.](#) (1994). Biochemical and histopathological changes in nasal epithelium of rats after 3-day intermittent exposure to formaldehyde and ozone alone or in combination. *Toxicol Lett* 72: 257-268. [http://dx.doi.org/10.1016/0378-4274\(94\)90037-x](http://dx.doi.org/10.1016/0378-4274(94)90037-x).
- [Cassee, FR; Groten, JP; Feron, VJ.](#) (1996b). Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. *Toxicol Sci* 29: 208-218. <http://dx.doi.org/10.1006/faat.1996.0024>.
- [Casset, A; Marchand, C; Purohit, A; le Calve, S; Uring-Lambert, B; Donnay, C; Meyer, P; de Blay, F.](#) (2006). Inhaled formaldehyde exposure: effect on bronchial response to mite allergen in sensitized asthma patients. *Allergy* 61: 1344-1350. <http://dx.doi.org/10.1111/j.1398-9995.2006.01174.x>.
- [Casset, A; Purohit, A; Marchand, C; Le Calve, S; Pauli, G; de Blay, F.](#) (2007). Inhaled formaldehyde and the bronchial response. *Rev Fr Allergol Immunol Clin* 47: 80-83. <http://dx.doi.org/10.1016/j.allerg.2006.10.009>.
- [CDC](#) (Centers for Disease Control and Prevention). (2011). Formaldehyde exposures during Brazilian blowout hair smoothing treatment at a hair salon - Ohio. (HETA-2011-0014-3147). Atlanta, GA: Department of Health and Human Services, CDC. <http://dx.doi.org/10.26616/NIOSH-HETA-2011-00143147>.
- [Ceder, R; Merne, M; Staab, C; al., e.](#) (2007a). The application of normal, SV40 T-antigen-immortalized and tumour-derived oral keratinocytes, under serum-free conditions, to the study of the probability of cancer progression as a result of environmental exposure to chemicals. *Altern Lab Anim* 35: 621-639.
- [Ceder, R; Merne, M; Staab, CA; Nilsson, JA; Höög, JO; Dressler, D; Engelhart, K; Grafström, RC.](#) (2007b). The application of normal, SV40 T-antigen-immortalised and tumour-derived oral keratinocytes, under serum-free conditions, to the study of the probability of cancer progression as a result of environmental exposure to chemicals. *Altern Lab Anim* 35: 621-639. <http://dx.doi.org/10.1177/026119290703500603>.
- [Cederbaum, Al; Qureshi, A.](#) (1982). Role of catalase and hydroxyl radicals in the oxidation of methanol by rat liver microsomes. *Biochem Pharmacol* 31: 329-335. [http://dx.doi.org/10.1016/0006-2952\(82\)90179-4](http://dx.doi.org/10.1016/0006-2952(82)90179-4).
- [Chanet, R; Izard, C; Moustacchi, E.](#) (1975). Genetic effects of formaldehyde in yeast. I. Influence of the growth stages on killing and recombination. *Mutat Res* 33: 179-186. [http://dx.doi.org/10.1016/0027-5107\(75\)90193-1](http://dx.doi.org/10.1016/0027-5107(75)90193-1).
- [Chang, JCF; Barrow, CS.](#) (1984). Sensory irritation tolerance and cross-tolerance in F-344 rats exposed to chlorine or formaldehyde gas. *Toxicol Appl Pharmacol* 76: 319-327. [http://dx.doi.org/10.1016/0041-008X\(84\)90013-9](http://dx.doi.org/10.1016/0041-008X(84)90013-9).
- [Chang, JCF; Gross, EA; Swenberg, JA; Barrow, CS.](#) (1983). Nasal cavity deposition, histopathology, and cell proliferation after single or repeated formaldehyde exposures in B6C3F1 mice and F-344 rats. *Toxicol Appl Pharmacol* 68: 161-176. [http://dx.doi.org/10.1016/0041-008x\(83\)90001-7](http://dx.doi.org/10.1016/0041-008x(83)90001-7).
- [Chang, JCF; Steinhagen, WH; Barrow, CS.](#) (1981). Effect of single or repeated formaldehyde exposure on minute volume of B6C3F1 mice and F-344 rats. *Toxicol Appl Pharmacol* 61: 451-459. [http://dx.doi.org/10.1016/0041-008x\(81\)90368-9](http://dx.doi.org/10.1016/0041-008x(81)90368-9).
- [Chang, M; Park, H; Ha, M; Hong, YC; Lim, YH; Kim, Y; Kim, YJ; Lee, D; Ha, EH.](#) (2017). The effect of prenatal TVOC exposure on birth and infantile weight: the Mothers and Children's Environmental Health study. *Pediatr Res* 82: 423-428. <http://dx.doi.org/10.1038/pr.2017.55>.
- [Chaw, YF; Crane, LE; Lange, P; Shapiro, R.](#) (1980). Isolation and identification of cross-links from formaldehyde-treated nucleic acids. *Biochemistry* 19: 5525-5531.
- [Checkoway, H; Dell, LD; Boffetta, P; Gallagher, AE; Crawford, L; Lees, PS; Mundt, KA.](#) (2015). Formaldehyde Exposure and Mortality Risks From Acute Myeloid Leukemia and Other Lymphohematopoietic Malignancies in the US National Cancer Institute Cohort Study of

Supplemental Information—Formaldehyde (Inhalation)

- Workers in Formaldehyde Industries. *J Occup Environ Med* 57: 785-794.
<http://dx.doi.org/10.1097/JOM.0000000000000466>.
- [Checkoway, H; Ray, RM; Lundin, JI; Astrakianakis, G; Seixas, NS; Camp, JE; Wernli, KJ; Fitzgibbons, ED; Li, W; Feng, Z; Gao, DL; Thomas, DB.](#) (2011). Lung cancer and occupational exposures other than cotton dust and endotoxin among women textile workers in Shanghai, China. *Occup Environ Med* 68: 425-429. <http://dx.doi.org/10.1136/oem.2010.059519>.
- [Chen, D; Fang, L; Mei, S; Li, H; Xu, X; Des Marais, TL; Lu, K; Liu, XS; Jin, C.](#) (2017). Regulation of Chromatin Assembly and Cell Transformation by Formaldehyde Exposure in Human Cells. *Environ Health Perspect* 125: 097019. <http://dx.doi.org/10.1289/EHP1275>.
- [Cheng, G; Shi, Y; Sturla, SJ; Jalas, J. R.; McIntee, EJ; Villalta, PW; Wang, M; Hecht, SS.](#) (2003). Reactions of formaldehyde plus acetaldehyde with deoxyguanosine and DNA: formation of cyclic deoxyguanosine adducts and formaldehyde cross-links. *Chem Res Toxicol* 16: 145-152.
<http://dx.doi.org/10.1021/tx025614r>.
- [Cheng, G; Wang, M; Upadhyaya, P; Villalta, PW; Hecht, SS.](#) (2008). Formation of formaldehyde adducts in the reactions of DNA and deoxyribonucleosides with alpha-acetates of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and N-nitrosodimethylamine (NDMA). *Chem Res Toxicol* 21: 746-751.
<http://dx.doi.org/10.1021/tx7003823>.
- [Cheng, J; Zhang, L; Tang, Y; Li, Z.](#) (2016). The toxicity of continuous long-term low-dose formaldehyde inhalation in mice. *Immunopharmacol Immunotoxicol* 38: 495-501.
<http://dx.doi.org/10.1080/08923973.2016.1248844>.
- [Cheng, YJ; Hildesheim, A; Hsu, MM; Chen, IH; Brinton, LA; Levine, PH; Chen, CJ; Yang, CS.](#) (1999). Cigarette smoking, alcohol consumption and risk of nasopharyngeal carcinoma in Taiwan. *Cancer Causes Control* 10: 201-207. <http://dx.doi.org/10.1023/A:1008893109257>.
- [Cheng, YS; Hansen, GK; Su, YF; Yeh, HC; Morgan, KT.](#) (1990). Deposition of ultrafine aerosols in rat nasal molds. *Toxicol Appl Pharmacol* 106: 222-233.
- [Cheng, Z; Li, Y; Liang, B; Wang, C.](#) (2004). [Investigation of formaldehyde level and health of personnel in clinical pathology]. *29*: 266-267.
- [Cheung C, WS; Beech, IB; Campbell, SA; Satherley, J; Schiffrin, DJ.](#) (1994). The effect of industrial biocides on sulphate-reducing bacteria under high pressure. *Int Biodeterior Biodegradation* 33: 299-310.
- [Chia, SE; Ong, CN; Foo, SC; Lee, HP.](#) (1992). Medical students' exposure to formaldehyde in a gross anatomy dissection laboratory. *J Am Coll Health* 41: 115-119.
<http://dx.doi.org/10.1080/07448481.1992.9936310>.
- [Chiazze, L, Jr; Watkins, DK; Fryar, C.](#) (1997). Historical cohort mortality study of a continuous filament fiberglass manufacturing plant. I. White men. *J Occup Environ Med* 39: 432-441.
<http://dx.doi.org/10.1097/00043764-199705000-00009>.
- [Choi, DW; Moon, KW; Byeon, SH; Lee, EI; Sul, DG; Lee, JH; Oh, EH; Kim, YH.](#) (2009). Indoor volatile organic compounds in atopy patients' houses in South Korea. *Indoor Built Environ* 18: 144-154.
<http://dx.doi.org/10.1177/1420326X08101945>.
- [Chonglei, L; Fan, W; Wei, L; Yihe, J.](#) (2012). Effects of Exposure to VOCs on Spatial Learning and Memory Capacity and the Expression of NMDA Receptor in Mice. *Journal of Animal and Veterinary Advances* 11: 3355-3364.
- [Ciftci, G; Aksoy, A; Cenesiz, S; Sogut, MU; Yarim, GF; Nisbet, C; Guvenc, D; Ertekin, A.](#) (2015). Therapeutic role of curcumin in oxidative DNA damage caused by formaldehyde. *Microsc Res Tech* 78: 391-395. <http://dx.doi.org/10.1002/jemt.22485>.
- [CIIT](#) (Chemical Industry Institute of Toxicology). (1999). Formaldehyde: Hazard characterization and dose-response assessment for carcinogenicity by the route of inhalation (revised edition). Research Triangle Park, NC.

- [Clement, PA; Stoop, AP; Kaufman, L.](#) (1987). The influence of formaldehyde on the nasal mucosa. *Rhinology* 25: 29-34.
- [Coggon, D; Harris, EC; Poole, J; Palmer, KT.](#) (2003). Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. *J Natl Cancer Inst* 95: 1608-1615. <http://dx.doi.org/10.1093/jnci/djg046>.
- [Coggon, D; Ntani, G; Harris, EC; Palmer, KT.](#) (2014). Upper Airway Cancer, Myeloid Leukemia, and Other Cancers in a Cohort of British Chemical Workers Exposed to Formaldehyde. *Am J Epidemiol* 179: 1301-1311. <http://dx.doi.org/10.1093/aje/kwu049>.
- [Cohen Hubal, EA; Schlosser, PM; Conolly, RB; Kimbell, JS.](#) (1997). Comparison of inhaled formaldehyde dosimetry predictions with DNA-protein cross-link measurements in the rat nasal passages. *Toxicol Appl Pharmacol* 143: 47-55. <http://dx.doi.org/10.1006/taap.1996.8076>.
- [Cohn, L; Elias, JA; Chupp, GL.](#) (2004). Asthma: mechanisms of disease persistence and progression [Review]. *Annu Rev Immunol* 22: 789-815. <http://dx.doi.org/10.1146/annurev.immunol.22.012703.104716>.
- [Comba, P; Barbieri, PG; Battista, G; Belli, S; Ponterio, F; Zanetti, D; Axelson, O.](#) (1992a). Cancer of the nose and paranasal sinuses in the metal industry: a case-control study. *Occup Environ Med* 49: 193-196. <http://dx.doi.org/10.1136/oem.49.3.193>.
- [Comba, P; Battista, G; Belli, S; Decapua, B; Merler, E; Orsi, D; Rodella, S; Vidigni, C; Axelson, O.](#) (1992b). A case-control study of cancer of the nose and paranasal sinuses and occupational exposures. *Am J Ind Med* 22: 511-520. <http://dx.doi.org/10.1002/ajim.4700220406>.
- [Cometto-Muñiz, JE; Hernández, SM.](#) (1990). Odorous and pungent attributes of mixed and unmixed odorants. *Percept Psychophys* 47: 391-399. <http://dx.doi.org/10.3758/bf03210879>.
- [Conaway, CC; Whysner, J; Verna, LK; Williams, GM.](#) (1996). Formaldehyde mechanistic data and risk assessment: Endogenous protection from DNA adduct formation [Review]. *Pharmacol Ther* 71: 29-55. [http://dx.doi.org/10.1016/0163-7258\(96\)00061-7](http://dx.doi.org/10.1016/0163-7258(96)00061-7).
- [Connor, TH; Barrie, MD; Theiss, JC; Matney, TS; Ward, JB.](#) (1983). Mutagenicity of formalin in the Ames assay. *Mutat Res Lett* 119: 145-149. [http://dx.doi.org/10.1016/0165-7992\(83\)90122-7](http://dx.doi.org/10.1016/0165-7992(83)90122-7).
- [Connor, TH; Theiss, JC; Hanna, HA; Monteith, DK; Matney, TS.](#) (1985a). Genotoxicity of organic chemicals frequently found in the air of mobile homes. *Toxicol Lett* 25: 33-40. [http://dx.doi.org/10.1016/0378-4274\(85\)90097-9](http://dx.doi.org/10.1016/0378-4274(85)90097-9).
- [Connor, TH; Ward, JB; Legator, MS.](#) (1985b). Absence of mutagenicity in the urine of autopsy service workers exposed to formaldehyde: Factors influencing mutagenicity testing of urine. *Int Arch Occup Environ Health* 56: 225-237. <http://dx.doi.org/10.1007/BF00396600>.
- [Conolly, RB.](#) (2002). The use of biologically based modeling in risk assessment [Review]. *Toxicology* 181-182: 275-279. [http://dx.doi.org/10.1016/S0300-483X\(02\)00295-0](http://dx.doi.org/10.1016/S0300-483X(02)00295-0).
- [Conolly, RB; Kimbell, JS; Janszen, D; Schlosser, PM; Kalisak, D; Preston, J; Miller, FJ.](#) (2003). Biologically motivated computational modeling of formaldehyde carcinogenicity in the F344 rat. *Toxicol Sci* 75: 432-447. <http://dx.doi.org/10.1093/toxsci/kfg182>.
- [Conolly, RB; Kimbell, JS; Janszen, D; Schlosser, PM; Kalisak, D; Preston, J; Miller, FJ.](#) (2004). Human respiratory tract cancer risks of inhaled formaldehyde: dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. *Toxicol Sci* 82: 279-296. <http://dx.doi.org/10.1093/toxsci/kfh223>.
- [Conolly, RB; Lilly, PD; Kimbell, JS.](#) (2000). Simulation modelling of the tissue disposition of formaldehyde to predict nasal DNA-protein cross-links in Fischer 344 rats, rhesus monkeys, and humans. *Environ Health Perspect* 108: 919-924. <http://dx.doi.org/10.2307/3454325>.
- [Conolly, RB; Lutz, WK.](#) (2004). Nonmonotonic dose-response relationships: Mechanistic basis, kinetic modeling, and implications for risk assessment. *Toxicol Sci* 77: 151-157. <http://dx.doi.org/10.1093/toxsci/kfh007>.

- Conolly, RB; Miller, FJ; Kimbell, JS; Janszen, D. (2009). Letter to the editor: Formaldehyde risk assessment [Letter]. *Ann Occup Hyg* 53: 181-184. <http://dx.doi.org/10.1093/annhyg/men084>.
- Coombs, KC; Chew, GL; Schaffer, C; Ryan, PH; Brokamp, C; Grinshpun, SA; Adamkiewicz, G; Chillrud, S; Hedman, C; Colton, M; Ross, J; Reponen, T. (2016). Indoor air quality in green-renovated vs. non-green low-income homes of children living in a temperate region of US (Ohio). *Sci Total Environ* 554: 178-185. <http://dx.doi.org/10.1016/j.scitotenv.2016.02.136>.
- Coon, RA; Jones, RA; Jenkins, LJ, Jr; Siegel, J. (1970). Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. *Toxicol Appl Pharmacol* 16: 646-655. [http://dx.doi.org/10.1016/0041-008X\(70\)90069-4](http://dx.doi.org/10.1016/0041-008X(70)90069-4).
- Corley, RA; Kabilan, S; Kuprat, AP; Carson, JP; Jacob, RE; Minard, KR; Teeguarden, JG; Timchalk, C; Pipavath, S; Glenn, R; Einstein, DR. (2015). Comparative risks of aldehyde constituents in cigarette smoke using transient computational fluid dynamics/physiologically based pharmacokinetic models of the rat and human respiratory tracts. *Toxicol Sci* 146: 65-88. <http://dx.doi.org/10.1093/toxsci/kfv071>.
- Cosma, GN; Marchok, AC. (1988). Benzo[a]pyrene- and formaldehyde-induced DNA damage and repair in rat tracheal epithelial cells. *Toxicology* 51: 309-320. [http://dx.doi.org/10.1016/0300-483X\(88\)90159-X](http://dx.doi.org/10.1016/0300-483X(88)90159-X).
- Cosma, GN; Wilhite, AS; Marchok, AC. (1988). The detection of DNA-protein cross-links in rat tracheal implants exposed in vivo to benzo[a]pyrene and formaldehyde. *Cancer Lett* 42: 13-21. [http://dx.doi.org/10.1016/0304-3835\(88\)90233-9](http://dx.doi.org/10.1016/0304-3835(88)90233-9).
- Costa, M; Zhitkovich, A; Harris, M; Paustenbach, D; Gargas, M. (1997). DNA-protein cross-links produced by various chemicals in cultured human lymphoma cells. *J Toxicol Environ Health* 50: 433-449. <http://dx.doi.org/10.1080/00984109708984000>.
- Costa, S; Carvalho, S; Costa, C; Coelho, P; Silva, S; Santos, LS; Gaspar, JF; Porto, B; Laffon, B; Teixeira, JP. (2015). Increased levels of chromosomal aberrations and DNA damage in a group of workers exposed to formaldehyde. *Mutagenesis* 30: 463-473. <http://dx.doi.org/10.1093/mutage/gev002>.
- Costa, S; Coelho, P; Costa, C; Silva, S; Mayan, O; Santos, LS; Gaspar, J; Teixeira, JP. (2008). Genotoxic damage in pathology anatomy laboratory workers exposed to formaldehyde. *Toxicology* 252: 40-48. <http://dx.doi.org/10.1016/j.tox.2008.07.056>.
- Costa, S; Costa, C; Madureira, J; Valdiglesias, V; Teixeira-Gomes, A; Guedes de Pinho, P; Laffon, B; Teixeira, JP. (2019). Occupational exposure to formaldehyde and early biomarkers of cancer risk, immunotoxicity and susceptibility. *Environ Res* 179: 108740. <http://dx.doi.org/10.1016/j.envres.2019.108740>.
- Costa, S; García-Lestón, J; Coelho, M; Coelho, P; Costa, C; Silva, S; Porto, B; Laffon, B; Teixeira, JP. (2013). Cytogenetic and immunological effects associated with occupational formaldehyde exposure. *J Toxicol Environ Health A* 76: 217-229. <http://dx.doi.org/10.1080/15287394.2013.757212>.
- Costa, S; Pina, C; Coelho, P; Costa, C; Silva, S; Porto, B; Laffon, B; Teixeira, JP. (2011). Occupational exposure to formaldehyde: genotoxic risk evaluation by comet assay and micronucleus test using human peripheral lymphocytes. *J Toxicol Environ Health A* 74: 1040-1051. <http://dx.doi.org/10.1080/15287394.2011.582293>.
- Coste, A; Rateau, JG; Roudot-Thoraval, F; Chapelin, C; Gilain, L; Poron, F; Peynegre, R; Bernaudin, JF; Escudier, E. (1996). Increased epithelial cell proliferation in nasal polyps. *Arch Otolaryngol Head Neck Surg* 122: 432-436. <http://dx.doi.org/10.1001/archotol.1996.01890160072013>.
- Cox, DR; Hinkley, DV. (1974). *Theoretical statistics*. London, UK: Chapman and Hall.
- Cox, DR; Oakes, D. (1984). *Analysis of survival data*. London, UK: Chapman and Hall.
- Craft, TR; Bermudez, E; Skopek, TR. (1987). Formaldehyde mutagenesis and formation of DNA-protein crosslinks in human lymphoblasts in vitro. *Mutat Res* 176: 147-155. [http://dx.doi.org/10.1016/0027-5107\(87\)90262-4](http://dx.doi.org/10.1016/0027-5107(87)90262-4).

- Crosby, RM; Richardson, KK; Craft, TR; Benforado, KB; Liber, HL; Skopek, TR. (1988). Molecular analysis of formaldehyde-induced mutations in human lymphoblasts and *E. coli*. *Environ Mol Mutagen* 12: 155-166. <http://dx.doi.org/10.1002/em.2860120202>.
- Crouch, EA. (1989). TOX-RISK: a program for fitting dose-response formulae and extrapolating between species. *Risk Anal* 9: 599-603. <http://dx.doi.org/10.1111/j.1539-6924.1989.tb01270.x>.
- Crump, K. (2002). Benchmark analysis. In A El-Shaarawi; WW Piegorsch (Eds.), *Encyclopedia of environmetrics* (pp. 163-170). West Sussex, UK: John Wiley & Sons Ltd. <http://dx.doi.org/10.1002/9780470057339.vab009>.
- Crump, KS. (1994a). Limitations of biological models of carcinogenesis for low-dose extrapolation. *Risk Anal* 14: 883-886. <http://dx.doi.org/10.1111/j.1539-6924.1994.tb00050.x>.
- Crump, KS. (1994b). Use of mechanistic models to estimate low-dose cancer risks. *Risk Anal* 14: 1033-1038.
- Crump, KS; Bussard, DA; Chen, C; Jinot, J; Subramaniam, R. (2014). The bottom-up approach does not necessarily bound low-dose risk [Letter]. *Regul Toxicol Pharmacol* 70: 735-736. <http://dx.doi.org/10.1016/j.yrtph.2014.10.008>.
- Crump, KS; Chen, C; Chiu, WA; Louis, TA; Portier, CJ; Subramaniam, RP; White, PD. (2010). What role for biologically based dose-response models in estimating low-dose risk? [Review]. *Environ Health Perspect* 118: 585-588. <http://dx.doi.org/10.1289/ehp.0901249>.
- Crump, KS; Chen, C; Fox, JF; Subramaniam, R. (2009). Reply to Conolly et al [Letter]. *Ann Occup Hyg* 53: 184-189. <http://dx.doi.org/10.1093/annhyg/men088>.
- Crump, KS; Chen, C; Fox, JF; Van Landingham, C; Subramaniam, R. (2008). Sensitivity analysis of biologically motivated model for formaldehyde-induced respiratory cancer in humans. *Ann Occup Hyg* 52: 481-495. <http://dx.doi.org/10.1093/annhyg/men038>.
- Crump, KS; Hoel, DG; Langley, CH; Peto, R. (1976). Fundamental carcinogenic processes and their implications for low dose risk assessment. *Cancer Res* 36: 2973-2979.
- Crump, KS; Subramaniam, RP; Van Landingham, CB. (2005). A numerical solution to the nonhomogeneous two-stage MVK model of cancer. *Risk Anal* 25: 921-926. <http://dx.doi.org/10.1111/j.1539-6924.2005.00651.x>.
- Cui, Y; Li, H; Wu, S; Zhao, R; Du, D; Ding, Y; Nie, H; Ji, HL. (2016). Formaldehyde impairs transepithelial sodium transport. *Sci Rep* 6: 35857. <http://dx.doi.org/10.1038/srep35857>.
- D'Errico, A; Pasian, S; Baratti, A; Zanelli, R; Alfonzo, S; Gilardi, L; Beatrice, F; Bena, A; Costa, G. (2009). A case-control study on occupational risk factors for sino-nasal cancer. *Occup Environ Med* 66: 448-455. <http://dx.doi.org/10.1136/oem.2008.041277>.
- da Silva, CM; Leal, MP; Brochetti, RA; Braga, T; Vitoretto, LB; Saraiva Camara, NO; Damazo, AS; Ligeiro-De-Oliveira, A; Chavantes, MC; Lino-Dos-Santos-Franco, A. (2015). Low Level Laser Therapy Reduces the Development of Lung Inflammation Induced by Formaldehyde Exposure. *PLoS ONE* 10: e0142816. <http://dx.doi.org/10.1371/journal.pone.0142816>.
- Dalbey, WE. (1982). Formaldehyde and tumors in hamster respiratory tract. *Toxicology* 24: 9-14. [http://dx.doi.org/10.1016/0300-483X\(82\)90058-0](http://dx.doi.org/10.1016/0300-483X(82)90058-0).
- Dallas, CE; Badeaux, P; Theiss, JC; Fairchild, EJ. (1989). The influence of inhaled formaldehyde on rat lung cytochrome P450. *Environ Res* 49: 50-59. [http://dx.doi.org/10.1016/S0013-9351\(89\)80021-0](http://dx.doi.org/10.1016/S0013-9351(89)80021-0).
- Dallas, CE; Mellard, DN; Theiss, JC; Pentecost, AR; Fairchild EJ, II. (1987). Distribution of DNA and RNA content in the bone marrow and alveolar macrophages of rats after subchronic inhalation of formaldehyde. *Environ Res* 43: 191-202.
- Dallas, CE; Scott, MJ; Ward, JB, Jr; Theiss, JC. (1992). Cytogenetic analysis of pulmonary lavage and bone marrow cells of rats after repeated formaldehyde inhalation. *J Appl Toxicol* 12: 199-203. <http://dx.doi.org/10.1002/jat.2550120309>.

Supplemental Information—Formaldehyde (Inhalation)

- [Dally, KA; Hanrahan, LP; Woodbury, MA; Kanarek, MS.](#) (1981). Formaldehyde exposure in nonoccupational environments. *Arch Environ Occup Health* 36: 277-284. <http://dx.doi.org/10.1080/00039896.1981.10667638>.
- [Dannemiller, KC; Murphy, JS; Dixon, SL; Pennell, KG; Suuberg, EM; Jacobs, DE; Sandel, M.](#) (2013). Formaldehyde concentrations in household air of asthma patients determined using colorimetric detector tubes. *Indoor Air* 23: 285-294. <http://dx.doi.org/10.1111/ina.12024>.
- [Dawicki, W; Marshall, JS.](#) (2007). New and emerging roles for mast cells in host defence [Review]. *Curr Opin Immunol* 19: 31-38. <http://dx.doi.org/10.1016/j.coi.2006.11.006>.
- [Day, JH; Lees, RE; Clark, RH; Pattee, PL.](#) (1984). Respiratory response to formaldehyde and off-gas of urea formaldehyde foam insulation. *Can Med Assoc J* 131: 1061-1065.
- [de Ceaurreiz, J; Micillino, JC; Bonnet, P; Guenier, JP.](#) (1981). [Prediction of the irritant effects of chemicals on the human respiratory tract: Advantages of an animal model]. *Cah Notes Doc* 102: 55-61.
- [De Flora, S.](#) (1981). Study of 106 organic and inorganic compounds in the Salmonella/microsome test. *Carcinogenesis* 2: 283-298. <http://dx.doi.org/10.1093/carcin/2.4.283>.
- [De Flora, S; Zancacchi, P; Camoirano, A; Bennicelli, C; Badolati, GS.](#) (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test [Review]. *Mutat Res* 133: 161-198. [http://dx.doi.org/10.1016/0165-1110\(84\)90016-2](http://dx.doi.org/10.1016/0165-1110(84)90016-2).
- [De Jong, WH; Arts, JHE; De Klerk, A; Schijf, MA; Ezendam, J; Kuper, CF; Van Loveren, H.](#) (2009). Contact and respiratory sensitizers can be identified by cytokine profiles following inhalation exposure. *Toxicology* 261: 103-111. <http://dx.doi.org/10.1016/j.tox.2009.04.057>.
- [de Serres, FJ; Brockman, HE.](#) (1999). Comparison of the spectra of genetic damage in formaldehyde-induced ad-3 mutations between DNA repair-proficient and -deficient heterokaryons of *Neurospora crassa*. *Mutat Res* 437: 151-163. [http://dx.doi.org/10.1016/S1383-5742\(99\)00081-2](http://dx.doi.org/10.1016/S1383-5742(99)00081-2).
- [de Serres, FJ; Brockman, HE; Hung, CY.](#) (1988). Effect of the homokaryotic state of the uvs-2 allele in *Neurospora crassa* on formaldehyde-induced killing and ad-3 mutation. *Mutat Res* 199: 235-242. [http://dx.doi.org/10.1016/0027-5107\(88\)90251-5](http://dx.doi.org/10.1016/0027-5107(88)90251-5).
- [De Stefani, E; Boffetta, P; Brennan, P; Deneo-Pellegrini, H; Ronco, A; Gutiérrez, LP.](#) (2005). Occupational exposures and risk of adenocarcinoma of the lung in Uruguay. *Cancer Causes Control* 16: 851-856. <http://dx.doi.org/10.1007/s10552-005-2819-4>.
- [de Vries, A; van Rijnsoever, C; Engels, F; Henricks, PA; Nijkamp, FP.](#) (2001). The role of sensory nerve endings in nerve growth factor-induced airway hyperresponsiveness to histamine in guinea-pigs. *Br J Pharmacol* 134: 771-776. <http://dx.doi.org/10.1038/sj.bjp.0704310>.
- [Dean, JH; Lauer, LD; House, RV; Murray, MJ; Stillman, WS; Irons, RD; Steinhagen, WH; Phelps, MC; Adams, DO.](#) (1984). Studies of immune function and host resistance in B6C3F1 mice exposed to formaldehyde. *Toxicol Appl Pharmacol* 72: 519-529. [http://dx.doi.org/10.1016/0041-008X\(84\)90129-7](http://dx.doi.org/10.1016/0041-008X(84)90129-7).
- [Dell, L; Teta, MJ.](#) (1995). Mortality among workers at a plastics manufacturing and research and development facility: 1946-1988. *Am J Ind Med* 28: 373-384. <http://dx.doi.org/10.1002/ajim.4700280307>.
- [Demkowicz-Dobrzanski, K; Castonguay, A.](#) (1992). Modulation by glutathione of DNA strand breaks induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and its aldehyde metabolites in rat hepatocytes. *Carcinogenesis* 13: 1447-1454.
- [Deng, J; Zhang, X; Liu, J; Wei, X; Liu, X; Ma, L; Zhao, Y; Li, Z.](#) (2020). [Effects of gaseous formaldehyde fluctuating exposure on medical students' subjective symptoms and pulmonary function]. *Weisheng Yanjiu* 49: 921-926. <http://dx.doi.org/10.19813/j.cnki.weishengyanjiu.2020.06.008>.
- [Dhreshwar, SS; Stella, VJ.](#) (2008). Your prodrug releases formaldehyde: should you be concerned? No! *J Pharm Sci* 97: 4184-4193. <http://dx.doi.org/10.1002/jps.21319>.
- [DHGC](#) (DuPont Haskell Global Centers). (2010). [Two Acute/ Subacute Inhalation toxicity studies].

- [Dickey, FH; Cleland, GH; Lotz, C.](#) (1949). The role of organic peroxides in the induction of mutations. *Proc Natl Acad Sci USA* 35: 581-586.
- [Dietrich, CJ; Richards, IS; Bernard, TE; Hammad, YY.](#) (1996). Human stress protein response to formaldehyde exposure. *Exp Toxicol Pathol* 48: 518-519. [http://dx.doi.org/10.1016/S0940-2993\(96\)80071-6](http://dx.doi.org/10.1016/S0940-2993(96)80071-6).
- [Dillon, D; Combes, R; Zeiger, E.](#) (1998). The effectiveness of Salmonella strains TA100, TA102 and TA104 for detecting mutagenicity of some aldehydes and peroxides. *Mutagenesis* 13: 19-26. <http://dx.doi.org/10.1093/mutage/13.1.19>.
- [Dingler, FA; Wang, M; Mu, A; Millington, CL; Oberbeck, N; Watcham, S; Pontel, LB; Kamimae-Lanning, AN; Langevin, F; Nadler, C; Cordell, RL; Monks, PS; Yu, R; Wilson, NK; Hira, A; Yoshida, K; Mori, M; Okamoto, Y; Okuno, Y; Muramatsu, H; Shiraishi, Y; Kobayashi, M; Moriguchi, T; Osumi, T; Kato, M; Miyano, S; Ito, E; Kojima, S; Yabe, H; Yabe, M; Matsuo, K; Ogawa, S; Göttgens, B; Hodkinson, MRG; Takata, M; Patel, KJ.](#) (2020). Two aldehyde clearance systems are essential to prevent lethal formaldehyde accumulation in mice and humans. *Mol Cell* 80: 996-1012.e1019. <http://dx.doi.org/10.1016/j.molcel.2020.10.012>.
- [Dinsdale, D; Riley, RA; Verschoyle, RD.](#) (1993). Pulmonary cytochrome P450 in rats exposed to formaldehyde vapor. *Environ Res* 62: 19-27. <http://dx.doi.org/10.1006/enrs.1993.1085>.
- [Divine, BJ; Barron, V.](#) (1987). Texaco mortality study: III. A cohort study of producing and pipeline workers. *Am J Ind Med* 11: 189-202. <http://dx.doi.org/10.1002/ajim.4700110208>.
- [Doll, R; Peto, R.](#) (1978). Cigarette smoking and bronchial carcinoma: Dose and time relationships among regular smoker and lifetime nonsmokers. *J Epidemiol Community Health* 32: 303-313.
- [Doolittle, DJ; Furlong, JW; Butterworth, BE.](#) (1985). Assessment of chemically induced DNA repair in primary cultures of human bronchial epithelial cells. *Toxicol Appl Pharmacol* 79: 28-38. [http://dx.doi.org/10.1016/0041-008X\(85\)90365-5](http://dx.doi.org/10.1016/0041-008X(85)90365-5).
- [Douglas, MP; Rogers, SO.](#) (1998). DNA damage caused by common cytological fixatives. *Mutat Res* 401: 77-88. [http://dx.doi.org/10.1016/S0027-5107\(97\)00314-X](http://dx.doi.org/10.1016/S0027-5107(97)00314-X).
- [Dragan, YP; Hully, J; Baker, K; Crow, R; Mass, MJ; Pitot, HC.](#) (1995). Comparison of experimental and theoretical parameters of the Moolgavkar-Venzon-Knudson incidence function for the stages of initiation and promotion in rat hepatocarcinogenesis. *Toxicology* 102: 161-175. [http://dx.doi.org/10.1016/0300-483X\(95\)03045-H](http://dx.doi.org/10.1016/0300-483X(95)03045-H).
- [Dresp, J; Bauchinger, M.](#) (1988). Direct analysis of the clastogenic effect of formaldehyde in unstimulated human lymphocytes by means of the premature chromosome condensation technique. *Mutat Res* 204: 349-352. [http://dx.doi.org/10.1016/0165-1218\(88\)90110-3](http://dx.doi.org/10.1016/0165-1218(88)90110-3).
- [Duan, J; Kang, J; Qin, W; Deng, T; Liu, H; Li, B; Yu, W; Gong, S; Yang, X; Chen, M.](#) (2018). Exposure to formaldehyde and diisononyl phthalate exacerbate neuroinflammation through NF- κ B activation in a mouse asthma model. *Ecotoxicol Environ Saf* 163: 356-364. <http://dx.doi.org/10.1016/j.ecoenv.2018.07.089>.
- [Duan, J; Xie, J; Deng, T; Xie, X; Liu, H; Li, B; Chen, M.](#) (2020). Exposure to both formaldehyde and high relative humidity exacerbates allergic asthma by activating the TRPV4-p38 MAPK pathway in Balb/c mice. *Environ Pollut* 256: 113375. <http://dx.doi.org/10.1016/j.envpol.2019.113375>.
- [Duan, YY.](#) (2011). [Effects of overexpression of heat shock protein 70 on the damage induced by formaldehyde in vitro]. *Zhonghua Laodong Weisheng Zhiyebing Zazhi* 29: 349-352.
- [Dumas, O; Boggs, KM; Quinot, C; Varraso, R; Zock, JP; Henneberger, PK; Speizer, FE; Le Moual, N; Camargo, CA.](#) (2020). Occupational exposure to disinfectants and asthma incidence in U.S. nurses: A prospective cohort study. *Am J Ind Med* 63: 44-50. <http://dx.doi.org/10.1002/ajim.23067>.

- [Dykewicz, MS; Patterson, R; Cugell, DW; Harris, KE; Wu, AF.](#) (1991). Serum IgE and IgG to formaldehyde-human serum albumin: Lack of relation to gaseous formaldehyde exposure and symptoms. *J Allergy Clin Immunol* 87: 48-57. [http://dx.doi.org/10.1016/0091-6749\(91\)90212-7](http://dx.doi.org/10.1016/0091-6749(91)90212-7).
- [ECHA](#) (European Chemicals Agency). (2012). Committee for risk assessment. RAC. Opinion proposing harmonized classification and labeling at EU level of formaldehyde. (CLH-O-0000003155-80-01/F). Helsinki, Finland. <https://echa.europa.eu/documents/10162/b8dfa022-9544-72e8-dcaa-7491dff3c0d5>.
- [Edling, C; Hellquist, H; Odkvist, L.](#) (1987a). Occupational formaldehyde exposure and the nasal mucosa. *Rhinology* 25: 181-187.
- [Edling, C; Hellquist, H; Odkvist, L.](#) (1988). Occupational exposure to formaldehyde and histopathological changes in the nasal mucosa. *Br J Ind Med* 45: 761-765. <http://dx.doi.org/10.1136/oem.45.11.761>.
- [Edling, C; Järnholm, B; Andersson, L; Axelsson, O.](#) (1987b). Mortality and cancer incidence among workers in an abrasive manufacturing industry. *Br J Ind Med* 44: 57-59. <http://dx.doi.org/10.1136/oem.44.1.57>.
- [Edrissi, B; Taghizadeh, K; Dedon, PC.](#) (2013a). Quantitative analysis of histone modifications: formaldehyde is a source of pathological n(6)-formyllysine that is refractory to histone deacetylases. *PLoS Genet* 9: e1003328. <http://dx.doi.org/10.1371/journal.pgen.1003328>.
- [Edrissi, B; Taghizadeh, K; Moeller, BC; Kracko, D; Doyle-Eisele, M; Swenberg, JA; Dedon, PC.](#) (2013b). Dosimetry of N⁶-formyllysine adducts following [¹³C²H₂]-formaldehyde exposures in rats. *Chem Res Toxicol* 26: 1421-1423. <http://dx.doi.org/10.1021/tx400320u>.
- [Edrissi, B; Taghizadeh, K; Moeller, BC; Yu, R; Kracko, D; Doyle-Eisele, M; Swenberg, JA; Dedon, PC.](#) (2017). N⁶-Formyllysine as a Biomarker of Formaldehyde Exposure: Formation and Loss of N⁶-Formyllysine in Nasal Epithelium in Long-Term, Low-Dose Inhalation Studies in Rats. *Chem Res Toxicol* 30: 1572-1576. <http://dx.doi.org/10.1021/acs.chemrestox.7b00075>.
- [Egle, JL, Jr.](#) (1972). Retention of inhaled formaldehyde, propionaldehyde, and acrolein in the dog. *Arch Environ Health* 25: 119-124. <http://dx.doi.org/10.1080/00039896.1972.10666147>.
- [EHS Consultants Ltd.](#) (EHS Consultants Limited). (1999). Consultancy study for indoor air pollution in offices and public places in Hong Kong (Agreement no. CE 14/95). Hong Kong: Environmental Protection Department.
- [El-Feky, AA; Kabbash, IA; Zayet, HH; El-Sallamy, RM.](#) (2020). Health disorders and safety measures among workers in Tanta Flax and Oil Company, Egypt. *Environ Sci Pollut Res Int* 28: 13981-13990. <http://dx.doi.org/10.1007/s11356-020-11588-0>.
- [Elenkov, IJ.](#) (2004). Glucocorticoids and the Th1/Th2 balance [Review]. *Ann N Y Acad Sci* 1024: 138-146. <http://dx.doi.org/10.1196/annals.1321.010>.
- [Emri, G; Schaefer, D; Held, B; Herbst, C; Zieger, W; Horkay, I; Bayerl, C.](#) (2004). Low concentrations of formaldehyde induce DNA damage and delay DNA repair after UV irradiation in human skin cells. *Exp Dermatol* 13: 305-315. <http://dx.doi.org/10.1111/j.0906-6705.2004.00157.x>.
- [Environment Canada](#) (Environment Canada). (2000). Priority substances list assessment report. Formaldehyde. Ottawa, Canada: Health Canada. <http://publications.gc.ca/collections/Collection/En40-215-50E.pdf>.
- [Eom, HJ; Liu, YD; Kwak, GS; Heo, M; Song, KS; Chung, YD; Chon, TS; Choi, J.](#) (2017). Inhalation toxicity of indoor air pollutants in *Drosophila melanogaster* using integrated transcriptomics and computational behavior analyses. *Sci Rep* 7: 46473. <http://dx.doi.org/10.1038/srep46473>.
- [Epstein, SS; Arnold, E; Andrea, J; Bass, W; Bishop, Y.](#) (1972). Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol Appl Pharmacol* 23: 288-325. [http://dx.doi.org/10.1016/0041-008X\(72\)90192-5](http://dx.doi.org/10.1016/0041-008X(72)90192-5).

- [Epstein, SS; Shafner, H.](#) (1968). Chemical mutagens in the human environment. *Nature* 219: 385-387.
<http://dx.doi.org/10.1038/219385a0>.
- [Erb, KJ; Le Gros, G.](#) (1996). The role of Th2 type CD4+ T cells and Th2 type CD8+ T cells in asthma [Review]. *Immunol Cell Biol* 74: 206-208. <http://dx.doi.org/10.1038/icb.1996.29>.
- [Erdei, E; Bobvos, J; Brózik, M; Páldy, A; Farkas, I; Vaskövi, E; Rudnai, P.](#) (2003). Indoor air pollutants and immune biomarkers among Hungarian asthmatic children. *Arch Environ Occup Health* 58: 337-347.
- [Ericson, A; Eriksson, M; Källén, B; Westerholm, P; Zetterström, R.](#) (1984). Delivery outcome of women working in laboratories during pregnancy. *Arch Environ Health* 39: 5-10.
- [Esterbauer, H; Cheeseman, KH; Dianzani, MU; Poli, G; Slater, TF.](#) (1982). Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe²⁺ in rat liver microsomes. *Biochem J* 208: 129-140.
- [Ezratty, V; Bonay, M; Neukirch, C; Orset-Guillossou, G; Dehoux, M; Koscielny, S; Cabanes, PA; Lambrozo, J; Aubier, M.](#) (2007). Effect of formaldehyde on asthmatic response to inhaled allergen challenge. *Environ Health Perspect* 115: 210-214. <http://dx.doi.org/10.1289/ehp.9414>.
- [Fajac, I; Braunstein, G; Ickovic, MR; Lacronique, J; Frossard, N.](#) (1995). Selective recruitment of eosinophils by substance P after repeated allergen exposure in allergic rhinitis. *Allergy* 50: 970-975. <http://dx.doi.org/10.1111/j.1398-9995.1995.tb02509.x>.
- [Falk, JE; Juto, JE; Stridh, G; Bylin, G.](#) (1994). Dose-response study of formaldehyde on nasal mucosa swelling. A study on residents with nasal distress at home. *Am J Rhinol Allergy* 8: 143-146.
<http://dx.doi.org/10.2500/105065894781874412>.
- [Fang, F; Quinlan, P; Ye, W; Barber, MK; Umbach, DM; Sandler, DP; Kamel, F.](#) (2009). Workplace exposures and the risk of amyotrophic lateral sclerosis. *Environ Health Perspect* 117: 1387-1392.
<http://dx.doi.org/10.1289/ehp.0900580>.
- [Fang, J; Li, DH; Yu, XQ; Lv, MQ; Bai, LZ; Du, LZ; Zhou, DX.](#) (2015). Formaldehyde exposure inhibits the expression of mammalian target of rapamycin in rat testis. *Toxicol Ind Health* 32: 1882-1890.
<http://dx.doi.org/10.1177/0748233715592992>.
- [Farrand, KF; Fridman, M; Stillman, IÖ; Schaumberg, DA.](#) (2017). Prevalence of diagnosed dry eye disease in the United States among adults aged 18 years and older. *Am J Ophthalmol* 182: 90-98.
<http://dx.doi.org/10.1016/j.ajo.2017.06.033>.
- [Fenech, M.](#) (1993). The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations [Review]. *Mutat Res* 285: 35-44.
- [Fenech, M.](#) (2000). The in vitro micronucleus technique [Review]. *Mutat Res* 455: 81-95.
- [Fenech, M.](#) (2007). Cytokinesis-block micronucleus cytome assay. *Nat Protoc* 2: 1084-1104.
<http://dx.doi.org/10.1038/nprot.2007.77>.
- [Fenech, M; Chang, WP; Kirsch-Volders, M; Holland, N; Bonassi, S; Zeiger, E; project, HM.](#) (2003). HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures [Comment]. *Mutat Res* 534: 65-75.
- [Fenech, M; Morley, AA.](#) (1985). Measurement of micronuclei in lymphocytes. *Mutat Res* 147: 29-36.
- [Fenech, M; Morley, AA.](#) (1986). Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo ageing and low dose X-irradiation. *Mutat Res* 161: 193-198.
- [Fennell, TR.](#) (1994). Development of methods for measuring biological markers of formaldehyde exposure (pp. 1-20; discussion 21-26). Cambridge, MA: Health Effects Institute.
- [Feron, VJ; Bruyntjes, JP; Woutersen, RA; Immel, HR; Appelman, LM.](#) (1988). Nasal tumours in rats after short-term exposure to a cytotoxic concentration of formaldehyde. *Cancer Lett* 39: 101-111.
[http://dx.doi.org/10.1016/0304-3835\(88\)90045-6](http://dx.doi.org/10.1016/0304-3835(88)90045-6).

Supplemental Information—Formaldehyde (Inhalation)

- [Feron, VJ; Immel, HR; Wilmer, JWGM; Woutersen, RA; Zwart, A.](#) (1987). Nasal Tumours in Rats after Severe Injury to the Nasal Mucosa and Exposure to Formaldehyde Vapour: Preliminary Results (pp. 8-12). (NIOSH/00176121). Feron, VJ; Immel, HR; Wilmer, JWGM; Woutersen, RA; Zwart, A.
- [Ferris, BG.](#) (1978). Epidemiology standardization project (American Thoracic Society). *Am Rev Respir Dis* 118: 1-120.
- [Fiddler, W; Miller, AJ; Pensabene, JW; Doerr, RC.](#) (1984). Investigation on the mutagenicity of N-nitrosothiazolidine using the Ames Salmonella test. In IK O'Neill; RC von Borstel; CT Miller; J Long; H Bartsch (Eds.), *N-nitroso compounds: Occurrence, biological effects and relevance to human cancer* (pp. 95-100). Lyon, France: International Agency for Research on Cancer.
- [Finkelman, FD.](#) (2007). Anaphylaxis: lessons from mouse models [Review]. *J Allergy Clin Immunol* 120: 506-515; quiz 516-507. <http://dx.doi.org/10.1016/j.jaci.2007.07.033>.
- [FJ, C.](#) (2009). Natural killer cells in infection and inflammation of the lung [Review]. *Immunology* 128: 151-163. <http://dx.doi.org/10.1111/j.1365-2567.2009.03167.x>.
- [Flamant-Hulin, M; Caillaud, D; Sacco, P; Pénard-Morand, C; Annesi-Maesano, I.](#) (2010). Air pollution and increased levels of fractional exhaled nitric oxide in children with no history of airway damage. *J Toxicol Environ Health A* 73: 272-283. <http://dx.doi.org/10.1080/15287390903249206>.
- [Fleig, I; Petri, N; Stocker, WG; Thiess, AM.](#) (1982). Cytogenetic analyses of blood lymphocytes of workers exposed to formaldehyde in formaldehyde manufacturing and processing. *J Occup Med* 24: 1009-1012.
- [Fleisher, JM.](#) (1987). Medical students' exposure to formaldehyde in gross anatomy laboratories. *N Y State J Med* 87: 385-388.
- [Fló-Neyret, C; Lorenzi-Filho, G; Macchione, M; Garcia, ML; Saldiva, PH.](#) (2001). Effects of formaldehyde on the frog's mucociliary epithelium as a surrogate to evaluate air pollution effects on the respiratory epithelium. *Braz J Med Biol Res* 34: 639-643. <http://dx.doi.org/10.1590/S0100-879X2001000500012>.
- [Fontignie-Houbrechts, N.](#) (1981). Genetic effects of formaldehyde in the mouse. *Mutat Res* 88: 109-114. [http://dx.doi.org/10.1016/0165-1218\(81\)90095-1](http://dx.doi.org/10.1016/0165-1218(81)90095-1).
- [Fontignie-Houbrechts, N; Moutschen-Dahmen, M; Degraeve, N; Gloor, H.](#) (1982). Genetic effects in the mouse of formaldehyde in combination with adenosine and hydrogen peroxide. *Mutat Res* 104: 371-376. [http://dx.doi.org/10.1016/0165-7992\(82\)90172-5](http://dx.doi.org/10.1016/0165-7992(82)90172-5).
- [Fornace, AJ.](#) (1982). Detection of DNA single-strand breaks produced during the repair of damage by DNA-protein cross-linking agents. *Cancer Res* 42: 145-149.
- [Fornace, AJ; Lechner, JF; Grafstrom, RC; Harris, CC.](#) (1982). DNA repair in human bronchial epithelial cells. *Carcinogenesis* 3: 1373-1377. <http://dx.doi.org/10.1093/carcin/3.12.1373>.
- [Fox, CH; Johnson, FB; Whiting, J; Roller, PP.](#) (1985). Formaldehyde fixation [Review]. *J Histochem Cytochem* 33: 845-853.
- [Franklin, P; Dingle, P; Stick, S.](#) (2000). Raised exhaled nitric oxide in healthy children is associated with domestic formaldehyde levels. *Am J Respir Crit Care Med* 161: 1575-1759. <http://dx.doi.org/10.1164/ajrccm.161.5.9905061>.
- [Franklin, P; Tan, M; Hemy, N; Hall, GL.](#) (2019). Maternal Exposure to Indoor Air Pollution and Birth Outcomes. *Int J Environ Res Public Health* 16. <http://dx.doi.org/10.3390/ijerph16081364>.
- [Franks, SJ.](#) (2005). A mathematical model for the absorption and metabolism of formaldehyde vapour by humans. *Toxicol Appl Pharmacol* 206: 309-320. <http://dx.doi.org/10.1016/j.taap.2004.11.012>.
- [Fransman, W; Mclean, D; Douwes, J; Demers, PA; Leung, V; Pearce, N.](#) (2003). Respiratory symptoms and occupational exposures in New Zealand plywood mill workers. *Ann Occup Hyg* 47: 287-295. <http://dx.doi.org/10.1093/annhyg/meg046>.
- [Frazelle, JH; Abernethy, DJ; Boreiko, CJ.](#) (1983). Weak promotion of C3H/10T1/2 cell transformation by repeated treatments with formaldehyde. *Cancer Res* 43: 3236-3239.

- [Fryzek, JP; Chadda, BK; Cohen, SS; Marano, D; White, K; Steinwandel, M; McLaughlin, JK. \(2005\). Retrospective cohort mortality study of workers engaged in motion picture film processing. J Occup Environ Med 47: 278-286. <http://dx.doi.org/10.1097/01.jom.0000155712.22617.42>.](#)
- [Fsadni, P; Bezzina, F; Fsadni, C; Montefort, S. \(2018\). Impact of School Air Quality on Children's Respiratory Health. Indian J Occup Environ Med 22: 156-162. \[http://dx.doi.org/10.4103/ijoom.IJOEM_95_18\]\(http://dx.doi.org/10.4103/ijoom.IJOEM_95_18\).](#)
- [Fujii, K; Tsuji, K; Matsuura, H; Okazaki, F; Takahashi, S; Arata, J; Iwatsuki, K. \(2005\). Effect of formaldehyde gas exposure in a murine allergic contact hypersensitivity model. Immunopharmacol Immunotoxicol 27: 163-175. <http://dx.doi.org/10.1081/IPH-51768>.](#)
- [Fujimaki, H; Kurokawa, Y; Kakeyama, M; Kunugita, N; Fueta, Y; Fukuda, T; Hori, H; Arashidani, K. \(2004a\). Inhalation of low-level formaldehyde enhances nerve growth factor production in the hippocampus of mice. Neuroimmunomodulation 11: 373-375. <http://dx.doi.org/10.1159/000080147>.](#)
- [Fujimaki, H; Kurokawa, Y; Kunugita, N; Kikuchi, M; Sato, F; Arashidani, K. \(2004b\). Differential immunogenic and neurogenic inflammatory responses in an allergic mouse model exposed to low levels of formaldehyde. Toxicology 197: 1-13. <http://dx.doi.org/10.1016/j.tox.2003.11.015>.](#)
- [Fujimaki, H; Shiraishi, F; Katayama, N. \(1992\). Enhancement of histamine release from rat peritoneal mast cells exposed to formaldehyde. Inhal Toxicol 4: 125-136. <http://dx.doi.org/10.3109/08958379209145309>.](#)
- [Galloway, SM; Bloom, AD; Resnick, M; Margolin, BH; Nakamura, F; Archer, P; Zeiger, E. \(1985\). Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ Mutagen 7: 1-51. <http://dx.doi.org/10.1002/em.2860070102>.](#)
- [Gamble, JF; McMichael, AJ; Williams, T; Battigelli, M. \(1976\). Respiratory function and symptoms: an environmental-epidemiological study of rubber workers exposed to a phenolformaldehyde type resin. Am Ind Hyg Assoc J 37: 499-513. <http://dx.doi.org/10.1080/0002889768507508>.](#)
- [Gammage, RB; Hawthorne, AR. \(1985\). Current status of measurement techniques and concentrations of formaldehyde in residences. In V Turoski \(Ed.\), Formaldehyde: Analytical Chemistry and Toxicology \(pp. 117-130\). Washington, DC: ACS Publications. <http://dx.doi.org/10.1021/ba-1985-0210.ch009>.](#)
- [García-Calderón, CB; Bejarano-García, JA; Tinoco-Gago, I; Castro, MJ; Moreno-Gordillo, P; Piruat, JI; Caballero-Velázquez, T; Pérez-Simón, JA; Rosado, IV. \(2018\). Genotoxicity of tetrahydrofolic acid to hematopoietic stem and progenitor cells. Cell Death Differ 25: 1967-1979. <http://dx.doi.org/10.1038/s41418-018-0089-4>.](#)
- [Gardner, MJ; Pannett, B; Winter, PD; Cruddas, AM. \(1993\). A cohort study of workers exposed to formaldehyde in the British chemical industry: An update. Br J Ind Med 50: 827-834. <http://dx.doi.org/10.1136/oem.50.9.827>.](#)
- [Gardner, RJ; Burgess, BA; Kennedy, GL, Jr. \(1985\). Sensory irritation potential of selected nasal tumorigens in the rat. Food Chem Toxicol 23: 87-92. \[http://dx.doi.org/10.1016/0278-6915\\(85\\)90225-X\]\(http://dx.doi.org/10.1016/0278-6915\(85\)90225-X\).](#)
- [Garrett, MH; Hooper, MA; Hooper, BM; Rayment, PR; Abramson, MJ. \(1999a\). Increased risk of allergy in children due to formaldehyde exposure in homes. Allergy 54: 330-337. <http://dx.doi.org/10.1034/j.1398-9995.1999.00763.x>.](#)
- [Garrett, MH; Hooper, MA; Hooper, BM; Rayment, PR; Abramson, MJ. \(1999b\). Increased risk of allergy in children due to formaldehyde exposure in homes. Errata \[Erratum\]. Allergy 54: 1327.](#)
- [Gasteiger, G; Rudensky, AY. \(2014\). Interactions between innate and adaptive lymphocytes \[Review\]. Nat Rev Immunol 14: 631-639. <http://dx.doi.org/10.1038/nri3726>.](#)

- [Gavala, ML; Bashir, H; Gern, JE.](#) (2013). Virus/Allergen Interactions in Asthma. *Curr Allergy Asthma Rep* 13: 298-307. <http://dx.doi.org/10.1007/s11882-013-0344-1>.
- [Gaylor, DW; Zheng, Q.](#) (1996). Risk assessment of nongenotoxic carcinogens based upon cell proliferation/death rates in rodents. *Risk Anal* 16: 221-225.
- [Ge, J; Yang, H; Lu, X; Wang, S; Zhao, Y; Huang, J; Xi, Z; Zhang, L; Li, R.](#) (2020a). Combined exposure to formaldehyde and PM2.5: Hematopoietic toxicity and molecular mechanism in mice. *Environ Int* 144: 106050. <http://dx.doi.org/10.1016/j.envint.2020.106050>.
- [Ge, P; Zhang, X; Yang, YQ; Lv, MQ; Zhou, DX.](#) (2020b). Long-term exposure to formaldehyde induced down-regulation of SPO11 in rats. *Inhal Toxicol* 33: 1-10. <http://dx.doi.org/10.1080/08958378.2020.1859652>.
- [Ge, S; Yan, B; Huang, J; Chen, Y; Chen, M; Yang, X; Wu, Y; Shen, D; Ma, P.](#) (2019). Diisodecyl phthalate aggravates the formaldehyde-exposure-induced learning and memory impairment in mice. *Food Chem Toxicol* 126: 152-161. <http://dx.doi.org/10.1016/j.fct.2019.02.024>.
- [Gee, IL; Watson, AFR; Tavernier, G; Stewart, LJ; Fletcher, G; Niven, RM.](#) (2005). Indoor air quality, environmental tobacco smoke and asthma: A case control study of asthma in a community population. *Indoor Built Environ* 14: 215-219. <http://dx.doi.org/10.1177/1420326X05054288>.
- [Gentry, PR; Rodricks, JV; Turnbull, D; Bachand, A; Van Landingham, C; Shipp, AM; Albertini, RJ; Irons, R.](#) (2013). Formaldehyde exposure and leukemia: Critical review and reevaluation of the results from a study that is the focus for evidence of biological plausibility [Review]. *Crit Rev Toxicol* 43: 661-670. <http://dx.doi.org/10.3109/10408444.2013.818618>.
- [Georgieva, AV; Kimbell, JS; Schlosser, PM.](#) (2003). A distributed-parameter model for formaldehyde uptake and disposition in the rat nasal lining. *Inhal Toxicol* 15: 1435-1463. <http://dx.doi.org/10.1080/08958370390249085>.
- [Gerberich, HR; Seaman, GC.](#) (2013). Formaldehyde. In *JI Kroshwitz; M Howe-Grant (Eds.), Kirk-Othmer encyclopedia of chemical technology* (4th ed., pp. 929-951). New York, NY: John Wiley & Sons.
- [Gerde, P; Cheng, YS; Medinsky, MA.](#) (1991). In vivo deposition of ultrafine aerosols in the nasal airway of the rat. *Toxicol Sci* 16: 330-336. [http://dx.doi.org/10.1016/0272-0590\(91\)90117-m](http://dx.doi.org/10.1016/0272-0590(91)90117-m).
- [Gérin, M; Siemiatycki, J; Nadon, L; Dewar, R; Krewski, D.](#) (1989). Cancer risks due to occupational exposure to formaldehyde: Results of a multi-site case-control study in Montreal. *Int J Cancer* 44: 53-58. <http://dx.doi.org/10.1002/ijc.2910440110>.
- [Ghelli, F; Buglisi, M; Bellisario, V; Santovito, A; Bono, R.](#) (2020). Formaldehyde in hospitals can still represent a risk factor. Oxidative stress and GSTT1 polymorphism [Abstract]. *Eur J Public Health* 30. <http://dx.doi.org/10.1093/eurpub/ckaa166.340>.
- [Giavina-Bianchi, P; Aun, MV; Takejima, P; Kalil, J; Agondi, RC.](#) (2016). United airway disease: current perspectives [Review]. *Journal of Asthma and Allergy* 9: 93-100. <http://dx.doi.org/10.2147/JAA.S81541>.
- [Gieroba, ZJ; Yu, YH; Blessing, WW.](#) (1994). Vasoconstriction induced by inhalation of irritant vapour is associated with appearance of Fos protein in C1 catecholamine neurons in rabbit medulla oblongata. *Brain Res* 636: 157-161. [http://dx.doi.org/10.1016/0006-8993\(94\)90192-9](http://dx.doi.org/10.1016/0006-8993(94)90192-9).
- [Gilbert, NL; Gauvin, D; Guay, M; Heroux, ME; Dupuis, G; Legris, M; Chan, CC; Dietz, RN; Levesque, B.](#) (2006). Housing characteristics and indoor concentrations of nitrogen dioxide and formaldehyde in Quebec City, Canada. *Environ Res* 102: 1-8. <http://dx.doi.org/10.1016/j.envres.2006.02.007>.
- [Gilbert, NL; Guay, M; David Miller, J; Judek, S; Chan, CC; Dales, RE.](#) (2005). Levels and determinants of formaldehyde, acetaldehyde, and acrolein in residential indoor air in Prince Edward Island, Canada. *Environ Res* 99: 11-17. <http://dx.doi.org/10.1016/j.envres.2004.09.009>.
- [Ginsberg, GL; Foos, BP; Firestone, MP.](#) (2005). Review and analysis of inhalation dosimetry methods for application to children's risk assessment [Review]. *J Toxicol Environ Health A* 68: 573-615. <http://dx.doi.org/10.1080/15287390590921793>.

- [Glaser, R; Kiecolt-Glaser, JK.](#) (2005). Stress-induced immune dysfunction: implications for health [Review]. *Nat Rev Immunol* 5: 243-251. <http://dx.doi.org/10.1038/nri1571>.
- [Gocke, E; King, MT; Eckhardt, K; Wild, D.](#) (1981). [Mutagenicity of cosmetics ingredients licensed by the European communities]. *Mutat Res* 90: 91-109. [http://dx.doi.org/10.1016/0165-1218\(81\)90072-0](http://dx.doi.org/10.1016/0165-1218(81)90072-0).
- [Gofmekler, VA.](#) (1968). Effect on embryonic development of benzene and formaldehyde in inhalation experiments. *Hyg Sanit* 33: 327-332.
- [Gofmekler, VA; Bonashevskaya, TI.](#) (1969). Experimental studies of teratogenic properties of formaldehyde, based on pathological investigations. *Hyg Sanit* 34: 266-268.
- [Golalipour, MJ; Azarhoush, R; Ghafari, S; Gharravi, AM; Fazeli, SA; Davarian, A.](#) (2007). Formaldehyde exposure induces histopathological and morphometric changes in the rat testis. *Folia Morphol (Warsz)* 66: 167-171.
- [Golalipour, MJ; Kord, H; Ghafari, S; Gharravi, AM; Davarian, A; Fazeli, SA; Azarhoush, R.](#) (2008). Morphometric alterations of the rat spleen following formaldehyde exposure. *Folia Morphol (Warsz)* 67: 19-23.
- [Goldmacher, VS; Thilly, WG.](#) (1983). Formaldehyde is mutagenic for cultured human cells. *Mutat Res* 116: 417-422. [http://dx.doi.org/10.1016/0165-1218\(83\)90080-0](http://dx.doi.org/10.1016/0165-1218(83)90080-0).
- [Goldsworthy, TL; Butterworth, BE; Maronpot, RR.](#) (1993). Concepts, labeling procedures, and design of cell proliferation studies relating to carcinogenesis [Review]. *Environ Health Perspect* 101 Suppl 5: 59-65. <http://dx.doi.org/10.1289/ehp.93101s559>.
- [Goldsworthy, TL; Morgan, KT; Popp, JA; Butterworth, BE.](#) (1991). Guidelines for measuring chemically induced cell proliferation in specific rodent target organs. *Prog Clin Biol Res* 369: 253-284.
- [Gomaa, M; Elmesallamy, GE; Sameer, MM.](#) (2012). Evaluation of genotoxic effects of formaldehyde in adult albino rats and its implication in case of human exposure. *Life Science Journal* 9: 3085-3093.
- [Gonzalez-Rivera, JC; Sherman, MW; Wang, DS; Chuvalo-Abraham, JCL; Hildebrandt Ruiz, L; Contreras, LM.](#) (2020). RNA oxidation in chromatin modification and DNA-damage response following exposure to formaldehyde. *Sci Rep* 10: 16545. <http://dx.doi.org/10.1038/s41598-020-73376-7>.
- [Gordon, CJ; Spencer, PJ; Hotchkiss, J; Miller, DB; Hinderliter, PM; Pauluhn, J.](#) (2008). Thermoregulation and its influence on toxicity assessment. *Toxicology* 244: 87-97. <http://dx.doi.org/10.1016/j.tox.2007.10.030>.
- [Górski, P; Krakowiak, A.](#) (1991). Formaldehyde--induced bronchial asthma--does it really exist? *Pol J Occup Med Environ Health* 4: 317-320.
- [Gostner, JM; Zeisler, J; Alam, MT; Gruber, P; Fuchs, D; Becker, K; Neubert, K; Kleinhapfl, M; Martini, S; Überall, F.](#) (2016). Cellular reactions to long-term volatile organic compound (VOC) exposures. *Sci Rep* 6: 37842. <http://dx.doi.org/10.1038/srep37842>.
- [Gottschling, LM; Beaulieu, HJ; Melvin, WW.](#) (1984). Monitoring of formic acid in urine of humans exposed to low levels of formaldehyde. *Am Ind Hyg Assoc J* 45: 19-23. <http://dx.doi.org/10.1080/15298668491399299>.
- [Graf, RA; Kater, SB; Gordon, H.](#) (1999). Prolonged Cytosolic Calcium Elevations in Growth Cones Contacting Muscle. *Dev Neurosci* 21: 409-416. <http://dx.doi.org/10.1159/000017408>.
- [Grafstrom, RC.](#) (1990). In vitro studies of aldehyde effects related to human respiratory carcinogenesis. *Mutat Res* 238: 175-184.
- [Grafstrom, RC; Fornace, A, Jr; Harris, CC.](#) (1984). Repair of DNA damage caused by formaldehyde in human cells. *Cancer Res* 44: 4323-4327.
- [Grafstrom, RC; Fornace, AJ, Jr; Autrup, H; Lechner, JF; Harris, CC.](#) (1983). Formaldehyde damage to DNA and inhibition of DNA repair in human bronchial cells. *Science* 220: 216-218. <http://dx.doi.org/10.1126/science.6828890>.

- [Grafström, RC; Hsu, IC; Harris, CC.](#) (1993). Mutagenicity of formaldehyde in Chinese hamster lung fibroblasts: Synergy with ionizing radiation and N-nitroso-N-methylurea. *Chem Biol Interact* 86: 41-49. [http://dx.doi.org/10.1016/0009-2797\(93\)90110-K](http://dx.doi.org/10.1016/0009-2797(93)90110-K).
- [Grafstrom, RC; Sundqvist, K; Dypbukt, JM; Hybbinette, SA; Harris, CC.](#) (1986). CYTOTOXIC AND GENOTOXIC EFFECTS OF ALDEHYDES IN CULTURED HUMAN BRONCHIAL CELLS (pp. BASEL). (BIOSIS/87/05969). Grafstrom, RC; Sundqvist, K; Dypbukt, JM; Hybbinette, SA; Harris, CC.
- [Grafstrom, RC; Willey, JC; Harris, CC.](#) (1985). PATHOBIOLOGICAL EFFECT OF TOBACCO SMOKE CONDENSATE FRACTIONS AND SMOKE-RELATED ALDEHYDES IN CULTURED NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS (pp. 6-13). (BIOSIS/85/10935). Grafstrom, RC; Willey, JC; Harris, CC.
- [Grafström, RC; Willey, JC; Sundqvist, K; Harris, CC.](#) (1986). Pathobiological effects of tobacco smoke-related aldehydes in cultured human bronchial epithelial cells. In D Hoffman; CC Harris (Eds.), *Mechanisms in Tobacco Carcinogenesis*, Banbury Report 23 (pp. 273-285). Cold Springs Harbor, NY: CSH Press.
- [Grammer, LC; Harris, KE; Shaughnessy, MA; Sparks, P; Ayars, GH; Altman, LC; Patterson, R.](#) (1990). Clinical and immunologic evaluation of 37 workers exposed to gaseous formaldehyde. *J Allergy Clin Immunol* 86: 177-181. [http://dx.doi.org/10.1016/S0091-6749\(05\)80063-6](http://dx.doi.org/10.1016/S0091-6749(05)80063-6).
- [Grasl-Kraupp, B; Luebeck, G; Wagner, A; Löw-Baselli, A; de Gunst, M; Waldhör, T; Moolgavkar, S; Schulte-Hermann, R.](#) (2000). Quantitative analysis of tumor initiation in rat liver: role of cell replication and cell death (apoptosis). *Carcinogenesis* 21: 1411-1421. <http://dx.doi.org/10.1093/carcin/21.7.1411>.
- [Graves, RJ; Callander, RD; Green, T.](#) (1994). The role of formaldehyde and S-chloromethylglutathione in the bacterial mutagenicity of methylene chloride. *Mutat Res* 320: 235-243. [http://dx.doi.org/10.1016/0165-1218\(94\)90050-7](http://dx.doi.org/10.1016/0165-1218(94)90050-7).
- [Graves, RJ; Trueman, P; Jones, S; Green, T.](#) (1996). DNA sequence analysis of methylene chloride-induced HPRT mutations in Chinese hamster ovary cells: Comparison with the mutation spectrum obtained for 1,2-dibromoethane and formaldehyde. *Mutagenesis* 11: 229-233. <http://dx.doi.org/10.1093/mutage/11.3.229>.
- [Green, DJ; Bascom, R; Healey, EM; Hebel, JR; Sauder, LR; Kulle, TJ.](#) (1989). Acute pulmonary response in healthy, nonsmoking adults to inhalation of formaldehyde and carbon. *J Toxicol Environ Health* 28: 261-275. <http://dx.doi.org/10.1080/15287398909531347>.
- [Green, DJ; Sauder, LR; Kulle, TJ; Bascom, R.](#) (1987). Acute response to 3.0 ppm formaldehyde in exercising healthy nonsmokers and asthmatics. *Am Rev Respir Dis* 135: 1261-1266. <http://dx.doi.org/10.1164/arrd.1987.135.6.1261>.
- [Greenfeder, S; Umland, SP; Cuss, FM; Chapman, RW; Egan, RW.](#) (2001). Th2 cytokines and asthma. The role of interleukin-5 in allergic eosinophilic disease [Review]. *Respir Res* 2: 71-79. <http://dx.doi.org/10.1186/rr41>.
- [Grimsrud, DT; Lipschutz, RD; Girman, JR.](#) (1983). Indoor air quality in energy efficient residences. In PJ Walsh; CS Dudley; ED Copenhaver (Eds.), *Indoor air quality* (pp. 69-85). Boca Raton, FL: CRC Press. <https://www.crcpress.com/Indoor-Air-Quality/Walsh-Dudley-Copenhaver/9780849350153>.
- [Gross, EA; Swenberg, JA; Fields, S; Popp, JA.](#) (1982). Comparative morphometry of the nasal cavity in rats and mice. *J Anat* 135: 83-88.
- [Groten, JP; Schoen, ED; van Bladeren, PJ; Kuper, CF; van Zorge, JA; Feron, VJ.](#) (1997). Subacute toxicity of a mixture of nine chemicals in rats: detecting interactive effects with a fractionated two-level factorial design. *Fundam Appl Toxicol* 36: 15-29. <http://dx.doi.org/10.1006/faat.1996.2281>.

- [Gu, Y; Fujimiya, Y; Kunugita, N.](#) (2008). Long-term exposure to gaseous formaldehyde promotes allergen-specific IgE-mediated immune responses in a murine model. *Hum Exp Toxicol* 27: 37-43. <http://dx.doi.org/10.1177/0960327108088973>.
- [Guilmette, RA; Cheng, YS; Griffith, WC.](#) (1997). Characterising the variability in adult human nasal airway dimensions. *Ann Occup Hyg* 41: 491-496.
- [Güleç, M; Songur, A; Sahin, S; Ozen, OA; Sarsilmaz, M; Akyol, O.](#) (2006). Antioxidant enzyme activities and lipid peroxidation products in heart tissue of subacute and subchronic formaldehyde-exposed rats: a preliminary study. *Toxicol Ind Health* 22: 117-124. <http://dx.doi.org/10.1191/0748233706th248oa>.
- [Guseva, VA.](#) (1973a). Study of the gonadotropic effect in male rats exposed to formaldehyde, supplied simultaneously in air and water. *Gig Sanit* 37: 102-103.
- [Guseva, VA.](#) (1973b). [Study of the gonadotropic effect in male rats under the effect of formaldehyde administered simultaneously with air and water]. *Gig Sanit* 37: 102-103.
- [Gustavsson, P; Jakobsson, R; Johansson, H; Lewin, F; Norell, S; Rutkvist, LE.](#) (1998). Occupational exposures and squamous cell carcinoma of the oral cavity, pharynx, larynx, and oesophagus: A case-control study in Sweden. *Occup Environ Med* 55: 393-400.
- [Guyton, AC.](#) (1991). Textbook of medical physiology (8th ed.). Philadelphia, PA: W.B. Saunders Co.
- [Hagiwara, M; Watanabe, E; Barrett, JC; Tsutsui, T.](#) (2006). Assessment of genotoxicity of 14 chemical agents used in dental practice: Ability to induce chromosome aberrations in Syrian hamster embryo cells. *Mutat Res* 603: 111-120. <http://dx.doi.org/10.1016/j.mrgentox.2005.08.011>.
- [Hagmar, L; Bellander, T; Englander, V; Ranstam, J; Attewell, R; Skerfving, S.](#) (1986). Mortality and cancer morbidity among workers in a chemical factory. *Scand J Work Environ Health* 12: 545-551. <http://dx.doi.org/10.5271/sjweh.2102>.
- [Hall, A; Harrington, JM; Aw, TC.](#) (1991). Mortality study of British pathologists. *Am J Ind Med* 20: 83-89. <http://dx.doi.org/10.1002/ajim.4700200108>.
- [Hamaguchi, F; Tsutui, T.](#) (2000). Assessment of genotoxicity of dental antiseptics: Ability of phenol, guaiacol, p-phenolsulfonic acid, sodium hypochlorite, p-chlorophenol, m-cresol or formaldehyde to induce unscheduled DNA synthesis in cultured Syrian hamster embryo cells. *Jpn J Pharmacol* 83: 273-276. <http://dx.doi.org/10.1254/jip.83.273>.
- [Hamelmann, E; Tateda, K; Oshiba, A; Gelfand, EW.](#) (1999). Role of IgE in the development of allergic airway inflammation and airway hyperresponsiveness--a murine model [Review]. *Allergy* 54: 297-305. <http://dx.doi.org/10.1034/j.1398-9995.1999.00085.x>.
- [Hamelmann, E; Vella, AT; Oshiba, A; Kappler, JW; Marrack, P; Gelfand, EW.](#) (1997). Allergic airway sensitization induces T cell activation but not airway hyperresponsiveness in B cell-deficient mice. *Proc Natl Acad Sci USA* 94: 1350-1355.
- [Han, RT; Back, SK; Lee, H; Lee, J; Kim, HY; Kim, HJ; Na, HS.](#) (2016). Formaldehyde-Induced Aggravation of Pruritus and Dermatitis Is Associated with the Elevated Expression of Th1 Cytokines in a Rat Model of Atopic Dermatitis. *PLoS ONE* 11: e0168466. <http://dx.doi.org/10.1371/journal.pone.0168466>.
- [Han, SP; Zhou, DX; Lin, P; Qin, Z; An, L; Zheng, LR; Lei, L.](#) (2015). Formaldehyde exposure induces autophagy in testicular tissues of adult male rats. *Environ Toxicol* 30: 323-331. <http://dx.doi.org/10.1002/tox.21910>.
- [Hanna, LM; Lou, SR; Su, S; Jarabek, AM.](#) (2001). Mass transport analysis: inhalation RfC methods framework for interspecies dosimetric adjustment. *Inhal Toxicol* 13: 437-463. <http://dx.doi.org/10.1080/08958370119198>.
- [Hanrahan, LP; Dally, KA; Anderson, HA; Kanarek, MS; Rankin, J.](#) (1984). Formaldehyde vapor in mobile homes: A cross sectional survey of concentrations and irritant effects. *Am J Public Health* 74: 1026-1027. <http://dx.doi.org/10.2105/ajph.74.9.1026>.

Supplemental Information—Formaldehyde (Inhalation)

- [Hansen, J; Olsen, JH.](#) (1995). Formaldehyde and cancer morbidity among male employees in Denmark. *Cancer Causes Control* 6: 354-360. <http://dx.doi.org/10.1007/BF00051411>.
- [Hansen, J; Olsen, JH; Larsen, AI.](#) (1994). Cancer morbidity among employees in a Danish pharmaceutical plant. *Int J Epidemiol* 23: 891-898. <http://dx.doi.org/10.1093/ije/23.5.891>.
- [Hardell, L; Johansson, B; Axelson, O.](#) (1982). Epidemiological study of nasal and nasopharyngeal cancer and their relation to phenoxy acid or chlorophenol exposure. *Am J Ind Med* 3: 247-257. <http://dx.doi.org/10.1002/ajim.4700030304>.
- [Hare, DA; Margosian, RL; Groah, WJ; 3rd, AS; Schweer, LG; Koontz, MD.](#) (1996). Evaluating the contribution of UF-bonded building materials to indoor formaldehyde levels in a newly constructed house.
- [Harkema, JR; Carey, SA; Wagner, JG.](#) (2006). The nose revisited: A brief overview of the comparative structure, function, and toxicologic pathology of the nasal epithelium [Review]. *Toxicol Pathol* 34: 252-269. <http://dx.doi.org/10.1080/01926230600713475>.
- [Harrington, JM; Oakes, D.](#) (1984). Mortality study of British pathologists 1974-80. *Occup Environ Med* 41: 188-191. <http://dx.doi.org/10.1136/oem.41.2.188>.
- [Harrington, JM; Shannon, HS.](#) (1975). Mortality study of pathologists and medical laboratory technicians. *Br Med J* 4: 329-332.
- [Harving, H; Korsgaard, J; Dahl, R; Pedersen, OF; Molhave, L.](#) (1986). Low concentrations of formaldehyde in bronchial asthma: a study of exposure under controlled conditions. *Br Med J* 293: 310.
- [Harving, H; Korsgaard, J; Pedersen, OF; Mølhave, L; Dahl, R.](#) (1990). Pulmonary function and bronchial reactivity in asthmatics during low-level formaldehyde exposure. *Lung* 168: 15-21. <http://dx.doi.org/10.1007/BF02719669>.
- [Haseman, JK.](#) (1995). Data analysis: statistical analysis and use of historical control data. *Regul Toxicol Pharmacol* 21: 52-59. <http://dx.doi.org/10.1006/rtp.1995.1009>.
- [Hastie, AT; Patrick, H; Fish, JE.](#) (1990). Inhibition and recovery of mammalian respiratory ciliary function after formaldehyde exposure. *Toxicol Appl Pharmacol* 102: 282-291. [http://dx.doi.org/10.1016/0041-008X\(90\)90027-R](http://dx.doi.org/10.1016/0041-008X(90)90027-R).
- [Hauptmann, M; Lubin Jay, H; Stewart, PA; Hayes, RB; Blair, A.](#) (2003). Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. *J Natl Cancer Inst* 95: 1615-1623. <http://dx.doi.org/10.1093/jnci/djg083>.
- [Hauptmann, M; Lubin, JH; Stewart, PA; Hayes, RB; Blair, A.](#) (2004a). Cancer mortality among workers in formaldehyde industries [Abstract]. *Ann Epidemiol* 14: 608. <http://dx.doi.org/10.1016/j.annepidem.2004.07.045>.
- [Hauptmann, M; Lubin, JH; Stewart, PA; Hayes, RB; Blair, A.](#) (2004b). Mortality from solid cancers among workers in formaldehyde industries. *Am J Epidemiol* 159: 1117-1130. <http://dx.doi.org/10.1093/aje/kwh174>.
- [Hauptmann, M; Stewart, PA; Lubin, JH; Beane Freeman, LE; Hornung, RW; Herrick, RF; Hoover, RN; Fraumeni, JF, Jr; Blair, A; Hayes, RB.](#) (2009). Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde. *J Natl Cancer Inst* 101: 1696-1708. <http://dx.doi.org/10.1093/jnci/djp416>.
- [Haworth, S; Lawlor, T; Mortelmans, K; Speck, W; Zeiger, E.](#) (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen* 5: 3-142. <http://dx.doi.org/10.1002/em.2860050703>.
- [Hawthorne, AR; Gammage, RB; Dudney, CS; Womack, DR; Morris, SA; Westley, RR; Gupta, DC.](#) (1983). Preliminary results of a forty-home indoor air pollutant monitoring study. In ER Frederick (Ed.), *Proceedings of the Air Pollution Control Association Specialty Conference: Measurement and Monitoring of Non-Criteria (TOXIC) Contaminants in Air*, Chicago, IL, March 22-24, 1983 (pp. 514-526). Philadelphia, PA: Air Pollution Control Association. <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/DE83009839.xhtml>.

Supplemental Information—Formaldehyde (Inhalation)

- [Hayashi, H; Kunugita, N; Arashidani, K; Fujimaki, H; Ichikawa, M.](#) (2004). Long-term exposure to low levels of formaldehyde increases the number of tyrosine hydroxylase-immunopositive periglomerular cells in mouse main olfactory bulb. *Brain Res* 1007: 192-197.
<http://dx.doi.org/10.1016/j.brainres.2003.12.052>.
- [Hayes, RB; Blair, A; Stewart, PA; Herrick, RF; Mahar, H.](#) (1990). Mortality of U.S. embalmers and funeral directors. *Am J Ind Med* 18: 641-652. <http://dx.doi.org/10.1002/ajim.4700180603>.
- [Hayes, RB; Gerin, M; Raatgever, JW; de Bruyn, A.](#) (1986a). Wood-related occupations, wood dust exposure, and sinonasal cancer. *Am J Epidemiol* 124: 569-577.
<http://dx.doi.org/10.1093/oxfordjournals.aje.a114429>.
- [Hayes, RB; Klein, S; Suruda, A; Schulte, P; Boeniger, M; Stewart, P; Livingston, GK; Oesch, F.](#) (1997). O6-alkylguanine DNA alkyltransferase activity in student embalmers. *Am J Ind Med* 31: 361-365.
[http://dx.doi.org/10.1002/\(SICI\)1097-0274\(199703\)31:3<361::AID-AJIM13>3.0.CO;2-Z](http://dx.doi.org/10.1002/(SICI)1097-0274(199703)31:3<361::AID-AJIM13>3.0.CO;2-Z).
- [Hayes, RB; Raatgever, JW; de Bruyn, A; Gerin, M.](#) (1986b). Cancer of the nasal cavity and paranasal sinuses, and formaldehyde exposure. *Int J Cancer* 37: 487-492.
<http://dx.doi.org/10.1002/ijc.2910370403>.
- [Hd'A, H; Casanova, M.](#) (1994). Nasal dosimetry of formaldehyde: Modeling site specificity and the effects of preexposure. *Inhal Toxicol* 6 supplement: 159-175.
- [He, HJ; Liu, HL; Wu, J; Lu, ZS; Yan, Y; Yang, X; Li, CM.](#) (2005). A study on the acute irritation responses and molecular mechanism of gaseous formaldehyde. In X Yang; B Zhao; R Zhao (Eds.), *Indoor Air 2005: Proceedings of the 10th International Conference on Indoor Air Quality and Climate*, vol 5 (pp. 3691-3695). Beijing, China: Tsinghua University Press.
<https://www.isiaq.org/docs/PDFs/3691.pdf>.
- [He, JL; Jin, LF; Jin, HY.](#) (1998). Detection of cytogenetic effects in peripheral lymphocytes of students exposed to formaldehyde with cytokinesis-blocked micronucleus assay. *Biomed Environ Sci* 11: 87-92.
- [Health Canada.](#) (2001). Priority substances list assessment report Formaldehyde. Hull, Quebec, Canada: Environment Canada and Health Canada.
- [Health Canada.](#) (2006). Residential indoor air quality guideline. Formaldehyde.
<http://healthycanadians.gc.ca/publications/healthy-living-vie-saine/formaldehyde/alt/formaldehyde-eng.pdf>.
- [Heck, H; Casanova-Schmitz, M; Dodd, PB; Schachter, EN; Witek, TJ; Tosun, T.](#) (1985). Formaldehyde (CH₂O) concentrations in the blood of humans and Fischer-344 rats exposed to CH₂O under controlled conditions. *AIHA J* 46: 1-3. <http://dx.doi.org/10.1080/15298668591394275>.
- [Heck, H; Casanova, M.](#) (1987). Isotope effects and their implications for the covalent binding of inhaled [3H]- and [14C]formaldehyde in the rat nasal mucosa. *Toxicol Appl Pharmacol* 89: 122-134.
[http://dx.doi.org/10.1016/0041-008X\(87\)90182-7](http://dx.doi.org/10.1016/0041-008X(87)90182-7).
- [Heck, H; Casanova, M.](#) (1999). Pharmacodynamics of formaldehyde: Applications of a model for the arrest of DNA replication by DNA-protein cross-links. *Toxicol Appl Pharmacol* 160: 86-100.
<http://dx.doi.org/10.1006/taap.1999.8764>.
- [Heck, H; Chin, TY; Schmitz, MC.](#) (1983). Distribution of [14C] formaldehyde in rats after inhalation exposure. In JE Gibson (Ed.), *Formaldehyde toxicity* (pp. 26-37). Washington, DC: Hemisphere Publishing.
- [Heck, H; Keller, DA.](#) (1988). Toxicology of formaldehyde. *ISI Atlas of Science: Pharmacology* 2: 5-9.
- [Heck, H; White, EL; Casanova-Schmitz, M.](#) (1982). Determination of formaldehyde in biological tissues by gas chromatography/mass spectrometry. *Biomed Mass Spectrom* 9: 347-353.
<http://dx.doi.org/10.1002/bms.1200090808>.

- [Hedberg, JJ; Höög, JO; Nilsson, JA; Xi, Z; Elfving, A; Grafström, RC.](#) (2000). Expression of alcohol dehydrogenase 3 in tissue and cultured cells from human oral mucosa. *Am J Pathol* 157: 1745-1755. [http://dx.doi.org/10.1016/S0002-9440\(10\)64811-0](http://dx.doi.org/10.1016/S0002-9440(10)64811-0).
- [Heineman, EF; Olsen, JH; Pottern, LM; Gomez, M; Raffn, E; Blair, A.](#) (1992). Occupational risk factors for multiple myeloma among Danish men. *Cancer Causes Control* 3: 555-568. <http://dx.doi.org/10.1007/BF00052753>.
- [Hemminki, K; Kyyrönen, P; Lindbohm, ML.](#) (1985). Spontaneous abortions and malformations in the offspring of nurses exposed to anaesthetic gases, cytostatic drugs, and other potential hazards in hospitals, based on registered information of outcome. *J Epidemiol Community Health* 39: 141-147. <http://dx.doi.org/10.1136/jech.39.2.141>.
- [Hemminki, K; Mutanen, P; Saloniemi, I; Niemi, ML; Vainio, H.](#) (1982). Spontaneous abortions in hospital staff engaged in sterilizing instruments with chemical agents. *J Occup Environ Med* 285: 1461-1463.
- [Henry, AJ.](#) (2008). CDC finds cause of health problems from emergency trailers. *Bull Am Meteorol Soc* 89: 1251-1252,1254.
- [Herbert, FA; Hessel, PA; Melenka, LS; Yoshida, K; Nakaza, M.](#) (1994). Respiratory consequences of exposure to wood dust and formaldehyde of workers manufacturing oriented strand board. *Arch Environ Health* 49: 465-470. <http://dx.doi.org/10.1080/00039896.1994.9955002>.
- [Herbert, FA; Hessel, PA; Melenka, LS; Yoshida, K; Nakaza, M.](#) (1995). Pulmonary effects of simultaneous exposures to MDI formaldehyde and wood dust on workers in an oriented strand board plant. *J Occup Environ Med* 37: 461-465. <http://dx.doi.org/10.1097/00043764-199504000-00018>.
- [Hernandez, O; Rhomberg, L; Hogan, K; Siegel-Scott, C; Lai, D; Grindstaff, G; Henry, M; Cotruvo, JA.](#) (1994). Risk assessment of formaldehyde. *J Hazard Mater* 39: 161-172. [http://dx.doi.org/10.1016/0304-3894\(94\)00067-0](http://dx.doi.org/10.1016/0304-3894(94)00067-0).
- [Hester, SD; Benavides, GB; Yoon, L; Morgan, KT; Zou, F; Barry, W; Wolf, DC.](#) (2003). Formaldehyde-induced gene expression in F344 rat nasal respiratory epithelium. *Toxicology* 187: 13-24. [http://dx.doi.org/10.1016/S0300-483X\(03\)00008-8](http://dx.doi.org/10.1016/S0300-483X(03)00008-8).
- [Hikiba, H; Watanabe, E; Barrett, JC; Tsutsui, T.](#) (2005). Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. *J Pharmacol Sci* 97: 146-152. <http://dx.doi.org/10.1254/jphs.fpj04044x>.
- [Hildesheim, A; Anderson, LM; Chen, CJ; Cheng, YJ; Brinton, LA; Daly, AK; Reed, CD; Chen, IH; Caporaso, NE; Hsu, MM; Chen, JY; Idle, JR; Hoover, RN; Yang, CS; Chhabra, SK.](#) (1997). CYP2E1 genetic polymorphisms and risk of nasopharyngeal carcinoma in Taiwan. *J Natl Cancer Inst* 89: 1207-1212. <http://dx.doi.org/10.1093/jnci/89.16.1207>.
- [Hildesheim, A; Dosemeci, M; Chan, CC; Chen, CJ; Cheng, YJ; Hsu, MM; Chen, IH; Mittl, BF; Sun, B; Levine, PH; Chen, JY; Brinton, LA; Yang, CS.](#) (2001). Occupational exposure to wood, formaldehyde, and solvents and risk of nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev* 10: 1145-1153.
- [Hildesheim, A; West, S; Deveyra, E; De Guzman, MF; Jurado, A; Jones, C; Imai, J; Hinuma, Y.](#) (1992). Herbal medicine use, Epstein-Barr virus, and risk of nasopharyngeal carcinoma. *Cancer Res* 52: 3048-3051.
- [Hill, AB.](#) (1965). The environment and disease: Association or causation? *Proc R Soc Med* 58: 295-300. <http://dx.doi.org/10.1177/003591576505800503>.
- [Ho, KF; Lee, SC; Tsai, WY.](#) (2006). Carbonyl compounds in the roadside environment of Hong Kong. *J Hazard Mater* 133: 24-29. <http://dx.doi.org/10.1016/j.jhazmat.2005.09.054>.
- [Hodgson, AT; Beal, D; Mcilvaine, JER.](#) (2002). Sources of formaldehyde, other aldehydes and terpenes in a new manufactured house. *Indoor Air* 12: 235-242. <http://dx.doi.org/10.1034/j.1600-0668.2002.01129.x>.

- Hodgson, AT; Rudd, AF; Beal, D; Chandra, S. (2000). Volatile organic compound concentrations and emission rates in new manufactured and site-built houses. *Indoor Air* 10: 178-192.
<http://dx.doi.org/10.1034/j.1600-0668.2000.010003178.x>.
- Hofmaier, S; Comberiati, P; Matricardi, PM. (2014). Immunoglobulin G in IgE-mediated allergy and allergen-specific immunotherapy [Review]. *Eur Ann Allergy Clin Immunol* 46: 6-11.
- Hogan, SP; Mould, AW; Young, JM; Rothenberg, ME; Ramsay, AJ; Matthaei, K; Young, IG; Foster, PS. (1998). Cellular and molecular regulation of eosinophil trafficking to the lung. *Immunol Cell Biol* 76: 454-460. <http://dx.doi.org/10.1046/j.1440-1711.1998.00766.x>.
- Holgate, ST; Samet, JM; Koren, HS; Maynard, RL. (1999). Air pollution and health. San Diego, CA: Academic Press.
- Holmes, BJ; Macary, PA; Noble, A; Kemeny, DM. (1997). Antigen-specific CD8+ T cells inhibit IgE responses and interleukin-4 production by CD4+ T cells. *Eur J Immunol* 27: 2657-2665.
<http://dx.doi.org/10.1002/eji.1830271027>.
- Holmström, M; Rosén, G; Wilhelmsson, B. (1991). Symptoms, airway physiology and histology of workers exposed to medium-density fiber board. *Scand J Work Environ Health* 17: 409-413.
<http://dx.doi.org/10.5271/sjweh.1685>.
- Holmstrom, M; Rynnel-Dagoo, B; Wilhelmsson, B. (1989a). Antibody production in rats after long-term exposure to formaldehyde. *Toxicol Appl Pharmacol* 100: 328-333.
[http://dx.doi.org/10.1016/0041-008X\(89\)90318-9](http://dx.doi.org/10.1016/0041-008X(89)90318-9).
- Holmström, M; Wilhelmsson, B. (1988). Respiratory symptoms and pathophysiological effects of occupational exposure to formaldehyde and wood dust. *Scand J Work Environ Health* 14: 306-311.
- Holmstrom, M; Wilhelmsson, B; Hellquist, H. (1989b). Histological changes in the nasal mucosa in rats after long-term exposure to formaldehyde and wood dust. *Acta Otolaryngol* 108: 274-283.
<http://dx.doi.org/10.3109/00016488909125528>.
- Holmstrom, M; Wilhelmsson, B; Hellquist, H; Rosen, G. (1989c). Histological changes in the nasal mucosa in persons occupationally exposed to formaldehyde alone and in combination with wood dust. *Acta Otolaryngol* 107: 120-129. <http://dx.doi.org/10.3109/00016488909127488>.
- Holness, DL; Nethercott, JR. (1989). Health status of funeral service workers exposed to formaldehyde. *Arch Environ Occup Health* 44: 222-228. <http://dx.doi.org/10.1080/00039896.1989.9935887>.
- Hoogenveen, RT; Clewell, HJ; Andersen, ME; Slob, W. (1999). An alternative exact solution of the two-stage clonal growth model of cancer. *Risk Anal* 19: 9-14. <http://dx.doi.org/10.1111/j.1539-6924.1999.tb00381.x>.
- Hopner, T; Knappe, J. (1974). Formate determination with formate dehydrogenase. In HU Bergmeyer (Ed.), *Methods of Enzymatic Analysis*, 2nd Ed (pp. 1551). New York: Academic Press.
- Horton, AW; Tye, R; Stemmer, KL. (1963). Experimental carcinogenesis of the lung: Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. *J Natl Cancer Inst* 30: 31-40.
<http://dx.doi.org/10.1093/jnci/30.1.31>.
- Horvath, EP, Jr; Anderson, H, Jr; Pierce, WE; Hanrahan, L; Wendlick, JD. (1988). Effects of formaldehyde on the mucous membranes and lungs: A study of an industrial population. *JAMA* 259: 701-707.
<http://dx.doi.org/10.1001/jama.1988.03720050037020>.
- Hosgood, HD, III; Zhang, L; Tang, X; Vermeulen, R; Hao, Z; Shen, M, in; Qiu, C; Ge, Y; Hua, M; Ji, Z; Li, S; Xiong, J, un; Reiss, B; Liu, S; Xin, KX; Azuma, M; Xie, Y; Freeman, LB; Ruan, X; Guo, W; Galvan, N, oe; Blair, A; Li, L; Huang, H; Smith, MT; Rothman, N; Lan, Q. (2013). Occupational exposure to formaldehyde and alterations in lymphocyte subsets. *Am J Ind Med* 56: 252-257.
<http://dx.doi.org/10.1002/ajim.22088>.
- Howarth, PH; Beckett, P; Dahl, R. (2000). The effect of long-acting beta2-agonists on airway inflammation in asthmatic patients [Review]. *Respir Med* 94 Suppl F: S22-S25.

- Hsu, NY; Lee, CC; Wang, JY; Li, YC; Chang, HW; Chen, CY; Bornehag, CG; Wu, PC; Sundell, J; Su, HJ. (2012). Predicted risk of childhood allergy, asthma and reported symptoms using measured phthalate exposure in dust and urine. *Indoor Air* 22: 186–199. <http://dx.doi.org/10.1111/j.1600-0668.2011.00753.x>.
- Huang, C; Liu, W; Cai, J; Wang, X; Zou, Z; Sun, CJ. (2017). Household formaldehyde exposure and its associations with dwelling characteristics, lifestyle behaviours, and childhood health outcomes in Shanghai, China. *Build Environ* 125: 143-152. <http://dx.doi.org/10.1016/j.buildenv.2017.08.042>.
- Huang, H; Hopkins, PB. (1993). DNA interstrand cross-linking by formaldehyde - nucleotide-sequence preference and covalent structure of the predominant cross-link formed in synthetic oligonucleotides. *J Am Chem Soc* 115: 9402-9408. <http://dx.doi.org/10.1021/ja00074a005>.
- Huang, HF; Solomon, MS; Hopkins, PB. (1992). Formaldehyde preferentially interstrand cross-links duplex DNA through deoxyadenosine residues at the sequence 5'-d(AT). *J Am Chem Soc* 114: 9240-9241. <http://dx.doi.org/10.1021/ja00049a097>.
- Huang, J; Lu, Y; Zhang, B; Yang, S; Zhang, Q; Cui, H; Lu, X; Zhao, Y; Yang, X; Li, R. (2019). Antagonistic effect of epigallocatechin-3-gallate on neurotoxicity induced by formaldehyde. *Toxicology* 412: 29-36. <http://dx.doi.org/10.1016/j.tox.2018.10.022>.
- Huber, M; Lohoff, M. (2015). Change of paradigm: CD8+ T cells as important helper for CD4+ T cells during asthma and autoimmune encephalomyelitis [Review]. 24: 8-15. <http://dx.doi.org/10.1007/s40629-015-0038-4>.
- Hulin, M; Caillaud, D; Annesi-Maesano, I. (2010). Indoor air pollution and childhood asthma: variations between urban and rural areas. *Indoor Air* 20: 502-514. <http://dx.doi.org/10.1111/j.1600-0668.2010.00673.x>.
- Hulsmann, AR; Dejongste, JC. (1996). Modulation of airway responsiveness by the airway epithelium in humans: Putative mechanisms. *Clin Exp Allergy* 26: 1236-1242.
- Hummel, T; Livermore, A. (2002). Intranasal chemosensory function of the trigeminal nerve and aspects of its relation to olfaction [Review]. *Int Arch Occup Environ Health* 75: 305-313. <http://dx.doi.org/10.1007/s00420-002-0315-7>.
- Hwang, G; Yoon, C; Choi, J. (2011). A case-control study: exposure assessment of VOCs and formaldehyde for asthma in children. *Aerosol Air Qual Res* 11: 908-914. <http://dx.doi.org/10.4209/aaqr.2011.05.0072>.
- IARC (International Agency for Research on Cancer). (1995). Wood dust and formaldehyde. Lyon, France. <http://monographs.iarc.fr/ENG/Monographs/vol62/index.php>.
- IARC (International Agency for Research on Cancer). (2006). Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol [IARC Monograph] (pp. USD 40). (CIS/07/01141). Lyon, France. <https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Formaldehyde-2-Butoxyethanol-And-1--Em-Tert-Em--Butoxypropan-2-ol-2006>.
- IARC, IAfRoC. (2006). IARC monographs on the evaluation of carcinogenic risks to humans - Formaldehyde, 2-butoxyethanol, and 1--butoxypropan-2-ol (pp. USD 40). (CIS/07/01141). International Agency for Research on Cancer (IARC).
- IARC (International Agency for Research on Cancer). (2012). Formaldehyde [IARC Monograph]. In A review of human carcinogens: Chemical agents and related occupations (pp. 401-435). Lyon, France. <http://monographs.iarc.fr/ENG/Monographs/vol100F/index.php>.
- Iarmarcovai, G; Botta, A; Orsiere, T. (2006). Number of centromeric signals in micronuclei and mechanisms of aneuploidy. *Toxicol Lett* 166: 1-10. <http://dx.doi.org/10.1016/j.toxlet.2006.05.015>.

- [Ibrahim, BS; Barioni, ED; Heluany, C; Braga, TT; Drewes, CC; Costa, SG; Saraiva Camara, NO; Poliselli Farsky, SH; Lino-Dos-Santos-Franco, A.](#) (2016). Beneficial effects of vitamin C treatment on pregnant rats exposed to formaldehyde: Reversal of immunosuppression in the offspring. *Toxicol Appl Pharmacol* 300: 77-81. <http://dx.doi.org/10.1016/j.taap.2016.03.010>.
- [ICRP](#) (International Commission on Radiological Protection). (1994). Human respiratory tract model for radiological protection. *Ann ICRP* 24.
- [Im, H; Oh, E; Mun, J; Khim, JY; Lee, E; Kang, HS; Kim, E; Kim, H; Won, NH; Kim, YH; Jung, WW; Sul, D.](#) (2006). Evaluation of toxicological monitoring markers using proteomic analysis in rats exposed to formaldehyde. *J Proteome Res* 5: 1354-1366. <http://dx.doi.org/10.1021/pr050437b>.
- [Imbus, HR; Tochilin, SJ.](#) (1988). Acute effect upon pulmonary function of low level exposure to phenol-formaldehyde-resin-coated wood. *AIHA J* 49: 434-437. <http://dx.doi.org/10.1080/15298668891380033>.
- [Inoue, K; Nishimukai, H; Yamasawa, K.](#) (1979). Purification and partial characterization of aldehyde dehydrogenase from human erythrocytes. *Biochim Biophys Acta* 569: 117-123. [http://dx.doi.org/10.1016/0005-2744\(79\)90046-9](http://dx.doi.org/10.1016/0005-2744(79)90046-9).
- [Ionescu, J; Marinescu, D; Tapu, V; Eskenasy, A.](#) (1978). Experimental chronic obstructive lung disease: I. Bronchopulmonary changes induced in rabbits by prolonged exposure to formaldehyde. *Morphol Embryol (Bucur)* 24: 233-242.
- [IPCS](#) (International Programme on Chemical Safety). (1989). Environmental Health Criteria 89: Formaldehyde [WHO EHC]. In *Environmental Health Criteria Vol 89. (EHC 89)*. Geneva, Switzerland: World Health Organization. <http://www.inchem.org/documents/ehc/ehc/ehc89.htm>.
- [IPCS](#) (International Programme on Chemical Safety). (2002). Concise International Chemical Assessment Document 40: Formaldehyde. Geneva, Switzerland: World Health Organization. <https://inchem.org/documents/cicads/cicads/cicad40.htm>.
- [IPCS](#) (International Programme on Chemical Safety). (2012). Guidance for immunotoxicity risk assessment for chemicals. (Harmonization Project Document No. 10). Geneva, Switzerland: World Health Organization. <https://apps.who.int/iris/handle/10665/330098>.
- [Isa, KNM; Hashim, Z; Jalaludin, J; Norback, D, an; Jabbar, MA; Hashim, JH.](#) (2020a). The Impact of Exposure to Indoor Pollutants on Allergy and Lung Inflammation among School Children in Selangor, Malaysia: An Evaluation Using Factor Analysis. *Aerosol Air Qual Res* 20: 2371-2383. <http://dx.doi.org/10.4209/aaqr.2020.03.0128>.
- [Isa, KNM; Hashim, Z; Jalaludin, J; Than, LTL; Hashim, JH.](#) (2020b). The effects of indoor pollutants exposure on allergy and lung inflammation: An activation state of neutrophils and eosinophils in sputum. *Int J Environ Res Public Health* 17: 5413. <http://dx.doi.org/10.3390/ijerph17155413>.
- [Ishidate, M, Jr; Sofuni, T; Yoshikawa, K.](#) (1981). Chromosomal aberration tests in vitro as a primary screening tool for environmental mutagens and/or carcinogens. In N Inui; T Kuroki; MA Yamada; C Heidelberger (Eds.), *Mutation, promotion and transformation in vitro* (pp. 95-108). Tokyo, Japan: Japan Scientific Societies Press.
- [Ito, K; Sakamoto, T; Hayashi, Y; Morishita, M; Shibata, E; Sakai, K; Takeuchi, Y; Torii, S.](#) (1996). Role of tachykinin and bradykinin receptors and mast cells in gaseous formaldehyde-induced airway microvascular leakage in rats. *Eur J Pharmacol* 307: 291-298. [http://dx.doi.org/10.1016/0014-2999\(96\)00285-3](http://dx.doi.org/10.1016/0014-2999(96)00285-3).
- [Jacobsen, EA; Lee, NA; Lee, JJ.](#) (2014). Re-defining the unique roles for eosinophils in allergic respiratory inflammation [Review]. *Clin Exp Allergy* 44: 1119-1136. <http://dx.doi.org/10.1111/cea.12358>.
- [Jaeger, RJ; Gearhart, JM.](#) (1982). Respiratory and metabolic response of rats and mice to formalin vapor. *Toxicology* 25: 299-309. [http://dx.doi.org/10.1016/0300-483X\(82\)90108-1](http://dx.doi.org/10.1016/0300-483X(82)90108-1).

- Jakab, GJ. (1992). Relationship between carbon black particulate-bound formaldehyde, pulmonary antibacterial defenses, and alveolar macrophage phagocytosis. *Inhal Toxicol* 4: 325-342. <http://dx.doi.org/10.3109/08958379209145312>.
- Jakab, MG; Klupp, T; Besenyei, K; Biro, A; Major, J; Tompa, A. (2010). Formaldehyde-induced chromosomal aberrations and apoptosis in peripheral blood lymphocytes of personnel working in pathology departments. *Mutat Res* 698: 11-17. <http://dx.doi.org/10.1016/j.mrgentox.2010.02.015>.
- Jakobsson, K; Mikoczy, Z; Skerfving, S. (1997). Deaths and tumours among workers grinding stainless steel: a follow up. *Occup Environ Med* 54: 825-829. <http://dx.doi.org/10.1136/oem.54.11.825>.
- Jantunen, K; Maki-Paakkanen, J; Norppa, H. (1986). Induction of chromosome aberrations by styrene and vinylacetate in cultured human lymphocytes: Dependence on erythrocytes. *Mutat Res* 159: 109-116. [http://dx.doi.org/10.1016/0027-5107\(86\)90119-3](http://dx.doi.org/10.1016/0027-5107(86)90119-3).
- Jensen, DE; Belka, GK; Du Bois, GC. (1998). S-Nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme. *Biochem J* 331: 659-668. <http://dx.doi.org/10.1042/bj3310659>.
- Jensen, KA; Kirk, I; Kølmark, G; Westergaard, M. (1951). Chemically induced mutations in *Neurospora*. *Cold Spring Harb Symp Quant Biol* 16: 245-261. <http://dx.doi.org/10.1101/SQB.1951.016.01.020>.
- Ji, Z; Li, X; Fromowitz, M; Mutter-Rottmayer, E; Tung, J; Smith, MT; Zhang, L. (2014). Formaldehyde induces micronuclei in mouse erythropoietic cells and suppresses the expansion of human erythroid progenitor cells. *Toxicol Lett* 224: 233-239. <http://dx.doi.org/10.1016/j.toxlet.2013.10.028>.
- Jia, X; Jia, Q; Zhang, Z; Gao, W; Zhang, X; Niu, Y; Meng, T; Feng, B; Duan, H; Ye, M; Dai, Y; Jia, Z; Zheng, Y. (2014). Effects of formaldehyde on lymphocyte subsets and cytokines in the peripheral blood of exposed workers. *PLoS ONE* 9: e104069. <http://dx.doi.org/10.1371/journal.pone.0104069>.
- Jiang, J; Zhou, CF; Gao, S; Tian, Y; Wang, C; Wang, L, i; Gu, HF; Tang, XQ. (2015). BDNF-TrkB Pathway Mediates Neuroprotection of Hydrogen Sulfide against Formaldehyde-Induced Toxicity to PC12 Cells. *PLoS ONE* 10: e0119478. <http://dx.doi.org/10.1371/journal.pone.0119478>.
- Jiang, S; Yu, L; Cheng, J; Leng, S; Dai, Y; Zhang, Y; Niu, Y; Yan, H; Qu, W; Zhang, C; Zhang, K; Yang, R; Zhou, L; Zheng, Y. (2010). Genomic damages in peripheral blood lymphocytes and association with polymorphisms of three glutathione S-transferases in workers exposed to formaldehyde. *Mutat Res* 695: 9-15. <http://dx.doi.org/10.1016/j.mrgentox.2009.09.011>.
- Jin, L; Lynch, J; Richardson, A; Lorkiewicz, P; Srivastava, S; Theis, W; Shirk, G; Hand, A; Bhatnagar, A; Srivastava, S; Conklin, DJ. (2021). Electronic Cigarette Solvents, Pulmonary Irritation and Endothelial Dysfunction: Role of Acetaldehyde and Formaldehyde. *Am J Physiol Heart Circ Physiol* 320: H1510-H1525. <http://dx.doi.org/10.1152/ajpheart.00878.2020>.
- Jinot, J; Beane-Freeman, L. (2014). Personal communication Jennifer Jinot and Laura Beane-Freeman. Available online at (accessed
- John, EM; Savitz, DA; Shy, CM. (1994). Spontaneous abortions among cosmetologists. *Epidemiology* 5: 147-155. <http://dx.doi.org/10.1097/00001648-199403000-00004>.
- Johnsen, RC; Baillie, DL. (1988). Formaldehyde mutagenesis of the eT1 balanced region in *Caenorhabditis elegans*: dose-response curve and the analysis of mutational events. *Mutat Res* 201: 137-147. [http://dx.doi.org/10.1016/0027-5107\(88\)90120-0](http://dx.doi.org/10.1016/0027-5107(88)90120-0).
- Joos, GF; Germonpre, PF; Pauwels, RA. (2000). Role of tachykinins in asthma [Review]. *Allergy* 55: 321-337. <http://dx.doi.org/10.1034/j.1398-9995.2000.00112.x>.
- Joos, GF; Germonpre, PR; Kips, JC; Peleman, RA; Pauwels, RA. (1994). SENSORY NEUROPEPTIDES AND THE HUMAN LOWER AIRWAYS - PRESENT STATE AND FUTURE-DIRECTIONS. *Eur Respir J* 7: 1161-1171.

- [Joos, GF; Kips, JC; Peleman, RA; Pauwels, RA.](#) (1995). TACHYKININ ANTAGONISTS AND THE AIRWAYS. Arch Int Pharmacodyn Ther 329: 205-219.
- [Juarez, E; Chambwe, N; Tang, W; Mitchell, AD; Owen, N; Kumari, A; Monnat, RJ; Mccullough, AK.](#) (2018). An RNAi screen in human cell lines reveals conserved DNA damage repair pathways that mitigate formaldehyde sensitivity. DNA Repair 72: 1-9. <http://dx.doi.org/10.1016/j.dnarep.2018.10.002>.
- [Jude, J; Koziol-White, C; Scala, J; Yoo, E; Jester, W; Maute, C; Dalton, P; Panettieri, R.](#) (2016). Formaldehyde Induces Rho-associated Kinase Activity to Evoke Airway Hyperresponsiveness. Am J Respir Cell Mol Biol 55: 542-553. <http://dx.doi.org/10.1165/rcmb.2015-0254OC>.
- [Jung, R; Engelhart, G; Herbolt, B; Jäckh, R; Müller, W.](#) (1992). Collaborative study of mutagenicity with Salmonella typhimurium TA102. Mutat Res 278: 265-270. [http://dx.doi.org/10.1016/S0165-1218\(10\)80006-0](http://dx.doi.org/10.1016/S0165-1218(10)80006-0).
- [Jung, W; Kim, E; Lee, E; Yun, H; Ju, H; Jeong, M; Hwang, K; Sul, D; Kang, H.](#) (2007). Formaldehyde exposure induces airway inflammation by increasing eosinophil infiltrations through the regulation of reactive oxygen species production. Environ Toxicol Pharmacol 24: 174-182. <http://dx.doi.org/10.1016/j.etap.2007.05.001>.
- [Kallen, RG; Jencks, WP.](#) (1966a). The dissociation constants of tetrahydrofolic acid. J Biol Chem 241: 5845-5850.
- [Kallen, RG; Jencks, WP.](#) (1966b). The mechanism of the condensation of formaldehyde with tetrahydrofolic acid. J Biol Chem 241: 5851-5863.
- [Kamata, E; Nakadate, M; Ogawa, Y; Kaneko, T; Kurokawa, Y; Yukawa, M.](#) (1996a). Acute inhalation toxicity study of formaldehyde in rats: Effect of vapor on the pulmonary surfactant. Oyo Yakuri 51: 33-37.
- [Kamata, E; Nakadate, M; Uchida, O; Ogawa, Y; Kaneko, T; Kurokawa, Y.](#) (1996b). Effects of formaldehyde vapor on the nasal cavity and lungs of F-344 rats. J Environ Pathol Toxicol Oncol 15: 1-8.
- [Kamata, E; Nakadate, M; Uchida, O; Ogawa, Y; Suzuki, S; Kaneko, T; Saito, M; Kurokawa, Y.](#) (1997). Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. J Toxicol Sci 22: 239-254.
- [Kane, LE; Alarie, Y.](#) (1977). Sensory irritation to formaldehyde and acrolein during single and repeated exposures in mice. Am Ind Hyg Assoc J 38: 509-522. <http://dx.doi.org/10.1080/0002889778507665>.
- [Kane, LE; Barrow, CS; Alarie, Y.](#) (1979). A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Am Ind Hyg Assoc J 40: 207-229. <http://dx.doi.org/10.1080/15298667991429516>.
- [Kang, J; Duan, J; Song, J; Luo, C; Liu, H; Li, B; Yang, X; Yu, W; Chen, M.](#) (2018). Exposure to a combination of formaldehyde and DINP aggravated asthma-like pathology through oxidative stress and NF-κB activation. Toxicology 404-405: 49-58. <http://dx.doi.org/10.1016/j.tox.2018.05.006>.
- [Kang, S, ukYun; North, J, in; Gaytán, J; Romero, W, oo; De La Rosa, VY; Loguinov, A; Smith, MT; Zhang, L; Vulpe, CD.](#) (2016). Functional Toxicogenomic Profiling Expands Insight into Modulators of Formaldehyde Toxicity in Yeast. Front Genet 7: 200. <http://dx.doi.org/10.3389/fgene.2016.00200>.
- [Kaplan, EL; Meier, P.](#) (1958). Nonparametric estimation from incomplete observations. J Am Stat Assoc 53: 457-481. <http://dx.doi.org/10.1080/01621459.1958.10501452>.
- [Kaplan, WD.](#) (1948). Formaldehyde as a mutagen in Drosophila. Science 108: 43. <http://dx.doi.org/10.1126/science.108.2793.43>.
- [Kastner, PE; Le Calvé, S; Zheng, W; Casset, A; Pons, F.](#) (2013). A dynamic system for single and repeated exposure of airway epithelial cells to gaseous pollutants. Toxicol In Vitro 27: 632-640. <http://dx.doi.org/10.1016/j.tiv.2012.11.011>.

- [Katsnelson, BA; Degtyareva, TD; Privalova, LI; Minigaliyeva, IA; Slyshkina, TV; Ryzhov, VV; Beresneva, OY, u. \(2013\). Attenuation of subchronic formaldehyde inhalation toxicity with oral administration of glutamate, glycine and methionine. *Toxicol Lett* 220: 181-186. <http://dx.doi.org/10.1016/j.toxlet.2013.04.024>.](#)
- [Kauppinen, T; Teschke, K; Savela, A; Kogevinas, M; Boffetta, P. \(1997\). International data base of exposure measurements in the pulp, paper and paper product industries. *Int Arch Occup Environ Health* 70: 119-127. <http://dx.doi.org/10.1007/s004200050195>.](#)
- [Keller, DA; Heck, H; Randall, HW; Morgan, KT. \(1990\). Histochemical localization of formaldehyde dehydrogenase in the rat. *Toxicol Appl Pharmacol* 106: 311-326. \[http://dx.doi.org/10.1016/0041-008x\\(90\\)90250-x\]\(http://dx.doi.org/10.1016/0041-008x\(90\)90250-x\).](#)
- [Kelly, TJ; Smith, DL; Satola, J. \(1999\). Emission rates of formaldehyde from materials and consumer products found in California homes. *Environ Sci Technol* 33: 81-88. <http://dx.doi.org/10.1021/es980592%2B>.](#)
- [Kennedy, G; Slaich, PK; Golding, BT; Watson, WP. \(1996\). Structure and mechanism of formation of a new adduct from formaldehyde and guanosine. *Chem Biol Interact* 102: 93-100. \[http://dx.doi.org/10.1016/S0009-2797\\(96\\)03737-4\]\(http://dx.doi.org/10.1016/S0009-2797\(96\)03737-4\).](#)
- [Kepler, GM; Richardson, RB; Morgan, KT; Kimbell, JS. \(1998\). Computer simulation of inspiratory nasal airflow and inhaled gas uptake in a rhesus monkey. *Toxicol Appl Pharmacol* 150: 1-11. <http://dx.doi.org/10.1006/taap.1997.8350>.](#)
- [Kerfoot, EJ; Mooney, TF. \(1975\). Formaldehyde and paraformaldehyde study in funeral homes. *Am Ind Hyg Assoc J* 36: 533-537. <http://dx.doi.org/10.1080/0002889758507286>.](#)
- [Kerns, WD; Pavkov, KL; Donofrio, DJ; Gralla, EJ; Swenberg, JA. \(1983\). Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Res* 43: 4382-4392.](#)
- [Khaliq, F; Tripathi, P. \(2009\). Acute effects of formalin on pulmonary functions in gross anatomy laboratory. *Indian J Physiol Pharmacol* 53: 93-96.](#)
- [Khamgaonkar, MB; Fulare, MB. \(1991\). Pulmonary effects of formaldehyde exposure--an environmental-epidemiological study. *Indian J Chest Dis Allied Sci* 33: 9-13.](#)
- [Khan, AH. \(1967\). The induction of crossing over in the absence of mutation. *Sind University Science Research Journal* 3: 103-106.](#)
- [Kiernan, JA. \(2000\). Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: What they are and what they do. *Microsc Today* 00-1: 8-12.](#)
- [Kilburn, KH. \(2000\). Indoor air effects after building renovation and in manufactured homes. *Am J Med Sci* 320: 249-254. <http://dx.doi.org/10.1097/00000441-200010000-00005>.](#)
- [Kilburn, KH; McKenzie, WN. \(1978\). Leukocyte recruitment to airways by aldehyde-carbon combinations that mimic cigarette smoke. *Lab Invest* 38: 134-142.](#)
- [Kilburn, KH; Seidman, BC; Warshaw, R. \(1985a\). Neurobehavioral and respiratory symptoms of formaldehyde and xylene exposure in histology technicians. *Arch Environ Occup Health* 40: 229-233. <http://dx.doi.org/10.1080/00039896.1985.10545924>.](#)
- [Kilburn, KH; Warshaw, R; Boylen, CT; Johnson, SJ; Seidman, B; Sinclair, R; Takaro, T. \(1985b\). Pulmonary and neurobehavioral effects of formaldehyde exposure. *Arch Environ Health* 40: 254-260. <http://dx.doi.org/10.1080/00039896.1985.10545928>.](#)
- [Kilburn, KH; Warshaw, R; Thornton, JC. \(1987\). Formaldehyde impairs memory, equilibrium, and dexterity in histology technicians: Effects which persist for days after exposure. *Arch Environ Occup Health* 42: 117-120. <http://dx.doi.org/10.1080/00039896.1987.9935806>.](#)
- [Kilburn, KH; Warshaw, R; Thornton, JC. \(1989a\). Pulmonary function in histology technicians compared with women from Michigan: effects of chronic low dose formaldehyde on a national sample of women. *Br J Ind Med* 46: 468-472. <http://dx.doi.org/10.1136/oem.46.7.468>.](#)

- Kilburn, KH; Warshaw, R; Thornton, JC; Husmark, I. (1989b). An examination of factors that could affect choice reaction time in histology technicians. *Am J Ind Med* 15: 679-686.
<http://dx.doi.org/10.1002/ajim.4700150607>.
- Kilburn, KH; Warshaw, RH. (1992). Neurobehavioral effects of formaldehyde and solvents on histology technicians: Repeated testing across time. *Environ Res* 58: 134-146.
[http://dx.doi.org/10.1016/S0013-9351\(05\)80210-5](http://dx.doi.org/10.1016/S0013-9351(05)80210-5).
- Kim, EM; Lee, HY; Lee, EH; Lee, KM; Park, M; Ji, KY; Jang, JH; Jeong, YH; Lee, KH; Yoon, IJ; Kim, SM; Jeong, MJ; Kim, KD; Kang, HS. (2013a). Formaldehyde exposure impairs the function and differentiation of NK cells. *Toxicol Lett* 223: 154-161. <http://dx.doi.org/10.1016/j.toxlet.2013.09.008>.
- Kim, H; Kim, YD; Cho, SH. (1999). Formaldehyde exposure levels and serum antibodies to formaldehyde-human serum albumin of Korean medical students. *Arch Environ Health* 54: 115-118.
<http://dx.doi.org/10.1080/00039899909602245>.
- Kim, HJ; Kim, CH; Kim, MJ; Ryu, JH; Seong, SY; Kim, S; Lim, SJ; Holtzman, MJ; Yoon, JH. (2015a). The Induction of Pattern-Recognition Receptor Expression against Influenza A Virus through Duox2-Derived Reactive Oxygen Species in Nasal Mucosa. *Am J Respir Cell Mol Biol* 53: 525-535.
<http://dx.doi.org/10.1165/rcmb.2014-0334OC>.
- Kim, JL; Elfman, L; Wieslander, G; Ferm, M; Torén, K; Norbäck, D. (2011). Respiratory health among Korean pupils in relation to home, school and outdoor environment. *J Korean Med Sci* 26: 166-173. <http://dx.doi.org/10.3346/jkms.2011.26.2.166>.
- Kim, JY; Jeong, MS; Park, KY; Seo, SJ. (2013b). Aggravation of atopic dermatitis-like symptoms by consecutive low concentration of formaldehyde exposure in NC/Nga mice [Letter]. *Exp Dermatol* 22: 219-221. <http://dx.doi.org/10.1111/exd.12092>.
- Kim, SM; Hwang, KA; Choi, DW; Choi, KC. (2018). The cigarette smoke components induced the cell proliferation and epithelial to mesenchymal transition via production of reactive oxygen species in endometrial adenocarcinoma cells. *Food Chem Toxicol* 121: 657-665.
<http://dx.doi.org/10.1016/j.fct.2018.09.023>.
- Kim, Y; Jekarl, DW; Kim, J; Kwon, A; Choi, H; Lee, S; Kim, YJ; Kim, HJ; Kim, Y; Oh, IH; Kim, M. (2015b). Genetic and epigenetic alterations of bone marrow stromal cells in myelodysplastic syndrome and acute myeloid leukemia patients. *Stem Cell Research* 14: 177-184.
<http://dx.doi.org/10.1016/j.scr.2015.01.004>.
- Kimbell, JS; Godo, MN; Gross, EA; Joyner, DR; Richardson, RB; Morgan, KT. (1997a). Computer simulation of inspiratory airflow in all regions of the F344 rat nasal passages. *Toxicol Appl Pharmacol* 145: 388-398. <http://dx.doi.org/10.1006/taap.1997.8206>.
- Kimbell, JS; Gross, EA; Joyner, DR; Godo, MN; Morgan, KT. (1993). Application of computational fluid dynamics to regional dosimetry of inhaled chemicals in the upper respiratory tract of the rat. *Toxicol Appl Pharmacol* 121: 253-263. <http://dx.doi.org/10.1006/taap.1993.1152>.
- Kimbell, JS; Gross, EA; Richardson, RB; Conolly, RB; Morgan, KT. (1997b). Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages. *Mutat Res* 380: 143-154.
[http://dx.doi.org/10.1016/S0027-5107\(97\)00132-2](http://dx.doi.org/10.1016/S0027-5107(97)00132-2).
- Kimbell, JS; Overton, JH; Subramaniam, RP; Schlosser, PM; Morgan, KT; Conolly, RB; Miller, FJ. (2001a). Dosimetry modeling of inhaled formaldehyde: Binning nasal flux predictions for quantitative risk assessment. *Toxicol Sci* 64: 111-121.
- Kimbell, JS; Subramaniam, RP. (2001). Use of computational fluid dynamics models for dosimetry of inhaled gases in the nasal passages [Review]. *Inhal Toxicol* 13: 325-334.
<http://dx.doi.org/10.1080/08958370120442>.

- [Kimbell, JS; Subramaniam, RP; Gross, EA; Schlosser, PM; Morgan, KT.](#) (2001b). Dosimetry modeling of inhaled formaldehyde: comparisons of local flux predictions in the rat, monkey, and human nasal passages. *Toxicol Sci* 64: 100-110.
- [Kimura, R; Kimoto, I; Takeda, M; Miyake, M; Sakamoto, T.](#) (2010). Alteration in airway microvascular leakage induced by sensorineural stimulation in rats exposed to inhaled formaldehyde. *Toxicol Lett* 199: 254-260. <http://dx.doi.org/10.1016/j.toxlet.2010.09.007>.
- [Kita, H; Oomichi, S.](#) (1974). Effect of air pollutants on ciliary activity of respiratory tract. *Bull Tokyo Med Dent Univ* 21: 327-343.
- [Kita, T; Fujimura, M; Myou, S; Ishiura, Y; Abo, M; Katayama, N; Nishitsuji, M; Yoshimi, Y; Nomura, S; Oribe, Y; Nakao, S.](#) (2003). Potentiation of allergic bronchoconstriction by repeated exposure to formaldehyde in guinea-pigs in vivo. *Clin Exp Allergy* 33: 1747-1753. <http://dx.doi.org/10.1111/j.1365-2222.2003.01826.x>.
- [Kitaev, EM; Savchenko, ON; Lovchikov, VA; Altukhov, VV; Vishnyakov, YS.](#) (1984). Razvitie zarodyshey i nekotorye pokazateli reproductivnoy funktsii u krys posle ingalyatsionnogo vozdeystviya formal'degida do oplodotvoreniya [Akush Ginekol 10: 49-52.
- [Kitaeva, L; Kitaev, E; Pimenova, M.](#) (1990). Cytopathic and cytogenetic effects of chronic inhalation of formaldehyde on the female rat's germ and marrow cells. *Tsitologiya* 32: 1212-1216.
- [Kitaeva, LV; Mikheeva, EA; Shelomova, LF; Shvartsman, P.](#) (1996). [Genotoxic effect of formaldehyde in somatic human cells in vivo]. *Genetika* 32: 1287-1290.
- [Klein, MD; Sinha, BK; Subramaniam, RP.](#) (2011). Statistical inferences from formaldehyde DNA-protein cross-link data: improving methods for characterization of uncertainty. *J Biopharm Stat* 21: 42-55. <http://dx.doi.org/10.1080/10543400903531601>.
- [Klein, S; Oesch, F.](#) (1990). A new assay for O6-alkylguanine-DNA-alkyltransferase to determine DNA repair capacities using lambda-phage DNA as substrate. *Carcinogenesis* 11: 1771-1774. <http://dx.doi.org/10.1093/carcin/11.10.1771>.
- [Kleinnijenhuis, AJ; Staal, YC; Duistermaat, E; Engel, R; Woutersen, RA.](#) (2013). The determination of exogenous formaldehyde in blood of rats during and after inhalation exposure. *Food Chem Toxicol* 52: 105-112. <http://dx.doi.org/10.1016/j.fct.2012.11.008>.
- [Kligerman, AD; Phelps, MC; Erexson, GL.](#) (1984). Cytogenetic analysis of lymphocytes from rats following formaldehyde inhalation. *Toxicol Lett* 21: 241-246. [http://dx.doi.org/10.1016/0378-4274\(84\)90079-1](http://dx.doi.org/10.1016/0378-4274(84)90079-1).
- [Knudsen, TB; Thomsen, SF; Nolte, H; Backer, V.](#) (2009). A population-based clinical study of allergic and non-allergic asthma. *J Asthma* 46: 91-94. <http://dx.doi.org/10.1080/02770900802524657>.
- [Kölmark, G; Westergaard, M.](#) (1953). Further studies on chemically induced reversions at the adenine locus of *Neurospora*. *Hereditas* 39: 209-224.
- [Kopf, M; Le Gros, G; Bachmann, M; Lamers, MC; Bluethmann, H; Köhler, G.](#) (1993). Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* 362: 245-248. <http://dx.doi.org/10.1038/362245a0>.
- [Korhonen, K; Liukkonen, T; Ahrens, W; Astrakianakis, G; Boffetta, P; Burdorf, A; Heederik, D; Kauppinen, T; Kogevinas, M; Osvoll, P; Rix, BA; Saalo, A; Sunyer, J; Szadkowska-Stanczyk, I; Teschke, K; Westberg, H; Widerkiewicz, K.](#) (2004). Occupational exposure to chemical agents in the paper industry. *Int Arch Occup Environ Health* 77: 451-460. <http://dx.doi.org/10.1007/s00420-004-0530-5>.
- [Koya, T; Miyahara, N; Takeda, K; Matsubara, S; Matsuda, H; Swasey, C; Balhorn, A; Dakhama, A; Gelfand, EW.](#) (2007). CD8+ T cell-mediated airway hyperresponsiveness and inflammation is dependent on CD4+IL-4+ T cells. *J Immunol* 179: 2787-2796. <http://dx.doi.org/10.4049/jimmunol.179.5.2787>.

- [Krakowiak, A; Górski, P; Pazdrak, K; Ruta, U.](#) (1998). Airway response to formaldehyde inhalation in asthmatic subjects with suspected respiratory formaldehyde sensitization. *Am J Ind Med* 33: 274-281. [http://dx.doi.org/10.1002/\(SICI\)1097-0274\(199803\)33:3<274::AID-AJIM9>3.0.CO;2-W](http://dx.doi.org/10.1002/(SICI)1097-0274(199803)33:3<274::AID-AJIM9>3.0.CO;2-W).
- [Kraneveld, AD; Nijkamp, FP.](#) (2001). Tachykinins and neuro-immune interactions in asthma [Review]. *Int Immunopharmacol* 1: 1629-1650. [http://dx.doi.org/10.1016/S1567-5769\(01\)00099-6](http://dx.doi.org/10.1016/S1567-5769(01)00099-6).
- [Kraneveld, AD; van der Kleij, HP; Kool, M; van Houwelingen, AH; Weitenberg, AC; Redegeld, FA; Nijkamp, FP.](#) (2002). Key role for mast cells in nonatopic asthma. *J Immunol* 169: 2044-2053. <http://dx.doi.org/10.4049/jimmunol.169.4.2044>.
- [Krewski, D; Crump, KS; Farmer, J; Gaylor, DW; Howe, R; Portier, C; Salsburg, D; Sielken, RL; Van Ryzin, J.](#) (1983). A comparison of statistical methods for low dose extrapolation utilizing time-to-tumour data. *Fundam Appl Toxicol* 3: 140-160. [http://dx.doi.org/10.1016/S0272-0590\(83\)80075-X](http://dx.doi.org/10.1016/S0272-0590(83)80075-X).
- [Kriebel, D; Myers, D; Cheng, M; Woskie, S; Cocanour, B.](#) (2001). Short-term effects of formaldehyde on peak expiratory flow and irritant symptoms. *Arch Environ Health* 56: 11-18. <http://dx.doi.org/10.1080/00039890109604049>.
- [Kriebel, D; Sama, SR; Cocanour, B.](#) (1993). Reversible pulmonary responses to formaldehyde. A study of clinical anatomy students. *Am Rev Respir Dis* 148: 1509-1515. http://dx.doi.org/10.1164/ajrccm/148.6_Pt_1.1509.
- [Krieger, NR; Megill, JR; Sterling, P.](#) (1983). Granule cells in the rat olfactory tubercle accumulate 3H-gamma-aminobutyric acid. *J Comp Neurol* 215: 465-471. <http://dx.doi.org/10.1002/cne.902150410>.
- [Krzyzanowski, M; Bateson, T.](#) (2023). Correspondence with Dr. Krzyzanowski seeking additional detail regarding HERO ID: 27351 (Krzyzanowski et al., 1990). Available online at (accessed
- [Krzyzanowski, M; Quackenboss, JJ; Lebowitz, MD.](#) (1990). Chronic respiratory effects of indoor formaldehyde exposure. *Environ Res* 52: 117-125. [http://dx.doi.org/10.1016/S0013-9351\(05\)80247-6](http://dx.doi.org/10.1016/S0013-9351(05)80247-6).
- [Kuehner, S; Holzmänn, K; Speit, G.](#) (2013). Characterization of formaldehyde's genotoxic mode of action by gene expression analysis in TK6 cells. *Arch Toxicol* 87: 1999-2012. <http://dx.doi.org/10.1007/s00204-013-1060-2>.
- [Kuehner, S; Schlaier, M; Schwarz, K; Speit, G.](#) (2012). Analysis of leukemia-specific aneuploidies in cultured myeloid progenitor cells in the absence and presence of formaldehyde exposure. *Toxicol Sci* 128: 72-78. <http://dx.doi.org/10.1093/toxsci/kfs126>.
- [Kulle, TJ.](#) (1993). Acute odor and irritation response in healthy nonsmokers with formaldehyde exposure. *Inhal Toxicol* 5: 323-332. <http://dx.doi.org/10.3109/08958379308998389>.
- [Kulle, TJ; Cooper, GP.](#) (1975). Effects of formaldehyde and ozone on the trigeminal nasal sensory system. *Arch Environ Occup Health* 30: 237-243.
- [Kulle, TJ; Sauder, LR; Hebel, JR; Green, DJ; Chatham, MD.](#) (1987). Formaldehyde dose-response in healthy nonsmokers. *J Air Pollut Control Assoc* 37: 919-924. <http://dx.doi.org/10.1080/08940630.1987.10466285>.
- [Kum, C; Kiral, F; Sekkin, S; Seyrek, K; Boyacioglu, M.](#) (2007a). Effects of xylene and formaldehyde inhalations on oxidative stress in adult and developing rats livers. *Exp Anim* 56: 35-42. <http://dx.doi.org/10.1538/expanim.56.35>.
- [Kum, C; Sekkin, S; Kiral, F; Akar, F.](#) (2007b). Effects of xylene and formaldehyde inhalations on renal oxidative stress and some serum biochemical parameters in rats. *Toxicol Ind Health* 23: 115-120. <http://dx.doi.org/10.1177/0748233707078218>.
- [Kumari, A; Lim, YX; Newell, AH; Olson, SB; Mccullough, AK.](#) (2012). Formaldehyde-induced genome instability is suppressed by an XPF-dependent pathway. *DNA Repair* 11: 236-246. <http://dx.doi.org/10.1016/j.dnarep.2011.11.001>.

- [Kunkler, PE; Ballard, CJ; Oxford, GS; Hurley, JH.](#) (2011). TRPA1 receptors mediate environmental irritant-induced meningeal vasodilatation. *Pain* 152: 38-44.
<http://dx.doi.org/10.1016/j.pain.2010.08.021>.
- [Kuo, HW; Jian, GJ; Chen, CL; Liu, CS; Lai, JS.](#) (1997). White blood cell count as an indicator of formaldehyde exposure. *Bull Environ Contam Toxicol* 59: 261-267.
<http://dx.doi.org/10.1007/s001289900473>.
- [Kuper, CF; van Oostrum, L; Ma-Hock, L; Durrer, S; Woutersen, RA.](#) (2011). Hyperplasia of the lymphoepithelium of NALT in rats but not in mice upon 28-day exposure to 15 ppm formaldehyde vapor. *Exp Toxicol Pathol* 63: 25-32. <http://dx.doi.org/10.1016/j.etp.2009.09.004>.
- [Kurtio, P; Norppa, H; Jarventaus, H; Sorsa, M; Kalliokoski, P.](#) (1993). Chromosome aberrations in peripheral lymphocytes of workers employed in the plywood industry. *Scand J Work Environ Health* 19: 132-134. <http://dx.doi.org/10.5271/sjweh.1495>.
- [Kushch, I; Schwarz, K; Schwentner, L; Baumann, B; Dzien, A; Schmid, A; Unterkofler, K; Gastl, G; Španel, P; Smith, D; Amann, A.](#) (2008). Compounds enhanced in a mass spectrometric profile of smokers' exhaled breath versus non-smokers as determined in a pilot study using PTR-MS. *J Breath Res* 2: 026002. <http://dx.doi.org/10.1088/1752-7155/2/2/026002>.
- [Kuykendall, JR; Bogdanffy, MS.](#) (1992). Efficiency of DNA-histone crosslinking induced by saturated and unsaturated aldehydes in vitro. *DNA Repair* 283: 131-136. [http://dx.doi.org/10.1016/0165-7992\(92\)90145-8](http://dx.doi.org/10.1016/0165-7992(92)90145-8).
- [Ladeira, C; Viegas, S; Carolino, E; Gomes, MC; Brito, M.](#) (2013). The influence of genetic polymorphisms in XRCC3 and ADH5 genes on the frequency of genotoxicity biomarkers in workers exposed to formaldehyde. *Environ Mol Mutagen* 54: 213-221. <http://dx.doi.org/10.1002/em.21755>.
- [Ladeira, C; Viegas, S; Carolino, E; Prista, J; Gomes, MC; Brito, M.](#) (2011). Genotoxicity biomarkers in occupational exposure to formaldehyde--the case of histopathology laboratories. *Mutat Res* 721: 15-20. <http://dx.doi.org/10.1016/j.mrgentox.2010.11.015>.
- [Laforest, L; Luce, D; Goldberg, P; Bégin, D; Gérin, M; Demers, PA; Brugère, J; Leclerc, A.](#) (2000). Laryngeal and hypopharyngeal cancers and occupational exposure to formaldehyde and various dusts: A case-control study in France. *Occup Environ Med* 57: 767-773.
<http://dx.doi.org/10.1136/oem.57.11.767>.
- [Lai, Y; Yu, R; Hartwell, HJ; Moeller, BC; Bodnar, WM; Swenberg, JA.](#) (2016). Measurement of Endogenous versus Exogenous Formaldehyde-Induced DNA-Protein Crosslinks in Animal Tissues by Stable Isotope Labeling and Ultrasensitive Mass Spectrometry. *Cancer Res* 76: 2652-2661.
<http://dx.doi.org/10.1158/0008-5472.CAN-15-2527>.
- [Lajoie, P; Aubin, D; Gingras, V; Daigneault, P; Ducharme, F; Gauvin, FD; Fugler, D; Leclerc, JM; Won, D; Won, D; Courteau, M; Gingras, S; Héroux, MÈ; Yang, W; Schleibinger, H.](#) (2014). The iVaire project - a randomized controlled study of the impact of ventilation on indoor air quality and the respiratory symptoms of asthmatic children in single family homes. *Indoor Air* 25: 582-597.
<http://dx.doi.org/10.1111/ina.12181>.
- [Lam, CW; Casanova, M; Heck, H.](#) (1985). Depletion of nasal mucosal glutathione by acrolein and enhancement of formaldehyde-induced DNA-protein cross-linking by simultaneous exposure to acrolein. *Arch Toxicol* 58: 67-71. <http://dx.doi.org/10.1007/bf00348311>.
- [Lamb, B; Westberg, H; Bryant, P; Dean, J; Mullins, S.](#) (1985). Air infiltration rates in pre- and post-weatherized houses. *J Air Pollut Control Assoc* 35: 545-551.
<http://dx.doi.org/10.1080/00022470.1985.10465931>.
- [Lambrecht, BN; Hammad, H.](#) (2012). The airway epithelium in asthma. *Nat Med* 18: 684-692.
<http://dx.doi.org/10.1038/nm.2737>.
- [Lan, Q; Smith, MT; Tang, X; Guo, W; Vermeulen, R; Ji, Z; Hu, W; Hubbard, AE; Min, S; Mchale, CM; Qiu, C; Liu, S; Reiss, B; Beane Freeman, L; Blair, A; Ge, Y; Xiong, J; Li, L; Rappaport, SM; Huang, H;](#)

- [Rothman, N; Zhang, L.](#) (2015). Chromosome-wide aneuploidy study (CWAS) of cultured circulating myeloid progenitor cells from workers occupationally exposed to formaldehyde. *Carcinogenesis* 36: 160-167. <http://dx.doi.org/10.1093/carcin/bgu229>.
- [Lang, I; Bruckner, T; Triebig, G.](#) (2008). Formaldehyde and chemosensory irritation in humans: A controlled human exposure study. *Regul Toxicol Pharmacol* 50: 23-36. <http://dx.doi.org/10.1016/j.yrtph.2007.08.012>.
- [Larsen, ST; Wolkoff, P; Hammer, M; Kofoed-Sørensen, V; Clausen, PA; Nielsen, GD.](#) (2013). Acute airway effects of airborne formaldehyde in sensitized and non-sensitized mice housed in a dry or humid environment. *Toxicol Appl Pharmacol* 268: 294-299. <http://dx.doi.org/10.1016/j.taap.2013.02.006>.
- [Lazenby, V; Hinwood, A; Callan, A; Franklin, P.](#) (2012). Formaldehyde personal exposure measurements and time weighted exposure estimates in children. *Chemosphere* 88: 966-973. <http://dx.doi.org/10.1016/j.chemosphere.2012.03.029>.
- [Lazutka, JR; Lekevicius, R; Dedonyte, V; Maciuleviciute-Gervers, L; Mierauskiene, J; Rudaitiene, S; Slapsyte, G.](#) (1999). Chromosomal aberrations and sister-chromatid exchanges in Lithuanian populations: Effects of occupational and environmental exposures. *Mutat Res Genet Toxicol Environ Mutagen* 445: 225-239. [http://dx.doi.org/10.1016/S1383-5718\(99\)00128-X](http://dx.doi.org/10.1016/S1383-5718(99)00128-X).
- [Le Curieux, F; Marzin, D; Erb, F.](#) (1993). Comparison of three short-term assays: Results on seven chemicals. Potential contribution to the control of water genotoxicity. *Mutat Res* 319: 223-236. [http://dx.doi.org/10.1016/0165-1218\(93\)90082-O](http://dx.doi.org/10.1016/0165-1218(93)90082-O).
- [Leal, MP; Brochetti, RA; Ignácio, A; Câmara, NOS; da Palma, RK; de Oliveira, LVF; de Fátima Teixeira da Silva, D; Lino-Dos-Santos-Franco, A.](#) (2018). Effects of formaldehyde exposure on the development of pulmonary fibrosis induced by bleomycin in mice. *Toxicol Rep* 5: 512-520. <http://dx.doi.org/10.1016/j.toxrep.2018.03.016>.
- [Leclerc, A; Martinez Cortes, M; Gérin, M; Luce, D; Brugère, J.](#) (1994). Sinonasal cancer and wood dust exposure: Results from a case-control study. *Am J Epidemiol* 140: 340-349. <http://dx.doi.org/10.1093/oxfordjournals.aje.a117256>.
- [Lee, HK; Alarie, Y; Karol, MH.](#) (1984). Induction of formaldehyde sensitivity in guinea pigs. *Toxicol Appl Pharmacol* 75: 147-155. [http://dx.doi.org/10.1016/0041-008X\(84\)90085-1](http://dx.doi.org/10.1016/0041-008X(84)90085-1).
- [Leikauf, GD.](#) (1992). Mechanisms of aldehyde-induced bronchial reactivity: Role of airway epithelium (pp. 1-35). (Research Report Number 49). Boston, MA: Health Effects Institute.
- [Lemus, R; Abdelghani, AA; Akers, TG; Horner, WE.](#) (1998). Potential health risks from exposure to indoor formaldehyde. *Rev Environ Health* 13: 91-98.
- [Leng, J; Liu, CW; Hartwell, HJ; Yu, R; Lai, Y; Bodnar, WM; Lu, K; Swenberg, JA.](#) (2019). Evaluation of inhaled low-dose formaldehyde-induced DNA adducts and DNA-protein cross-links by liquid chromatography-tandem mass spectrometry. *Arch Toxicol* 93: 763-773. <http://dx.doi.org/10.1007/s00204-019-02393-x>.
- [Levandowski, RA; Ou, DW; Jackson, GG.](#) (1986). Acute-phase decrease of T lymphocyte subsets in rhinovirus infection. *J Infect Dis* 153: 743-748. <http://dx.doi.org/10.1093/infdis/153.4.743>.
- [Levine, RJ; Andjelkovich, DA; Shaw, LK.](#) (1984a). The mortality of Ontario (Canada) undertakers and a review of a formaldehyde-related mortality studies. *J Occup Med* 26: 740-746.
- [Levine, RJ; Andjelkovich, DA; Shaw, LK.](#) (1984b). The mortality of Ontario undertakers and a review of formaldehyde-related mortality studies [Review]. *J Occup Environ Med* 26: 740-746. <http://dx.doi.org/10.1097/00043764-198410000-00014>.
- [Levine, RJ; Dalcorsio, RD; Blunden, PB; Battigelli, MC.](#) (1984c). The effects of occupational exposure on the respiratory health of West Virginia morticians. *J Occup Med* 26: 91-98.

- [Levy, S; Nocentini, S; Billardon, C.](#) (1983). Induction of cytogenetic effects in human fibroblast cultures after exposure to formaldehyde or X-rays. *Mutat Res* 119: 309-317.
[http://dx.doi.org/10.1016/0165-7992\(83\)90179-3](http://dx.doi.org/10.1016/0165-7992(83)90179-3).
- [Li, AM; Fung, CK; Yu, IT; Goggins, WB; Chan, GY; Chan, CK; Lau, AP; Leung, JO.](#) (2019). Associations of wheeze during the first 18 months of life with indoor nitrogen dioxide, formaldehyde, and family history of asthma: a prospective cohort study. *Hong Kong Med J* 25 Suppl 3: 20-23.
- [Li, F; Qin, Y; Gong, S; Zhang, H; Ding, S.](#) (2020). Learning and memory impairment of mice caused by gaseous formaldehyde. *Environ Res* 184: 109318.
<http://dx.doi.org/10.1016/j.envres.2020.109318>.
- [Li, G; Yang, J; Ling, S.](#) (2015). Formaldehyde exposure alters miRNA expression profiles in the olfactory bulb. *Inhal Toxicol* 27: 1-7. <http://dx.doi.org/10.3109/08958378.2015.1062580>.
- [Li, GY; Lee, HY; Choi, YJ; Lee, MO; Shin, HS; Kim, HY; Lee, SB; Lee, BH.](#) (2008). Changes in the Expression of Ras-family Genes in Rats Exposed to Formaldehyde by Inhalation. *Toxicological Research* 24: 201-206. <http://dx.doi.org/10.5487/TR.2008.24.3.201>.
- [Li, L; Hua, L; He, Y; Bao, Y.](#) (2017). Differential effects of formaldehyde exposure on airway inflammation and bronchial hyperresponsiveness in BALB/c and C57BL/6 mice. *PLoS ONE* 12: e0179231.
<http://dx.doi.org/10.1371/journal.pone.0179231>.
- [Li, L; Xia, Y; Nguyen, A; Lai, YH; Feng, L; Mosmann, TR; Lo, D.](#) (1999). Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potently induces eotaxin expression by airway epithelial cells. *J Immunol* 162: 2477-2487.
- [Li, R; Lu, ZS; Qiao, Y; Yao, HC; Yu, FF; Yang, X.](#) (2004). Study on the formaldehyde-induced DNA damage with comet assay. *Shi Yan Sheng Wu Xue Bao* 37: 262-268.
- [Li, W; Ray, RM; Gao, DL; Fitzgibbons, ED; Seixas, NS; Camp, JE; Wernli, KJ; Astrakianakis, G; Feng, Z; Thomas, DB; Checkoway, H.](#) (2006). Occupational risk factors for nasopharyngeal cancer among female textile workers in Shanghai, China. *Occup Environ Med* 63: 39-44.
<http://dx.doi.org/10.1136/oem.2005.021709>.
- [Li, Y; Song, Z; Ding, Y; Xin, Y, e; Wu, T; Su, T, ao; He, R; Tai, F; Lian, Z.](#) (2016). Effects of formaldehyde exposure on anxiety-like and depression-like behavior, cognition, central levels of glucocorticoid receptor and tyrosine hydroxylase in mice. *Chemosphere* 144: 2004-2012.
<http://dx.doi.org/10.1016/j.chemosphere.2015.10.102>.
- [Liao, S; Jiang, L; Zhang, X.](#) (2010). [Effects of inhaled formaldehyde on learning and memory and expression of CaMK II in hippocampus of Wistar rats of different ages]. *Chongqing Yike Daxue Xuebao* 35: 342-344.
- [Liber, HL; Benforado, K; Crosby, RM; Simpson, D; Skopek, TR.](#) (1989). Formaldehyde-induced and spontaneous alterations in human hprt DNA sequence and mRNA expression. *Mutat Res* 226: 31-37. [http://dx.doi.org/10.1016/0165-7992\(89\)90089-4](http://dx.doi.org/10.1016/0165-7992(89)90089-4).
- [LICM](#) (Lithium Ion Cell Manufacturers' Coalition). (2006). Studies on formation and repair of formaldehyde-damaged DNA by detection of DNA-protein crosslinks and DNA breaks. *Front Biosci* 11: 991-997. <http://dx.doi.org/10.2741/1856>.
- [LICM](#) (Lithium Ion Cell Manufacturers' Coalition). (2008). Effect of inhaled formaldehyde on learning and memory of mice. *Indoor Air* 18: 77-83. <http://dx.doi.org/10.1111/j.1600-0668.2008.00524.x>.
- [Liebling, T; Rosenman, KD; Pastides, H; Griffith, RG; Lemeshow, S.](#) (1984). Cancer mortality among workers exposed to formaldehyde. *Am J Ind Med* 5: 423-428.
<http://dx.doi.org/10.1002/ajim.4700050602>.
- [Lilly, CM; Hall, AE; Rodger, IW; Kobzik, L; Haley, KJ; Drazen, JM.](#) (1995). Substance P-induced histamine release in tracheally perfused guinea pig lungs. *J Appl Physiol* (1985) 78: 1234-1241.
<http://dx.doi.org/10.1152/jappl.1995.78.4.1234>.

- [Lima, LF; Murta, GL; Bandeira, AC; Nardeli, CR; Lima, WG; Bezerra, FS.](#) (2015). Short-term exposure to formaldehyde promotes oxidative damage and inflammation in the trachea and diaphragm muscle of adult rats. *Ann Anat* 202: 45-51. <http://dx.doi.org/10.1016/j.aanat.2015.08.003>.
- [Lin, D; Guo, Y; Yi, J; Kuang, D, an; Li, X; Deng, H; Huang, K, un; Guan, L, ei; He, Y; Zhang, X; Hu, D, ie; Zhang, Z; Zheng, H; Zhang, X; Mchale, CM; Zhang, L; Wu, T.](#) (2013). Occupational exposure to formaldehyde and genetic damage in the peripheral blood lymphocytes of plywood workers. *J Occup Health* 55: 284-291. <http://dx.doi.org/10.1539/joh.12-0288-OA>.
- [Lin, Z; Luo, W; Li, H; Zhang, Y.](#) (2005). The effect of endogenous formaldehyde on the rat aorta endothelial cells. *Toxicol Lett* 159: 134-143. <http://dx.doi.org/10.1016/j.toxlet.2005.05.003>.
- [Lindbohm, ML; Hemminki, K; Bonhomme, MG; Anttila, A; Rantala, K; Heikkila, P; Rosenberg, MJ.](#) (1991). Effects of paternal occupational exposure on spontaneous abortions. *Am J Public Health* 81: 1029-1033. <http://dx.doi.org/10.2105/ajph.81.8.1029>.
- [Lindstrom, AB; Proffitt, D; Fortune, CR.](#) (1995). Effects of modified residential construction on indoor air quality. *Indoor Air* 5: 258-269. <http://dx.doi.org/10.1111/j.1600-0668.1995.00005.x>.
- [Lino-Dos-Santos-Franco, A; Amemiya, RM; de Oliveira, AP; Damazo, AS; Breithaupt-Faloppa, AC; Vitoretti, LB; Acceturi, BG; Tavares-De-Lima, W.](#) (2013a). The putative role of ovary removal and progesterone when considering the effect of formaldehyde exposure on lung inflammation induced by ovalbumin. *Clinics* 68: 1528-1536. [http://dx.doi.org/10.6061/clinics/2013\(12\)09](http://dx.doi.org/10.6061/clinics/2013(12)09).
- [Lino-Dos-Santos-Franco, A; Amemiya, RM; Ligeiro de Oliveira, AP; Breithaupt-Faloppa, AC; Damazo, AS; Oliveira-Filho, RM; Tavares-De-Lima, W.](#) (2011a). Differential effects of female sex hormones on cellular recruitment and tracheal reactivity after formaldehyde exposure. *Toxicol Lett* 205: 327-335. <http://dx.doi.org/10.1016/j.toxlet.2011.06.023>.
- [Lino-Dos-Santos-Franco, A; Correa-Costa, M; Durão, AC; de Oliveira, AP; Breithaupt-Faloppa, AC; Bertoni, J; Oliveira-Filho, RM; Câmara, NO; Marcourakis, T; Tavares-De-Lima, W.](#) (2011b). Formaldehyde induces lung inflammation by an oxidant and antioxidant enzymes mediated mechanism in the lung tissue. *Toxicol Lett* 207: 278-285. <http://dx.doi.org/10.1016/j.toxlet.2011.09.026>.
- [Lino-Dos-Santos-Franco, A; Domingos, HV; de Oliveira, AP; Breithaupt-Faloppa, AC; Peron, JP; Bolonheis, S; Muscará, MN; Oliveira-Filho, RM; Vargaftig, BB; Tavares-De-Lima, W.](#) (2010). Differential effects of formaldehyde exposure on the cell influx and vascular permeability in a rat model of allergic lung inflammation. *Toxicol Lett* 197: 211-218.
- [Lino-Dos-Santos-Franco, A; Gimenes-Júnior, JA; Ligeiro-De-Oliveira, AP; Breithaupt-Faloppa, AC; Acceturi, BG; Vitoretti, LB; Machado, ID; Oliveira-Filho, RM; Farsky, SHP; Moriya, HT; Tavares-De-Lima, W.](#) (2013b). Formaldehyde inhalation reduces respiratory mechanics in a rat model with allergic lung inflammation by altering the nitric oxide/cyclooxygenase-derived products relationship. *Food Chem Toxicol* 59: 731-738. <http://dx.doi.org/10.1016/j.fct.2013.07.027>.
- [Lino dos Santos Franco, A; Damazo, AS; Beraldo de Souza, HR; Domingos, HV; Oliveira-Filho, RM; Oliani, SM; Costa, SK; Tavares de Lima, W.](#) (2006). Pulmonary neutrophil recruitment and bronchial reactivity in formaldehyde-exposed rats are modulated by mast cells and differentially by neuropeptides and nitric oxide. *Toxicol Appl Pharmacol* 214: 35-42. <http://dx.doi.org/10.1016/j.taap.2005.11.014>.
- [Lino dos Santos Franco, A; Domingos, HV; Damazo, AS; Breithaupt-Faloppa, AC; de Oliveira, AP; Costa, SK; Oliani, SM; Oliveira-Filho, RM; Vargaftig, BB; Tavares-De-Lima, W.](#) (2009). Reduced allergic lung inflammation in rats following formaldehyde exposure: Long-term effects on multiple effector systems. *Toxicology* 256: 157-163. <http://dx.doi.org/10.1016/j.tox.2008.11.011>.
- [Liteplo, RG; Meek, ME.](#) (2003). Inhaled formaldehyde: Exposure estimation, hazard characterization, and exposure-response analysis [Review]. *J Toxicol Environ Health B Crit Rev* 6: 85-114. <http://dx.doi.org/10.1080/10937400306480>.

- [Liu, D; Zheng, Y; Li, B; Yao, H; Li, R; Zhang, Y; Yang, X.](#) (2011). Adjuvant effects of gaseous formaldehyde on the hyper-responsiveness and inflammation in a mouse asthma model immunized by ovalbumin. *J Immunotoxicol* 8: 305-314. <http://dx.doi.org/10.3109/1547691X.2011.600738>.
- [Liu, KS; Huang, FY; Hayward, SB; Wesolowski, J; Sexton, K.](#) (1991). Irritant effects of formaldehyde exposure in mobile homes. *Environ Health Perspect* 94: 91-94. <http://dx.doi.org/10.2307/3431298>.
- [Liu, L; Huang, Y; Feng, X; Chen, J; Duan, Y.](#) (2019). Overexpressed Hsp70 alleviated formaldehyde-induced apoptosis partly via PI3K/Akt signaling pathway in human bronchial epithelial cells. *Environ Toxicol* 34: 495-504. <http://dx.doi.org/10.1002/tox.22703>.
- [Liu, QB; Wang, W; Jing, W.](#) (2018a). Indoor air pollution aggravates asthma in Chinese children and induces the changes in serum level of miR-155. *Int J Environ Health Res* 29: 1-9. <http://dx.doi.org/10.1080/09603123.2018.1506569>.
- [Liu, QP; Zhou, DX; Lv, MQ; Ge, P; Li, YX; Wang, SJ.](#) (2018b). Formaldehyde inhalation triggers autophagy in rat lung tissues. *Toxicol Ind Health* 74: 2337-2347. <http://dx.doi.org/10.1177/0748233718796347>.
- [Liu, W; Zhang, J; Hashim, JH; Jalaludin, J; Hashim, Z; Goldstein, BD.](#) (2003). Mosquito coil emissions and health implications. *Environ Health Perspect* 111: 1454-1460. <http://dx.doi.org/10.1289/ehp.6286>.
- [Liu, Y, i; Ye, Z; Luo, H; Sun, M; Li, M, i; Fan, D; Chui, D.](#) (2009a). Inhalative formaldehyde exposure enhances aggressive behavior and disturbs monoamines in frontal cortex synaptosome of male rats. *Neurosci Lett* 464: 113-116. <http://dx.doi.org/10.1016/j.neulet.2009.06.037>.
- [Liu, Y; Ye, Z; Yang, H; Zhou, L; Fan, D; He, S; Chui, D.](#) (2010). Disturbances of soluble N-ethylmaleimide-sensitive factor attachment proteins in hippocampal synaptosomes contribute to cognitive impairment after repetitive formaldehyde inhalation in male rats. *Neuroscience* 169: 1248-1254. <http://dx.doi.org/10.1016/j.neuroscience.2010.05.061>.
- [Liu, Y; Yu, D; Xiao, S.](#) (2017). Effects of chronic exposure to Formaldehyde on micronucleus rate of bone marrow cells in male mice. *J Pak Med Assoc* 67: 933-935.
- [Liu, YR; Zhou, Y; Qiu, W; Zeng, JY; Shen, LL; Li, AP; Zhou, JW.](#) (2009b). Exposure to formaldehyde induces heritable DNA mutations in mice. *J Toxicol Environ Health A* 72: 767-773. <http://dx.doi.org/10.1080/15287390902841615>.
- [Löfstedt, H; Westberg, H; Seldén, AI; Bryngelsson, IL; Svartengren, M.](#) (2011a). Respiratory symptoms and lung function in foundry workers using the hot box method: A 4-year follow-up. *J Occup Environ Med* 53: 1425-1429. <http://dx.doi.org/10.1097/JOM.0b013e3182363c17>.
- [Löfstedt, H; Westberg, H; Seldén, AI; Lundholm, C; Svartengren, M.](#) (2009). Respiratory symptoms and lung function in foundry workers exposed to low molecular weight isocyanates. *Am J Ind Med* 52: 455-463. <http://dx.doi.org/10.1002/ajim.20693>.
- [Löfstedt, H; Westberg, H; Seldén, AI; Rudblad, S; Bryngelsson, IL; Ngo, Y; Svartengren, M.](#) (2011b). Nasal and ocular effects in foundry workers using the hot box method. *J Occup Environ Med* 53: 43-48. <http://dx.doi.org/10.1097/JOM.0b013e318181ff05cc>.
- [Logue, J; Barrick, M; Jessup, G.](#) (1986). Mortality of radiologists and pathologists in the radiation registry of physicians. *J Occup Med* 28: 91-99.
- [Lorenti Garcia, C; Mechilli, M; Proietti De Santis, L; Schinoppi, A; Kobos, K; Palitti, F.](#) (2009). Relationship between DNA lesions, DNA repair and chromosomal damage induced by acetaldehyde. *Mutat Res* 662: 3-9. <http://dx.doi.org/10.1016/j.mrfmmm.2008.11.008>.
- [Lourenço, O; Fonseca, AM; Taborda-Barata, L.](#) (2016). Human CD8+ T Cells in Asthma: Possible Pathways and Roles for NK-Like Subtypes [Review]. *7*: 638. <http://dx.doi.org/10.3389/fimmu.2016.00638>.

- Lovreglio, P; Carrus, A; Iavicoli, S; Drago, I; Persechino, B; Soleo, L. (2009). Indoor formaldehyde and acetaldehyde levels in the province of Bari, South Italy, and estimated health risk. *J Environ Monit* 11: 955-961. <http://dx.doi.org/10.1039/b819843h>.
- Lu, K. (2009) Molecular binding of formaldehyde to dna and proteins. (Doctoral Dissertation). University of North Carolina at Chapel Hill, Chapel Hill, NC.
- Lu, K; Boysen, G; Gao, L; Collins, LB; Swenberg, JA. (2008). Formaldehyde-induced histone modifications in vitro. *Chem Res Toxicol* 21: 1586-1593. <http://dx.doi.org/10.1021/tx8000576>.
- Lu, K; Collins, LB; Ru, H; Bermudez, E; Swenberg, JA. (2010a). Distribution of DNA adducts caused by inhaled formaldehyde is consistent with induction of nasal carcinoma but not leukemia. *Toxicol Sci* 116: 441-451. <http://dx.doi.org/10.1093/toxsci/kfq061>.
- Lu, K; Craft, S; Nakamura, J; Moeller, BC; Swenberg, JA. (2012a). Use of LC-MS/MS and stable isotopes to differentiate hydroxymethyl and methyl DNA adducts from formaldehyde and nitrosodimethylamine. *Chem Res Toxicol* 25: 664-675. <http://dx.doi.org/10.1021/tx200426b>.
- Lu, K; Gul, H; Upton, PB; Moeller, BC; Swenberg, JA. (2012b). Formation of hydroxymethyl DNA adducts in rats orally exposed to stable isotope labeled methanol. *Toxicol Sci* 126: 28-38. <http://dx.doi.org/10.1093/toxsci/kfr328>.
- Lu, K; Moeller, B; Doyle-Eisele, M; McDonald, J; Swenberg, JA. (2011). Molecular dosimetry of N2-hydroxymethyl-dG DNA adducts in rats exposed to formaldehyde. *Chem Res Toxicol* 24: 159-161. <http://dx.doi.org/10.1021/tx1003886>.
- Lu, Y; Wang, D; Ma, C; Yang, H. (2010b). The effect of activated carbon adsorption on the photocatalytic removal of formaldehyde. *Build Environ* 45: 615-621. <http://dx.doi.org/10.1016/j.buildenv.2009.07.019>.
- Lu, Z; Li, CM; Qiao, Y; Liu, Y; Yan, Y; Yang, X. (2005). Type II vanilloid receptor signaling system: One of the possible mechanisms for the rise in asthma cases. *Front Biosci* 10: 2527-2533. <http://dx.doi.org/10.2741/1717>.
- Luce, D; Gérin, M; Leclerc, A; Morcet, JF; Brugère, J; Goldberg, M. (1993). Sinonasal cancer and occupational exposure to formaldehyde and other substances. *Int J Cancer* 53: 224-231. <http://dx.doi.org/10.1002/ijc.2910530209>.
- Luce, D; Leclerc, A; Bégin, D; Demers, PA; Gérin, M; Orłowski, E; Kogevinas, M; Belli, S; Bugel, I; Bolm-Audorff, U; Brinton, LA; Comba, P; Hardell, L; Hayes, RB; Magnani, C; Merler, E; Preston-Martin, S; Vaughan, TL; Zheng, W; Boffetta, P. (2002). Sinonasal cancer and occupational exposures: a pooled analysis of 12 case-control studies. *Cancer Causes Control* 13: 147-157. <http://dx.doi.org/10.1023/A:1014350004255>.
- Luce, D; Leclerc, A; Morcet, JF; Casal-Lareo, A; Gérin, M; Brugère, J; Haguenoer, JM; Goldberg, M. (1992). Occupational risk factors for sinonasal cancer: A case-control study in France. *Am J Ind Med* 21: 163-175. <http://dx.doi.org/10.1002/ajim.4700210206>.
- Luebeck, EG; Buchmann, A; Stinchcombe, S; Moolgavkar, SH; Schwarz, M. (2000). Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on initiation and promotion of GST-P-positive foci in rat liver: A quantitative analysis of experimental data using a stochastic model. *Toxicol Appl Pharmacol* 167: 63-73. <http://dx.doi.org/10.1006/taap.2000.8980>.
- Luebeck, EG; Grasl-Kraupp, B; Timmermann-Trosiener, I; Bursch, W; Schulte-Hermann, R; Moolgavkar, SH. (1995). Growth kinetics of enzyme-altered liver foci in rats treated with phenobarbital or alpha-hexachlorocyclohexane. *Toxicol Appl Pharmacol* 130: 304-315. <http://dx.doi.org/10.1006/taap.1995.1035>.
- Lundberg, JM; Saria, A. (1983). Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants [Letter]. *Nature* 302: 251-253. <http://dx.doi.org/10.1038/302251a0>.

- [Luo, YL; Guo, HM; Zhang, YL; Chen, PX; Zhu, YX; Huang, JH; Zhou, WL.](#) (2013). Cellular mechanism underlying formaldehyde-stimulated Cl⁻ secretion in rat airway epithelium. *PLoS ONE* 8: e54494. <http://dx.doi.org/10.1371/journal.pone.0054494>.
- [Lyapina, M; Zhelezova, G; Petrova, E; Boev, M.](#) (2004). Flow cytometric determination of neutrophil respiratory burst activity in workers exposed to formaldehyde. *Int Arch Occup Environ Health* 77: 335-340. <http://dx.doi.org/10.1007/s00420-004-0516-3>.
- [Ma, H; Song, X; Zhang, W; Ling, X; Yang, X; Wu, W; Lou, K; Xu, H.](#) (2020). Formaldehyde inhibits development of T lymphocytes in mice. *Toxicol Environ Chem* 102: 473-489. <http://dx.doi.org/10.1080/02772248.2020.1815202>.
- [Ma, TH; Harris, MM.](#) (1988). Review of the genotoxicity of formaldehyde [Review]. *Mutat Res Rev Genet Toxicol* 196: 37-59. [http://dx.doi.org/10.1016/0165-1110\(88\)90027-9](http://dx.doi.org/10.1016/0165-1110(88)90027-9).
- [Macedo, R; Gomes, F; Leal, M; Barioni, E; Braga, T; Camara, N; Farsky, S; Franco, ALD.](#) (2016a). Low level laser treatment reduces oxidative stress induced by formaldehyde exposure by the modulation of gene expression of oxidant and antioxidant enzymes in the lung tissue [Abstract]. *Lasers Surg Med* 48: 58. <http://dx.doi.org/10.1002/lsm.22485>.
- [Macedo, RS; Leal, MP; Braga, TT; Barioni, ED; Duro, S; Ratto Tempestini Horliana, AC; Saraiva Camara, NO; Marcourakis, T; Poliselli Farsky, SH; Lino-Dos-Santos-Franco, A.](#) (2016b). Photobiomodulation Therapy Decreases Oxidative Stress in the Lung Tissue after Formaldehyde Exposure: Role of Oxidant/Antioxidant Enzymes. *Mediators Inflamm* 2016: 9303126. <http://dx.doi.org/10.1155/2016/9303126>.
- [Mackenzie, RA; Burke, D; Skuse, NF; Lethlean, AK.](#) (1975). Fibre function and perception during cutaneous nerve block. *J Neurol Neurosurg Psychiatry* 38: 865-873. <http://dx.doi.org/10.1136/jnnp.38.9.865>.
- [Mackerer, CR; Angelosanto, FA; Blackburn, GR; Schreiner, CA.](#) (1996). Identification of formaldehyde as the metabolite responsible for the mutagenicity of methyl tertiary-butyl ether in the activated mouse lymphoma assay. *Proc Soc Exp Biol Med* 212: 338-341. <http://dx.doi.org/10.3181/00379727-212-44023>.
- [Macpherson, LJ; Xiao, B; Kwan, KY; Petrus, MJ; Dubin, AE; Hwang, S; Cravatt, B; Corey, DP; Patapoutian, A.](#) (2007). An ion channel essential for sensing chemical damage. *J Neurosci* 27: 11412-11415. <http://dx.doi.org/10.1523/JNEUROSCI.3600-07.2007>.
- [Maddalena, RL; Russell, M; Sullivan, DP; Apte, MG.](#) (2008). Interim report: VOC and aldehyde emissions in four FEMA temporary housing units. *Maddalena, RL; Russell, M; Sullivan, DP; Apte, MG.*
- [Madison, RE; Broughton, A; Thrasher, JD.](#) (1991). Immunologic biomarkers associated with an acute exposure to exothermic byproducts of a ureaformaldehyde spill. *Environ Health Perspect* 94: 219-223. <http://dx.doi.org/10.2307/3431314>.
- [Madureira, J; Paciência, I; Cavaleiro-Rufo, J; de Oliveira Fernandes, E.](#) (2016). Indoor pollutant exposure among children with and without asthma in Porto, Portugal, during the cold season. *Environ Sci Pollut Res Int* 23: 20539-20552. <http://dx.doi.org/10.1007/s11356-016-7269-x>.
- [Madureira, J; Paciência, I; Ramos, E; Barros, H; Pereira, C; Teixeira, JP; Fernandes, E.](#) (2015). Children's Health and Indoor Air Quality in Primary Schools and Homes in Portugal-Study Design. *J Toxicol Environ Health A* 78: 915-930. <http://dx.doi.org/10.1080/15287394.2015.1048926>.
- [Maes, T; Joos, GF; Brusselle, GG.](#) (2012). Targeting interleukin-4 in asthma: lost in translation? [Review]. *Am J Respir Cell Mol Biol* 47: 261-270. <http://dx.doi.org/10.1165/rcmb.2012-0080TR>.
- [Maffei, F; Angelini, S; Forti, GC; Lodi, V; Violante, FS; Mattioli, S; Hrelia, P.](#) (2002). Micronuclei frequencies in hospital workers occupationally exposed to low levels of ionizing radiation: influence of smoking status and other factors. *Mutagenesis* 17: 405-409. <http://dx.doi.org/10.1093/mutage/17.5.405>.

- [Magaña-Schwencke, N; Ekert, B.](#) (1978). Biochemical analysis of damage induced in yeast by formaldehyde. II. Induction of cross-links between DNA and protein. *Mutat Res* 51: 11-19. [http://dx.doi.org/10.1016/0027-5107\(78\)90003-9](http://dx.doi.org/10.1016/0027-5107(78)90003-9).
- [Magaña-Schwencke, N; Ekert, B; Moustacchi, E.](#) (1978). Biochemical analysis of damage induced in yeast by formaldehyde. I. Induction of single-strand breaks in DNA and their repair. *Mutat Res* 50: 181-193. [http://dx.doi.org/10.1016/0027-5107\(78\)90023-4](http://dx.doi.org/10.1016/0027-5107(78)90023-4).
- [Magaña-Schwencke, N; Moustacchi, E.](#) (1980). Biochemical analysis of damage induced in yeast by formaldehyde III. Repair of induced cross-links between DNA and proteins in the wild-type and in excision-deficient strains. *Mutat Res* 70: 29-35. [http://dx.doi.org/10.1016/0027-5107\(80\)90055-X](http://dx.doi.org/10.1016/0027-5107(80)90055-X).
- [Magnani, C; Comba, P; Ferraris, F; Ivaldi, C; Meneghin, M; Terracini, B.](#) (1993). A case-control study of carcinomas of the nose and paranasal sinuses in the woolen textile manufacturing industry. *Arch Environ Health* 48: 94-97. <http://dx.doi.org/10.1080/00039896.1993.9938401>.
- [Maiellaro, M; Correa-Costa, M; Vitoretti, LB; Gimenes Junior, JA; Saraiva Camara, NO; Tavares-De-Lima, W; Poliselli Farsky, SH; Lino-Dos-Santos-Franco, A.](#) (2014). Exposure to low doses of formaldehyde during pregnancy suppresses the development of allergic lung inflammation in offspring. *Toxicol Appl Pharmacol* 278: 266-274. <http://dx.doi.org/10.1016/j.taap.2014.05.003>.
- [Maiellaro, M; Macedo, RS; Mendes, E; Tavares-De-Lima, W; Ferreira, CM; Lino-Dos-Santos-Franco, A.](#) (2016). High dose of formaldehyde exposure during pregnancy increases neutrophils lung influx evoked by ovalbumin in the offspring. *Inflamm Res* 65: 179-181. <http://dx.doi.org/10.1007/s00011-015-0901-2>.
- [Main, DM; Hogan, TJ.](#) (1983). Health effects of low level exposure to formaldehyde. *J Occup Environ Med* 25: 896-900. <http://dx.doi.org/10.1097/00043764-198312000-00013>.
- [Malaka, T; Kodama, AM.](#) (1990). Respiratory health of plywood workers occupationally exposed to formaldehyde. *Arch Environ Health* 45: 288-294. <http://dx.doi.org/10.1080/00039896.1990.10118748>.
- [Malek, FA; Möritz, KU; Fanghänel, J.](#) (2003a). Formaldehyde inhalation & open field behaviour in rats. *Indian J Med Res* 118: 90-96.
- [Malek, FA; Möritz, KU; Fanghänel, J.](#) (2003b). A study on specific behavioral effects of formaldehyde in the rat. *J Exp Anim Sci* 42: 160-170. [http://dx.doi.org/10.1016/S0939-8600\(03\)80009-3](http://dx.doi.org/10.1016/S0939-8600(03)80009-3).
- [Malek, FA; Möritz, KU; Fanghänel, J.](#) (2003c). A study on the effect of inhalative formaldehyde exposure on water labyrinth test performance in rats. *Ann Anat* 185: 277-285. [http://dx.doi.org/10.1016/S0940-9602\(03\)80040-7](http://dx.doi.org/10.1016/S0940-9602(03)80040-7).
- [Malek, FA; Möritz, KU; Fanghänel, J.](#) (2004). Effects of a single inhalative exposure to formaldehyde on the open field behavior of mice. *Int J Hyg Environ Health* 207: 151-158. <http://dx.doi.org/10.1078/1438-4639-00268>.
- [Malker, HSR; Mclaughlin, JK; Weiner, JA; Silverman, DT; Blot, WJ; JLE, E; Fraumeni, J, r, J. F.](#) (1990). Occupational risk factors for nasopharyngeal cancer in Sweden. *Br J Ind Med* 47: 213-214. <http://dx.doi.org/10.1136/oem.47.3.213>.
- [Mallet, ML.](#) (2002). Pathophysiology of accidental hypothermia [Review]. *QJM* 95: 775-785. <http://dx.doi.org/10.1093/qjmed/95.12.775>.
- [Mallia, P; Message, SD; Contoli, M; Gray, K; Telcian, A; Laza-Stanca, V; Papi, A; Stanciu, LA; Elkin, S; Kon, OM; Johnson, M; Johnston, SL.](#) (2014). Lymphocyte subsets in experimental rhinovirus infection in chronic obstructive pulmonary disease. *Respir Med* 108: 78-85. <http://dx.doi.org/10.1016/j.rmed.2013.09.010>.
- [Marcon, A; Fracasso, ME; Marchetti, P; Doria, D; Girardi, P; Guarda, L; Pesce, G; Pironi, V; Ricci, P; de Marco, R.](#) (2014). Outdoor formaldehyde and NO2 exposures and markers of genotoxicity in

- children living near chipboard industries. *Environ Health Perspect* 122: 639-645.
<http://dx.doi.org/10.1289/ehp.1307259>.
- Marinari, UM; Ferro, M; Sciaba, L; Finollo, R; Bassi, AM; Brambilla, G. (1984). DNA-damaging activity of biotic and xenobiotic aldehydes in Chinese hamster ovary cells. *Cell Biochem Funct* 2: 243-248.
<http://dx.doi.org/10.1002/cbf.290020411>.
- Marks, GB; Ezz, W; Aust, N; Toelle, BG; Xuan, W; Belousova, E; Cosgrove, C; Jalaludin, B; Smith, WT. (2010). Respiratory health effects of exposure to low-NOx unflued gas heaters in the classroom: A double-blind, cluster-randomized, crossover study. *Environ Health Perspect* 118: 1476-1482.
<http://dx.doi.org/10.1289/ehp.1002186>.
- Marnett, LJ; Hurd, HK; Hollstein, MC; Levin, DE; Esterbauer, H; Ames, BN. (1985). Naturally occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. *Mutat Res Fundam Mol Mech Mutagen* 148: 25-34. [http://dx.doi.org/10.1016/0027-5107\(85\)90204-0](http://dx.doi.org/10.1016/0027-5107(85)90204-0).
- Maronpot, RR; Miller, RA; Clarke, WJ; Westerberg, RB; Decker, JR; Moss, OR. (1986). Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. *Toxicology* 41: 253-266.
[http://dx.doi.org/10.1016/0300-483X\(86\)90180-0](http://dx.doi.org/10.1016/0300-483X(86)90180-0).
- Marsh, G; Youk, A; Stone, R; Buchanich, J; Gula, M; Smith, T; Quinn, M. (2001). Historical cohort study of US man-made vitreous fiber production workers: I. 1992 fiberglass cohort follow-up: Initial findings. *J Occup Environ Med* 43: 741-756. <http://dx.doi.org/10.1097/00043764-200109000-00004>.
- Marsh, GM; Morfeld, P; Zimmerman, SD; Liu, Y; Balmert, LC. (2016). An updated re-analysis of the mortality risk from nasopharyngeal cancer in the National Cancer Institute formaldehyde worker cohort study. *J Occup Med Toxicol* 11: 8. <http://dx.doi.org/10.1186/s12995-016-0097-6>.
- Marsh, GM; Youk, AO; Buchanich, JM; Cassidy, LD; Lucas, LJ; Esmen, NA; Gathuru, IM. (2002). Pharyngeal cancer mortality among chemical plant workers exposed to formaldehyde. *Toxicol Ind Health* 18: 257-268. <http://dx.doi.org/10.1191/0748233702th149oa>.
- Marsh, GM; Youk, AO; Buchanich, JM; Erdal, S; Esmen, NA. (2007). Work in the metal industry and nasopharyngeal cancer mortality among formaldehyde-exposed workers. *Regul Toxicol Pharmacol* 48: 308-319. <http://dx.doi.org/10.1016/j.yrtph.2007.04.006>.
- Martin, CN; McDermid, AC; Garner, RC. (1978). Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells. *Cancer Res* 38: 2621-2627.
- Martin, WJ. (1990). A teratology study of inhaled formaldehyde in the rat. *Reprod Toxicol* 4: 237-239.
[http://dx.doi.org/10.1016/0890-6238\(90\)90065-4](http://dx.doi.org/10.1016/0890-6238(90)90065-4).
- Matanoski, GM. (1989). Risks of pathologists exposed to formaldehyde (final report). (DHHS Grant No. 5 R01-OH-01511-03). Baltimore, MD: Johns Hopkins University Department of Epidemiology.
<https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=PB91173682>.
- Matsunaga, I; Miyake, Y; Yoshida, T; Miyamoto, S; Ohya, Y; Sasaki, S; Tanaka, K; Oda, H; Ishiko, O; Hirota, Y; Group, OMaCHS. (2008). Ambient formaldehyde levels and allergic disorders among Japanese pregnant women: Baseline data from the Osaka maternal and child health study. *Ann Epidemiol* 18: 78-84. <http://dx.doi.org/10.1016/j.annepidem.2007.07.095>.
- Matsuoka, T; Takaki, A; Ohtaki, H; Shioda, S. (2010). Early changes to oxidative stress levels following exposure to formaldehyde in ICR mice. *J Toxicol Sci* 35: 721-730.
<http://dx.doi.org/10.2131/jts.35.721>.
- Mauderly, JL. (1986). Respiration of F344 rats in nose-only inhalation exposure tubes. *J Appl Toxicol* 6: 25-30. <http://dx.doi.org/10.1002/jat.2550060106>.
- Mayer, AK; Dalpke, AH. (2007). Regulation of local immunity by airway epithelial cells [Review]. *Arch Immunol Ther Exp* 55: 353-362. <http://dx.doi.org/10.1007/s00005-007-0041-7>.

- [Mayr, SI; Hafizovic, K; Waldfahrer, F; Iro, H; Kütting, B.](#) (2010). Characterization of initial clinical symptoms and risk factors for sinonasal adenocarcinomas: results of a case-control study. *Int Arch Occup Environ Health* 83: 631-638. <http://dx.doi.org/10.1007/s00420-009-0479-5>.
- [Mazzone, SB; Undem, BJ.](#) (2016). Vagal afferent innervation of the airways in health and disease [Review]. *Physiol Rev* 96: 975-1024. <http://dx.doi.org/10.1152/physrev.00039.2015>.
- [McGhee, JD; von Hippel, PH.](#) (1975a). Formaldehyde as a probe of DNA structure. I. Reaction with exocyclic amino groups of DNA bases. *Biochemistry* 14: 1281-1296. <http://dx.doi.org/10.1021/bi00677a029>.
- [McGhee, JD; von Hippel, PH.](#) (1975b). Formaldehyde as a probe of DNA structure. II. Reaction with endocyclic imino groups of DNA bases. *Biochemistry* 14: 1297-1303. <http://dx.doi.org/10.1021/bi00677a030>.
- [Mcnamara, CR; Mandel-Brehm, J; Bautista, DM; Siemens, J, an; Deranian, KL; Zhao, M; Hayward, NJ; Chong, JA; Julius, D; Moran, MM; Fanger, CM.](#) (2007). TRPA1 mediates formalin-induced pain. *Proc Natl Acad Sci USA* 104: 13525-13530. <http://dx.doi.org/10.1073/pnas.0705924104>.
- [Medoff, BD; Thomas, SY; Banerji, A; Wain, JC; Zhang, H; Lilly, CM; Ginns, LC; Luster, AD.](#) (2005). Pathogenic T-cell recruitment into the airway in human disease. *Ann N Y Acad Sci* 220-241. <http://dx.doi.org/10.1196/annals.1358.026>.
- [Mei, YF; Duan, CL; Li, XX; Zhao, Y; Cao, FH; Shang, S; Ding, SM; Yue, XP; Gao, G; Yang, H; Shen, LX; Feng, XY; Jia, JP; Tong, ZQ; Yang, X.](#) (2016). Reduction of Endogenous Melatonin Accelerates Cognitive Decline in Mice in a Simulated Occupational Formaldehyde Exposure Environment. *Int J Environ Res Public Health* 13. <http://dx.doi.org/10.3390/ijerph13030258>.
- [Meister, A; Anderson, ME.](#) (1983). Glutathione [Review]. *Annu Rev Biochem* 52: 711-760. <http://dx.doi.org/10.1146/annurev.bi.52.070183.003431>.
- [Meng, F; Bermudez, E; McKinzie, PB; Andersen, ME; III, CH; Parsons, BL.](#) (2010). Measurement of tumor-associated mutations in the nasal mucosa of rats exposed to varying doses of formaldehyde. *Regul Toxicol Pharmacol* 57: 274-283. <http://dx.doi.org/10.1016/j.yrtph.2010.03.007>.
- [Merk, O; Speit, G.](#) (1998). Significance of formaldehyde-induced DNA-protein crosslinks for mutagenesis. *Environ Mol Mutagen* 32: 260-268. [http://dx.doi.org/10.1002/\(SICI\)1098-2280\(1998\)32:3<260::AID-EM9>3.0.CO;2-M](http://dx.doi.org/10.1002/(SICI)1098-2280(1998)32:3<260::AID-EM9>3.0.CO;2-M).
- [Merk, O; Speit, G.](#) (1999). Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. *Environ Mol Mutagen* 33: 167-172. [http://dx.doi.org/10.1002/\(sici\)1098-2280\(1999\)33:2<167::aid-em9>3.0.co;2-d](http://dx.doi.org/10.1002/(sici)1098-2280(1999)33:2<167::aid-em9>3.0.co;2-d).
- [Merler, E; Baldasseroni, A; Iaria, R; Faravelli, P; Agostini, R; Pisa, R; Berrino, F.](#) (1986). On the causal association between exposure to leather dust and nasal cancer: Further evidence from a case-control study. *Br J Ind Med* 43: 91-95. <http://dx.doi.org/10.1136/oem.43.2.91>.
- [Message, SD; Laza-Stanca, V; Mallia, P; Parker, HL; Zhu, J, ie; Kebabdz, T; Contoli, M; Sanderson, G; Kon, O, nnM; Papi, A; Jeffery, PK; Stanciu, LA; Johnston, SL.](#) (2008). Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci USA* 105: 13562-13567. <http://dx.doi.org/10.1073/pnas.0804181105>.
- [Meyers, AR; Pinkerton, LE; Hein, MJ.](#) (2013). Cohort mortality study of garment industry workers exposed to formaldehyde: Update and internal comparisons. *Am J Ind Med* 56: 1027-1039. <http://dx.doi.org/10.1002/ajim.22199>.
- [Mi, L; Sui, J; Wu, Y; Liang, G; Zhang, Y; Pu, Y; Tian, Y; Liu, S; Jiang, L.](#) (2019). Bioinspired in vitro lung airway model for inflammatory analysis via hydrophobic nanochannel membrane with joint three-phase interface. *Anal Chem* 91: 15804-15810. <http://dx.doi.org/10.1021/acs.analchem.9b04114>.

- [Mi, YH; Norbäck, D; Tao, J; Mi, YL; Ferm, M. \(2006\). Current asthma and respiratory symptoms among pupils in Shanghai, China: Influence of building ventilation, nitrogen dioxide, ozone, and formaldehyde in classrooms. Indoor Air 16: 454-464. <http://dx.doi.org/10.1111/j.1600-0668.2006.00439.x>.](#)
- [Migliore, L; Ventura, L; Barale, R; Loprieno, N; Castellino, S; Pulci, R. \(1989\). Micronuclei and nuclear anomalies induced in the gastro-intestinal epithelium of rats treated with formaldehyde. Mutagenesis 4: 327-334. <http://dx.doi.org/10.1093/mutage/4.5.327>.](#)
- [Miller, FJ; Conolly, RB; Kimbell, JS. \(2017\). An updated analysis of respiratory tract cells at risk for formaldehyde carcinogenesis. Inhal Toxicol 29: 586-597. <http://dx.doi.org/10.1080/08958378.2018.1430191>.](#)
- [Milton, DK; Wypij, D; Kriebel, D; Walters, MD; Hammond, SK; Evans, JS. \(1996\). Endotoxin exposure-response in a fiberglass manufacturing facility. Am J Ind Med 29: 3-13. \[http://dx.doi.org/10.1002/\\(SICI\\)1097-0274\\(199601\\)29:1<3::AID-AJIM2>3.0.CO;2-V\]\(http://dx.doi.org/10.1002/\(SICI\)1097-0274\(199601\)29:1<3::AID-AJIM2>3.0.CO;2-V\).](#)
- [Mittrücker, HW; Visekruna, A; Huber, M. \(2014\). Heterogeneity in the differentiation and function of CD8⁺ T cells \[Review\]. Arch Immunol Ther Exp 62: 449-458. <http://dx.doi.org/10.1007/s00005-014-0293-y>.](#)
- [Miyachi, T; Tsutsui, T. \(2005\). Ability of 13 chemical agents used in dental practice to induce sister-chromatid exchanges in Syrian hamster embryo cells. Odontology 93: 24-29. <http://dx.doi.org/10.1007/s10266-005-0055-8>.](#)
- [Moeller, BC; Lu, K; Doyle-Eisele, M; McDonald, J; Gigliotti, A; Swenberg, JA. \(2011\). Determination of N2-hydroxymethyl-dG adducts in the nasal epithelium and bone marrow of nonhuman primates following 13CD2-formaldehyde inhalation exposure. Chem Res Toxicol 24: 162-164. <http://dx.doi.org/10.1021/tx1004166>.](#)
- [Mohammad `pour, AA; Maleki, M. \(2011\). Effect of formaldehyde exposure on pulmonary function tests of veterinary students in anatomy laboratory. Journal of Applied Animal Research 39: 114-116. <http://dx.doi.org/10.1080/09712119.2011.558678>.](#)
- [Möhner, M; Liu, Y; Marsh, GM. \(2019\). New insights into the mortality risk from nasopharyngeal cancer in the national cancer institute formaldehyde worker cohort study. J Occup Med Toxicol 14: 4. <http://dx.doi.org/10.1186/s12995-019-0224-2>.](#)
- [Monfared, AL. \(2012\). Histomorphological and ultrastructural changes of the placenta in mice exposed to formaldehyde. Toxicol Ind Health 30: 174-181. <http://dx.doi.org/10.1177/0748233712452603>.](#)
- [Monteiro-Riviere, NA; Popp, JA. \(1986\). Ultrastructural evaluation of acute nasal toxicity in the rat respiratory epithelium in response to formaldehyde gas. Fundam Appl Toxicol 6: 251-262. \[http://dx.doi.org/10.1016/0272-0590\\(86\\)90238-1\]\(http://dx.doi.org/10.1016/0272-0590\(86\)90238-1\).](#)
- [Monticello, TM; Gross, EA; Morgan, KT. \(1993\). Cell Proliferation and Nasal Carcinogenesis. Environ Health Perspect 101: 121. <http://dx.doi.org/10.2307/3431854>.](#)
- [Monticello, TM; Miller, FJ; Morgan, KT. \(1991\). Regional increases in rat nasal epithelial cell proliferation following acute and subchronic inhalation of formaldehyde. Toxicol Appl Pharmacol 111: 409-421. \[http://dx.doi.org/10.1016/0041-008X\\(91\\)90246-B\]\(http://dx.doi.org/10.1016/0041-008X\(91\)90246-B\).](#)
- [Monticello, TM; Morgan, KT. \(1994\). Cell proliferation and formaldehyde-induced respiratory carcinogenesis \[Review\]. Risk Anal 14: 313-319. <http://dx.doi.org/10.1111/j.1539-6924.1994.tb00246.x>.](#)
- [Monticello, TM; Morgan, KT. \(1997\). Chemically-induced nasal carcinogenesis and epithelial cell proliferation: A brief review \[Review\]. Mutat Res Fundam Mol Mech Mutagen 380: 33-41. \[http://dx.doi.org/10.1016/S0027-5107\\(97\\)00125-5\]\(http://dx.doi.org/10.1016/S0027-5107\(97\)00125-5\).](#)
- [Monticello, TM; Morgan, KT; Everitt, JI; Popp, JA. \(1989\). Effects of formaldehyde gas on the respiratory tract of rhesus monkeys: Pathology and cell proliferation. Am J Pathol 134: 515-527.](#)

Supplemental Information—Formaldehyde (Inhalation)

- [Monticello, TM; Morgan, KT; Hurtt, ME.](#) (1990). Unit length as the denominator for quantitation of cell proliferation in nasal epithelia. *Toxicol Pathol* 18: 24-31.
<http://dx.doi.org/10.1177/019262339001800104>.
- [Monticello, TM; Swenberg, JA; Gross, EA; Leininger, JR; Kimbell, JS; Seilkop, S; Starr, TB; Gibson, JE; Morgan, KT.](#) (1996). Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. *Cancer Res* 56: 1012-1022.
- [Moolgavkar, SH.](#) (1994). Biological models of carcinogenesis and quantitative cancer risk assessment. *Risk Anal* 14: 879-882. <http://dx.doi.org/10.1111/j.1539-6924.1994.tb00049.x>.
- [Moolgavkar, SH; Dewanji, A; Venzon, DJ.](#) (1988). A stochastic two-stage model for cancer risk assessment. I. The hazard function and the probability of tumor. *Risk Anal* 8: 383-392.
<http://dx.doi.org/10.1111/j.1539-6924.1988.tb00502.x>.
- [Moolgavkar, SH; Knudson, AJ.](#) (1981). Mutation and cancer: A model for human carcinogenesis. *J Natl Cancer Inst* 66: 1037-1052.
- [Moolgavkar, SH; Luebeck, EG.](#) (1992). Interpretation of labeling indices in the presence of cell death. *Carcinogenesis* 13: 1007-1010. <http://dx.doi.org/10.1093/carcin/13.6.1007>.
- [Moolgavkar, SH; Luebeck, EG; Krewski, D; Zielinski, JM.](#) (1993). Radon, cigarette smoke, and lung cancer: A re-analysis of the Colorado Plateau uranium miners' data. *Epidemiology* 4: 204-217.
- [Moolgavkar, SH; Venzon, DJ.](#) (1979). Two-event models for carcinogenesis: Incidence curves for childhood and adult tumors. *Math Biosci* 47: 55-77. [http://dx.doi.org/10.1016/0025-5564\(79\)90005-1](http://dx.doi.org/10.1016/0025-5564(79)90005-1).
- [Moorman, JE; Akinbami, LJ; Bailey, CM; Zahran, HS; King, ME; Johnson, CA; Liu, X.](#) (2012). National surveillance of asthma: United States, 2001-2010. In *Vital & Health Statistics Series 3: Analytical and Epidemiological Studies*, no 35. Hyattsville, MD: U.S. Department of Health and Human Services, Public Health Service, National Center for Health Statistics.
https://www.cdc.gov/nchs/data/series/sr_03/sr03_035.pdf.
- [Morgan, DL; Dixon, D; King, DH; Travlos, GS; Herbert, RA; French, JE; Tokar, EJ; Waalkes, MP; Jokinen, MP.](#) (2017). NTP research report on absence of formaldehyde-induced neoplasia in Trp53 haploinsufficient mice exposed by inhalation. (Research Report 3). Research Triangle Park, NC: National Toxicology Program. <http://dx.doi.org/10.22427/NTP-RR-3>.
- [Morgan, KT.](#) (1983). Localization of areas of inhibition of nasal mucociliary function in rats following in vivo exposure to formaldehyde. *Am Rev Respir Dis* 127: 166.
- [Morgan, KT.](#) (1997). A brief review of formaldehyde carcinogenesis in relation to rat nasal pathology and human health risk assessment [Review]. *Toxicol Pathol* 25: 291-305.
<http://dx.doi.org/10.1177/019262339702500307>.
- [Morgan, KT; Gross, EA; Patterson, DL.](#) (1986a). Distribution, progression, and recovery of acute formaldehyde-induced inhibition of nasal mucociliary function in F-344 rats. *Toxicol Appl Pharmacol* 86: 448-456. [http://dx.doi.org/10.1016/0041-008X\(86\)90372-8](http://dx.doi.org/10.1016/0041-008X(86)90372-8).
- [Morgan, KT; Jiang, XZ; Starr, TB; Kerns, WD.](#) (1986b). More precise localization of nasal tumors associated with chronic exposure of F-344 rats to formaldehyde gas. *Toxicol Appl Pharmacol* 82: 264-271. [http://dx.doi.org/10.1016/0041-008X\(86\)90201-2](http://dx.doi.org/10.1016/0041-008X(86)90201-2).
- [Morgan, KT; Kimbell, JS; Monticello, TM; Patra, AL; Fleishman, A.](#) (1991). Studies of inspiratory airflow patterns in the nasal passages of the F344 rat and rhesus monkey using nasal molds: Relevance to formaldehyde toxicity. *Toxicol Appl Pharmacol* 110: 223-240.
[http://dx.doi.org/10.1016/S0041-008X\(05\)80005-5](http://dx.doi.org/10.1016/S0041-008X(05)80005-5).
- [Morgan, KT; Patterson, DL; Gross, EA.](#) (1984). Frog palate mucociliary apparatus: Structure, function, and response to formaldehyde gas. *Fundam Appl Toxicol* 4: 58-68. [http://dx.doi.org/10.1016/0272-0590\(84\)90219-7](http://dx.doi.org/10.1016/0272-0590(84)90219-7).

- [Morgan, KT; Patterson, DL; Gross, EA.](#) (1986c). Responses of the nasal mucociliary apparatus of F-344 rats to formaldehyde gas. *Toxicol Appl Pharmacol* 82: 1-13. [http://dx.doi.org/10.1016/0041-008X\(86\)90431-X](http://dx.doi.org/10.1016/0041-008X(86)90431-X).
- [Morgan, KT; Patterson, DL; Gross, EA.](#) (1983). Formaldehyde and the nasal mucociliary apparatus. In JJ Clary; JE Gibson; RS Waritz (Eds.), *Formaldehyde: toxicology, epidemiology, mechanisms* (pp. 193-209). New York, NY: Marcel Dekker, Inc.
- [Mori, M; Matsumoto, Y; Kushino, N; Morimatsu, Y; Hoshiko, M; Saga, T; Yamaki, K; Ishitake, T.](#) (2016). Comparison of subjective symptoms associated with exposure to low levels of formaldehyde between students enrolled and not enrolled in a gross anatomy course. *Environ Health Prev Med* 21: 34-41. <http://dx.doi.org/10.1007/s12199-015-0497-8>.
- [Morita, T; Asano, N; Awogi, T; Sasaki, YF; Sato, S; Shimada, H; Sutou, S; Suzuki, T; Wakata, A; Sofuni, T; Hayashi, M.](#) (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS [Review]. *Mutat Res* 389: 3-122. [http://dx.doi.org/10.1016/S1383-5718\(96\)00070-8](http://dx.doi.org/10.1016/S1383-5718(96)00070-8).
- [Moser, B; Bodrogi, F; Eibl, G; Lechner, M; Rieder, J; Lirk, P.](#) (2005). Mass spectrometric profile of exhaled breath - field study by PTR-MS. *Respir Physiol Neurobiol* 145: 295-300. <http://dx.doi.org/10.1016/j.resp.2004.02.002>.
- [Mostafa, GA.](#) (2009). Neurogenic inflammation and allergy [Review]. *Egypt J Pediatr Allergy Immunol* 7: 45-58.
- [Mueller, JU; Bruckner, T; Triebig, G.](#) (2013). Exposure study to examine chemosensory effects of formaldehyde on hyposensitive and hypersensitive males. *Int Arch Occup Environ Health* 86: 107-117. <http://dx.doi.org/10.1007/s00420-012-0745-9>.
- [Müller, B; Schulz, G; Mehlin, A; Herzen, J; Lang, S; Holme, M; Zanette, I; Hieber, S; Deyhle, H; Beckmann, F; Pfeiffer, F; Weitkamp, T.](#) (2012). Grating-based tomography of human tissues. In A Momose; W Yashiro (Eds.), *Proceedings of the International Workshop on X-Ray and Neutron Phase Imaging with Gratings* (pp. 107-112). Melville, NY: American Institute of Physics. <http://dx.doi.org/10.1063/1.4742277>.
- [Müller, W; Engelhart, G; Herbold, B; Jäckh, R; Jung, R.](#) (1993). Evaluation of mutagenicity testing with *Salmonella typhimurium* TA102 in three different laboratories. *Environ Health Perspect* 101: 33-36. <http://dx.doi.org/10.1289/ehp.101-1521147>.
- [Mundt, KA; Gallagher, AE; Dell, LD; Natelson, EA; Boffetta, P; Gentry, PR.](#) (2017). Does occupational exposure to formaldehyde cause hematotoxicity and leukemia-specific chromosome changes in cultured myeloid progenitor cells? [Review]. *Crit Rev Toxicol* 47: 1-11. <http://dx.doi.org/10.1080/10408444.2017.1301878>.
- [Murta, GL; Duarte Campos, KK; Balthar Bandeira, A; Diniz, MF; Costa, G; Costa, DC; Talvani, A; Lima, WG; Bezerra, FS.](#) (2016). Oxidative effects on lung inflammatory response in rats exposed to different concentrations of formaldehyde. *Environ Pollut* 211: 206-213. <http://dx.doi.org/10.1016/j.envpol.2015.12.054>.
- [Musak, L; Smerhovsky, Z; Halasova, E; Osina, O; Letkova, L; Vodickova, L; Polakova, V; Buchancova, J; Hemminki, K; Vodicka, P.](#) (2013). Chromosomal damage among medical staff occupationally exposed to volatile anesthetics, antineoplastic drugs, and formaldehyde. *Scand J Work Environ Health* 39: 618-630. <http://dx.doi.org/10.5271/sjweh.3358>.
- [NAC/AEGL](#) (National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances). (2008). Interim acute exposure guideline levels (AEGLs) for formaldehyde (CAS Reg. No. 50-00-0). Washington, DC: National Advisory Committee for Acute Exposure Guideline Levels. https://www.epa.gov/sites/production/files/2014-07/documents/formaldehyde_tsd_interim_07_2008.v1_0.pdf.

- [Nadin, SB; Vargas-Roig, LM; Ciocca, DR.](#) (2001). A silver staining method for single-cell gel assay. *J Histochem Cytochem* 49: 1183-1186. <http://dx.doi.org/10.1177/002215540104900912>.
- [Nakamura, J; Holley, DW; Kawamoto, T; Bultman, SJ.](#) (2020). The failure of two major formaldehyde catabolism enzymes (ADH5 and ALDH2) leads to partial synthetic lethality in C57BL/6 mice. *Genes Environ* 42: 21. <http://dx.doi.org/10.1186/s41021-020-00160-4>.
- [Nalivaiko, E; De Pasquale, CG; Blessing, WW.](#) (2003). Electrocardiographic changes associated with the nasopharyngeal reflex in conscious rabbits: Vago-sympathetic co-activation. *Auton Neurosci* 105: 101-104. [http://dx.doi.org/10.1016/s1566-0702\(03\)00048-1](http://dx.doi.org/10.1016/s1566-0702(03)00048-1).
- [NASEM](#) (National Academies of Sciences, Engineering, and Medicine). (2023). Review of EPA's 2022 Draft Formaldehyde Assessment. Washington, DC. <https://nap.nationalacademies.org/catalog/27153/review-of-epas-2022-draft-formaldehyde-assessment>.
- [Natarajan, AT; Darroudi, F; Bussman, CJM; van Kesteren-Van Leeuwen, AC.](#) (1983). Evaluation of the mutagenicity of formaldehyde in mammalian cytogenetic assays in vivo and vitro. *Mutat Res* 122: 355-360. [http://dx.doi.org/10.1016/0165-7992\(83\)90019-2](http://dx.doi.org/10.1016/0165-7992(83)90019-2).
- [National Toxicology Program](#) (NTP). (2005). Control data from NTP technical report series NOs. 210, 267, 272, 306, 311, 314, 329, 346, 363, 371, 375, 377, 379, 380, 386, 421, 437, 440, 447, 448, 450, 451, 453, 454, 461, 462, 466, 467, 468, 471, 472, 475, 482, 484, 486, 487, 490, 492, 499, 500, 507, 513, 515, 519 and control data from the wollastonite calcium silicates study. https://ntp.niehs.nih.gov/go/historical_controls.
- [Nazarpourvar-Noshadi, M; Dolatabadi, JEN; Rasoulzadeh, Y; Mohammadian, Y; Shanehbandi, D.](#) (2020). Apoptosis and DNA damage induced by silica nanoparticles and formaldehyde in human lung epithelial cells. *Environ Sci Pollut Res Int* 27: 18592-18601. <http://dx.doi.org/10.1007/s11356-020-08191-8>.
- [NCHS](#) (National Center for Health Statistics). (2006). Chartbook on Trends in the Health of Americans. Hyattsville, MD: National Center for Health Statistics, Centers for Disease Control and Prevention. [http://www.cdc.gov/nchs/data/06.pdf](http://www.cdc.gov/nchs/data/hus/06.pdf).
- [NCHS](#) (National Center for Health Statistics). (2009). Deaths: Final data for 2006. *Natl Vital Stat Rep* 57: 1-134.
- [Neamtiu, IA; Lin, S; Chen, ML; Roba, C; Csobod, E, va; Gurzau, ES.](#) (2019). Assessment of formaldehyde levels in relation to respiratory and allergic symptoms in children from Alba County schools, Romania. *Environ Monit Assess* 191: 591. <http://dx.doi.org/10.1007/s10661-019-7768-6>.
- [NEG](#) (Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals). (2003). Formaldehyde. Stockholm, Sweden: National Institute for Working Life. http://ebib.arbetslivsinstitutet.se/ah/2003/ah2003_11.pdf.
- [Neghab, M; Delikhoon, M; Norouzian Baghani, A; Hassanzadeh, J.](#) (2017). Exposure to Cooking Fumes and Acute Reversible Decrement in Lung Functional Capacity. *Int J Occup Environ Med* 8: 207-216. <http://dx.doi.org/10.15171/ijoem.2017.1100>.
- [Neghab, M; Soltanzadeh, A; Choobineh, A.](#) (2011). Respiratory morbidity induced by occupational inhalation exposure to formaldehyde. *Ind Health* 49: 89-94. <http://dx.doi.org/10.2486/indhealth.MS1197>.
- [Neuss, S; Moepps, B; Speit, G.](#) (2010a). Exposure of human nasal epithelial cells to formaldehyde does not lead to DNA damage in lymphocytes after co-cultivation. *Mutagenesis* 25: 359-364. <http://dx.doi.org/10.1093/mutage/geq013>.
- [Neuss, S; Speit, G.](#) (2008). Further characterization of the genotoxicity of formaldehyde in vitro by the sister chromatid exchange test and co-cultivation experiments. *Mutagenesis* 23: 355-357. <http://dx.doi.org/10.1093/mutage/gen025>.

Supplemental Information—Formaldehyde (Inhalation)

- [Neuss, S; Zeller, J; Ma-Hock, L; Speit, G.](#) (2010b). Inhalation of formaldehyde does not induce genotoxic effects in broncho-alveolar lavage (BAL) cells of rats. *Mutat Res Genet Toxicol Environ Mutagen* 695: 61-68. <http://dx.doi.org/10.1016/j.mrgentox.2009.12.001>.
- [Nielsen, GD.](#) (1991). Mechanisms of activation of the sensory irritant receptor by airborne chemicals [Review]. *Crit Rev Toxicol* 21: 183-208. <http://dx.doi.org/10.3109/10408449109089879>.
- [Nielsen, GD; Hougaard, KS; Larsen, ST; Hammer, M; Wolkoff, P; Clausen, PA; Wilkins, CK; Alarie, Y.](#) (1999). Acute airway effects of formaldehyde and ozone in BALB/c mice. *Hum Exp Toxicol* 18: 400-409. <http://dx.doi.org/10.1191/096032799678840246>.
- [Nielsen, GD; Wolkoff, P; Alarie, Y.](#) (2007). Sensory irritation: risk assessment approaches [Review]. *Regul Toxicol Pharmacol* 48: 6-18. <http://dx.doi.org/10.1016/j.yrtph.2006.11.005>.
- [Niinimaa, V; Cole, P; Mintz, S; Shephard, RJ.](#) (1981). Oronasal distribution of respiratory airflow. *Respir Physiol* 43: 69-75. [http://dx.doi.org/10.1016/0034-5687\(81\)90089-X](http://dx.doi.org/10.1016/0034-5687(81)90089-X).
- [Nilsson, JA; Hedberg, JJ; Vondracek, M; Staab, CA; Hansson, A; Hoog, JO; Grafstrom, RC.](#) (2004). Alcohol dehydrogenase 3 transcription associates with proliferation of human oral keratinocytes. *Cell Mol Life Sci* 61: 610-617. <http://dx.doi.org/10.1007/s00018-003-3433-9>.
- [NIOSH](#) (National Institute for Occupational Safety and Health). (1977). NIOSH manual of analytical methods: Second edition, volume 2, part II: Standards completion program validated methods. In NIOSH manual of analytical methods: Second edition, volume 2, part II: Standards completion program validated methods. (DHEW (NIOSH) Publication No. 77-157-B). Cincinnati, OH.
- [NIOSH](#) (National Institute for Occupational Safety and Health). (1994). Formaldehyde method 3500. In NIOSH manual of analytical methods (4th ed., pp. 3500-3501 - 3500-3504). (DHHS/NIOSH Pub. 84-100). Washington, DC. <https://www.cdc.gov/niosh/docs/2003-154/pdfs/3500.pdf>.
- [NLM](#) (National Library of Medicine). (2019). PubChem: Hazardous Substance Data Bank: Formaldehyde, 50-00-0. Available online at <https://pubchem.ncbi.nlm.nih.gov/compound/712#source=HSDB> (accessed
- [Nockher, WA; Renz, H.](#) (2006). Neurotrophins and asthma: Novel insight into neuroimmune interaction. *J Allergy Clin Immunol* 117: 67-71. <http://dx.doi.org/10.1016/j.jaci.2005.08.029>.
- [Norback, D; Bjornsson, E; Janson, C; Widstrom, J; Boman, G.](#) (1995). Asthmatic symptoms and volatile organic compounds, formaldehyde, and carbon dioxide in dwellings. *Occup Environ Med* 52: 388-395. <http://dx.doi.org/10.1136/oem.52.6.388>.
- [Norbäck, D; Hashim, JH; Hashim, Z; Ali, F.](#) (2017). Volatile organic compounds (VOC), formaldehyde and nitrogen dioxide (NO₂) in schools in Johor Bahru, Malaysia: Associations with rhinitis, ocular, throat and dermal symptoms, headache and fatigue. *Sci Total Environ* 592: 153-160. <http://dx.doi.org/10.1016/j.scitotenv.2017.02.215>.
- [Norback, D; Walinder, R; Wieslander, G; Smedje, G; Erwall, C; Venge, P.](#) (2000). Indoor air pollutants in schools: nasal patency and biomarkers in nasal lavage. *Allergy* 55: 163-170. <http://dx.doi.org/10.1034/j.1398-9995.2000.00353.x>.
- [Norsted, SW; Kozinetz, CA; Annegers, JF.](#) (1985). Formaldehyde complaint investigations in mobile homes by the Texas Department of Health. *Environ Res* 37: 93-100. [http://dx.doi.org/10.1016/0013-9351\(85\)90052-0](http://dx.doi.org/10.1016/0013-9351(85)90052-0).
- [Novak, JP; Fedoroff, S.](#) (1996). Cell cycle time of murine neopallial cells in vitro. *J Neurosci Res* 45: 75-84. [http://dx.doi.org/10.1002/\(SICI\)1097-4547\(19960701\)45:1<75::AID-JNR7>3.0.CO;2-E](http://dx.doi.org/10.1002/(SICI)1097-4547(19960701)45:1<75::AID-JNR7>3.0.CO;2-E).
- [NRC](#) (National Research Council). (1988). Health risks of radon and other internally deposited alpha emitters: BEIR IV. Washington, DC. http://books.nap.edu/openbook.php?record_id=1026&page=R1.
- [NRC](#) (National Research Council). (2011). Review of the Environmental Protection Agency's draft IRIS assessment of formaldehyde (pp. 1-194). Washington, DC: The National Academies Press. <http://dx.doi.org/10.17226/13142>.

Supplemental Information—Formaldehyde (Inhalation)

- [NRC](#) (National Research Council). (2014). Review of the Formaldehyde Assessment in the National Toxicology Program 12th Report on Carcinogens. Washington (DC): National Academies Press (US). <http://dx.doi.org/10.17226/18948>.
- [NTP](#) (National Toxicology Program). (2010). Final report on carcinogens. Background document for formaldehyde [NTP] (pp. i-512).
- [NTP](#) (National Toxicology Program). (2011a). Report on carcinogens. Twelfth Edition, 2011 (12th ed., pp. 507). (NTP TR 581 126). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB2011111646.xhtml>.
- [NTP](#) (National Toxicology Program). (2011b). Twelfth Report On Carcinogens, 2011. [CBRNIA-1953235] (pp. 499). <https://www.dtic.mil/DOAC/document?document=CBRNIA-1953235&collection=ac-tems&contentType=PDF&citationFormat=1f>.
- [NTP](#) (National Toxicology Program). (2014). 13th Report on carcinogens [NTP]. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service.
- [Nunn, AJ; Craigen, AA; Darbyshire, JH; Venables, KM; Taylor, AJN](#). (1990). Six year follow up of lung function in men occupationally exposed to formaldehyde. *Br J Ind Med* 47: 747-752. <http://dx.doi.org/10.1136/oem.47.11.747>.
- [O'Connor, PM; Fox, BW](#). (1987). Comparative studies of DNA cross-linking reactions following methylene dimethanesulphonate and its hydrolytic product, formaldehyde. *Cancer Chemother Pharmacol* 19: 11-15. <http://dx.doi.org/10.1007/BF00296247>.
- [O'Connor, TM; O'Halloran, DJ; Shanahan, F](#). (2000). The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia [Review]. *QJM* 93: 323-333.
- [O'Donnell, R; Breen, D; Wilson, S; Djukanovic, R](#). (2006). Inflammatory cells in the airways in COPD [Review]. *Thorax* 61: 448-454. <http://dx.doi.org/10.1136/thx.2004.024463>.
- [O'Donovan, MR; Mee, CD](#). (1993). Formaldehyde is a bacterial mutagen in a range of Salmonella and Escherichia indicator strains. *Mutagenesis* 8: 577-581. <http://dx.doi.org/10.1093/mutage/8.6.577>.
- [Obe, G; Beek, B](#). (1979). Mutagenic activity of aldehydes. *Drug Alcohol Depend* 4: 91-94. [http://dx.doi.org/10.1016/0376-8716\(79\)90044-9](http://dx.doi.org/10.1016/0376-8716(79)90044-9).
- [Odeigah, PGC](#). (1997). Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. *Mutat Res Genet Toxicol Environ Mutagen* 389: 141-148. [http://dx.doi.org/10.1016/S1383-5718\(96\)00136-2](http://dx.doi.org/10.1016/S1383-5718(96)00136-2).
- [Odkvist, LM; Edling, C; Hellquist, H](#). (1985). Influence of vapours on the nasal mucosa among industry workers. *Rhinology* 23: 121-127.
- [OECD](#) (Organisation for Economic Co-operation and Development). (2009). Series on testing and assesment: Number 39. Guidance document on acute inhalation toxicity testing [OECD SIDS]. (ENV/JM/MONO(2009)28; JT03268144). Paris, Fr. <http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono%282009%2928&doclanguage=en>.
- [Oerstavik, D; Hongslo, JK](#). (1985). Mutagenicity of endodontic sealers. *Biomaterials* 6: 129-132.
- [Offermann, FJ; Robertson, J; Springer, D; Brennan, S; Woo, T](#). (2008). Window usage, ventilation, and formaldehyde concentrations in new california homes: Summer field sessions. Paper presented at ASHRAE IAQ 2007Baltimore, MD.
- [Ohmichi, K; Komiyama, M; Matsuno, Y; Sawabe, Y; Miyaso, H; Fukata, H; Ohmichi, M; Kadota, T; Nomura, F; Moria, C](#). (2006). Relationship between exposure to formaldehyde and immunoglobulin E (IgE) production during the gross anatomy laboratory. *J Health Sci* 52: 642-647. <http://dx.doi.org/10.1248/jhs.52.642>.

- [Ohta, T; Watanabe-Akanuma, M; Tokishita, S; Yamagata, H.](#) (1999). Mutation spectra of chemical mutagens determined by Lac+ reversion assay with Escherichia coli WP3101P-WP3106P tester strains. *Mutat Res Genet Toxicol Environ Mutagen* 440: 59-74.
- [Ohtsuka, R; Shuto, Y; Fujie, H; Takeda, M; Harada, T; Itagaki, S.](#) (1997). Response of respiratory epithelium of BN and F344 rats to formaldehyde inhalation. *Exp Anim* 46: 279-286. <http://dx.doi.org/10.1538/expanim.46.279>.
- [Ohtsuka, R; Shutoh, Y; Fujie, H; Yamaguchi, S; Takeda, M; Harada, T; Doi, K.](#) (2003). Rat strain difference in histology and expression of Th1- and Th2-related cytokines in nasal mucosa after short-term formaldehyde inhalation. *Exp Toxicol Pathol* 54: 287-291. <http://dx.doi.org/10.1078/0940-2993-00266>.
- [Olin, KL; Cherr, GN; Rifkin, E; Keen, CL.](#) (1996). The effects of some redox-active metals and reactive aldehydes on DNA-protein cross-links in vitro. *Toxicology* 110: 1-8. [http://dx.doi.org/10.1016/0300-483X\(96\)03318-5](http://dx.doi.org/10.1016/0300-483X(96)03318-5).
- [Olive, PL; Banath, JP.](#) (2006). The comet assay: A method to measure DNA damage in individual cells. *Nat Protoc* 1: 23-29. <http://dx.doi.org/10.1038/nprot.2006.5>.
- [Ollerenshaw, SL; Jarvis, D; Sullivan, CE; Woolcock, AJ.](#) (1991). Substance P immunoreactive nerves in airways from asthmatics and nonasthmatics. *Eur Respir J* 4: 673-682.
- [Olsen, JH; Asnaes, S.](#) (1986a). Formaldehyde and risk of squamous cell carcinoma of the sino nasal cavities. *Occup Environ Med* 43: 769-774.
- [Olsen, JH; Asnaes, S.](#) (1986b). Formaldehyde and the risk of squamous cell carcinoma of the sinonasal cavities. *Br J Ind Med* 43: 769-744. <http://dx.doi.org/10.1136/oem.43.11.769>.
- [Olsen, JH; Dossing, M.](#) (1982). Formaldehyde induced symptoms in day care centers. *AIHA J* 43: 366-370. <http://dx.doi.org/10.1080/15298668291409866>.
- [Olsen, JH; Jensen, SP; Hink, M; Faurbo, K; Breum, NO; Jensen, ON.](#) (1984). Occupational formaldehyde exposure and increased nasal cancer risk in man. *Int J Cancer* 34: 639-644. <http://dx.doi.org/10.1002/ijc.2910340509>.
- [Orsiere, T; Sari-Minodier, I; Iarmarcovai, G; Botta, A.](#) (2006). Genotoxic risk assessment of pathology and anatomy laboratory workers exposed to formaldehyde by use of personal air sampling and analysis of DNA damage in peripheral lymphocytes. *Mutat Res Genet Toxicol Environ Mutagen* 605: 30-41. <http://dx.doi.org/10.1016/j.mrgentox.2006.01.006>.
- [Orstavik, D; Hongslo, JK.](#) (1985). Mutagenicity of endodontic sealers. *Biomaterials* 6: 129-132. [http://dx.doi.org/10.1016/0142-9612\(85\)90076-6](http://dx.doi.org/10.1016/0142-9612(85)90076-6).
- [Ostojić, L; Bradarić, A; Mise, K; Ostojić, Z; Lovrić, J; Petrović, P; Ujević, A; Erceg, M; Janković, S; Tocilj, J.](#) (2006). Pulmonary function in persons who are professionally exposed to formaldehyde fumes. *Coll Antropol* 30: 507-511.
- [Ott, MG; Teta, J; Greenberg, HL.](#) (1989). Lymphatic and hematopoietic tissue cancer in a chemical manufacturing environment. *Am J Ind Med* 16: 631-644. <http://dx.doi.org/10.1002/ajim.4700160603>.
- [Overton, JH; Kimbell, JS; Miller, FJ.](#) (2001). Dosimetry modeling of inhaled formaldehyde: The human respiratory tract. *Toxicol Sci* 64: 122-134. <http://dx.doi.org/10.1093/toxsci/64.1.122>.
- [Ozen, OA; Akpolat, N; Songur, A; Kuş, I; Zararsiz, I; Ozaçmak, VH; Sarsilmaz, M.](#) (2005). Effect of formaldehyde inhalation on Hsp70 in seminiferous tubules of rat testes: An immunohistochemical study. *Toxicol Ind Health* 21: 249-254. <http://dx.doi.org/10.1191/0748233705th235oa>.
- [Ozen, OA; Kus, MA; Kus, I; Alkoc, OA; Songur, A.](#) (2008). Protective effects of melatonin against formaldehyde-induced oxidative damage and apoptosis in rat testes: An immunohistochemical and biochemical study. *Sys Biol Reprod Med* 54: 169-176. <http://dx.doi.org/10.1080/19396360802422402>.

- Ozen, OA; Songue, A; Sars, M; Yaman, M; Kus, I. (2003a). Changes of zinc, copper, and iron levels in the lung of male rats after subacute (4-week) and subchronic (13-week) exposure to formaldehyde. *J Trace Elem Exp Med* 16: 67-74. <http://dx.doi.org/10.1002/jtra.10026>.
- Ozen, OA; Songur, A; Sarsilmaz, M; Yaman, M; Kuş, I. (2003b). Zinc, copper and iron concentrations in cerebral cortex of male rats exposed to formaldehyde inhalation. *J Trace Elem Med Biol* 17: 207-209. [http://dx.doi.org/10.1016/S0946-672X\(03\)80027-5](http://dx.doi.org/10.1016/S0946-672X(03)80027-5).
- Ozen, OA; Yaman, M; Sarsilmaz, M; Songur, A; Kus, I. (2002). Testicular zinc, copper and iron concentrations in male rats exposed to subacute and subchronic formaldehyde gas inhalation. *J Trace Elem Med Biol* 16: 119-122. [http://dx.doi.org/10.1016/S0946-672X\(02\)80038-4](http://dx.doi.org/10.1016/S0946-672X(02)80038-4).
- Pala, M; Ugolini, D; Ceppi, M; Rizzo, F; Maiorana, L; Bolognesi, C; Schilirò, T; Gilli, G; Bigatti, P; Bono, R; Vecchio, D. (2008). Occupational exposure to formaldehyde and biological monitoring of Research Institute workers. *Cancer Detect Prev* 32: 2008. <http://dx.doi.org/10.1016/j.cdp.2008.05.003>.
- Palczynski, C; Krakowiak, A; Hanke, W; Walusiak, J; Gorski, P. (1999). Indoor formaldehyde exposure and airway allergic diseases. *Int Rev Allergol Clin Immunol* 5: 65-69.
- Pandey, MK. (2013). Molecular Basis for Downregulation of C5a-Mediated Inflammation by IgG1 Immune Complexes in Allergy and Asthma. *Curr Allergy Asthma Rep* 13: 596-606. <http://dx.doi.org/10.1007/s11882-013-0387-3>.
- Park, J; Yang, H; Song, MK; Kim, D; Lee, K. (2020). Formaldehyde exposure induces regulatory T cell-mediated immunosuppression via calcineurin-NFAT signalling pathway. *Sci Rep* 10: 17023. <http://dx.doi.org/10.1038/s41598-020-72502-9>.
- Partanen, T; Kauppinen, T; Hernberg, S; Nickels, J; Luukkonen, R; Hakulinen, T; Pukkala, E. (1990). Formaldehyde exposure and respiratory cancer among woodworkers - an update. *Scand J Work Environ Health* 16: 394-400. <http://dx.doi.org/10.5271/sjweh.1766>.
- Parthasarathy, S; Maddalena, RL; Russell, ML; Apte, MG. (2011). Effect of Temperature and Humidity on Formaldehyde Emissions in Temporary Housing Units. *J Air Waste Manag Assoc* 61: 689-695. <http://dx.doi.org/10.3155/1047-3289.61.6.689>.
- Paul, WE; Brown, M; Hornbeck, P; Mizuguchi, J; Ohara, J; Rabin, E; Snapper, C; Tsang, W. (1987). Regulation of B-lymphocyte activation, proliferation, and differentiation [Review]. *Ann N Y Acad Sci* 505: 82-89. <http://dx.doi.org/10.1111/j.1749-6632.1987.tb51284.x>.
- Pauluhn, J. (1998). Hazard identification and risk assessment of pyrethroids in the indoor environment. *Appl Occup Environ Hyg* 13: 469-478.
- Pauluhn, J. (2008). Inhalation toxicology: Methodological and regulatory challenges. *Exp Toxicol Pathol* 60: 111-124. <http://dx.doi.org/10.1016/j.etp.2008.01.013>.
- Pauluhn, J. (2018). Upper respiratory tract nociceptor stimulation and stress response following acute and repeated Cyfluthrin inhalation in normal and pregnant rats: Physiological rat-specific adaptations can easily be misunderstood as adversities [Review]. *Toxicol Lett* 282: 8-24. <http://dx.doi.org/10.1016/j.toxlet.2017.10.003>.
- Payani, S; Mamatha, C; Chandraprakash, C; Bhaskar, M. (2019). Protective role of (Bronco-T) against formaldehyde induced antioxidant, oxidative and histopathological changes in lung of male Wistar rats. *Toxicol Rep* 6: 718-726. <http://dx.doi.org/10.1016/j.toxrep.2019.07.002>.
- Pazdrak, K; Gorski, P; Krakowiak, A; Ruta, U. (1993). Changes in nasal lavage fluid due to formaldehyde inhalation. *Int Arch Occup Environ Health* 64: 515-519. <http://dx.doi.org/10.1007/BF00381101>.
- Peddada, SD; Dinse, GE; Kissling, GE. (2007). Incorporating historical control data when comparing tumor incidence rates. *J Am Stat Assoc* 102: 1212-1220. <http://dx.doi.org/10.1198/016214506000001356>.

- [Peddada, SD; Kissling, GE.](#) (2006). A survival-adjusted quantal-response test for analysis of tumor incidence rates in animal carcinogenicity studies. *Environ Health Perspect* 114: 537-541. <http://dx.doi.org/10.1289/ehp.8590>.
- [Peng, G; Yang, X; Zhao, W; Sun, J; Cao, Y; Xu, Q; Yuan, J; Ding, S.](#) (2006). Gaseous formaldehyde-induced DNA-protein crosslinks in liver, kidney and testicle of Kunming mice. *Life Science Journal* 3: 82-87.
- [Percy, C; Stanek, E, III; Gloeckler, L.](#) (1981). Accuracy of cancer death certificates and its effect on cancer mortality statistics. *Am J Public Health* 71: 242-250. <http://dx.doi.org/10.2105/AJPH.71.3.242>.
- [Percy, CL; Miller, BA; Gloeckler Ries, LA.](#) (1990). Effect of changes in cancer classification and the accuracy of cancer death certificates on trends in cancer mortality. *Ann N Y Acad Sci* 609: 87-99. <http://dx.doi.org/10.1111/j.1749-6632.1990.tb32059.x>.
- [Perry, P; Wolff, S.](#) (1974). New Giemsa method for the differential staining of sister chromatids. *Nature* 251: 156-158.
- [Persoz, C; Achard, S; Leleu, C; Momas, I; Seta, N.](#) (2010). An in vitro model to evaluate the inflammatory response after gaseous formaldehyde exposure of lung epithelial cells. *Toxicol Lett* 195: 99-105. <http://dx.doi.org/10.1016/j.toxlet.2010.03.003>.
- [Persoz, C; Achard, S; Momas, I; Seta, N.](#) (2012). Inflammatory response modulation of airway epithelial cells exposed to formaldehyde. *Toxicol Lett* 211: 159-163. <http://dx.doi.org/10.1016/j.toxlet.2012.03.799>.
- [Persoz, C; Leleu, C; Achard, S; Fasseu, M; Menotti, J; Meneceur, P; Momas, I; Derouin, F; Seta, N.](#) (2011). Sequential air-liquid exposure of human respiratory cells to chemical and biological pollutants. *Toxicol Lett* 207: 53-59. <http://dx.doi.org/10.1016/j.toxlet.2011.07.028>.
- [Pesch, B; Pierl, CB; Gebel, M; Gross, I; Becker, D; Johnen, G; Rihs, HP; Donhuijsen, K; Lepentsiotis, V; Meier, M; Schulze, J; Brüning, T.](#) (2008). Occupational risks for adenocarcinoma of the nasal cavity and paranasal sinuses in the German wood industry. *Occup Environ Med* 65: 191-196. <http://dx.doi.org/10.1136/oem.2007.033886>.
- [Petteffi, GP; Basso da Silva, L; Antunes, MV; Wilhelm, C; Valandro, ET; Glaeser, J; Kaefer, D; Linden, R.](#) (2015). Evaluation of genotoxicity in workers exposed to low levels of formaldehyde in a furniture manufacturing facility. *Toxicol Ind Health* 32: 1763-1773. <http://dx.doi.org/10.1177/0748233715584250>.
- [Peters, TL; Kamel, F; Lundholm, C; Feychting, M; Weibull, CE; Sandler, DP; Wiebert, P; Sparén, P; Ye, W; Fang, F.](#) (2017). Occupational exposures and the risk of amyotrophic lateral sclerosis. *Occup Environ Med* 74: 87-92. <http://dx.doi.org/10.1136/oemed-2016-103700>.
- [Pickrell, JA; Griffis, LC; Mokler, BV; Kanapilly, GM; Hobbs, CH.](#) (1984). Formaldehyde release from selected consumer products: influence of chamber loading, multiple products, relative humidity, and temperature. *Environ Sci Technol* 18: 682-686. <http://dx.doi.org/10.1021/es00127a009>.
- [Pickrell, JA; Mokler, BV; Griffis, LC; Hobbs, CH.](#) (1983). Formaldehyde release rate coefficients from selected consumer products. *Environ Sci Technol* 17: 753-757. <http://dx.doi.org/10.1021/es00118a012>.
- [Pierce, JS; Abelmann, A; Spicer, LJ; Adams, RE; Glynn, ME; Neier, K; Finley, BL; Gaffney, SH.](#) (2011). Characterization of formaldehyde exposure resulting from the use of four professional hair straightening products. *J Occup Environ Hyg* 8: 686-699. <http://dx.doi.org/10.1080/15459624.2011.626259>.
- [Pinkerton, LE; Hein, MJ; Meyers, A; Kamel, F.](#) (2013). Assessment of ALS mortality in a cohort of formaldehyde-exposed garment workers. *Am J Epidemiol* 178: 353-355. <http://dx.doi.org/10.3109/21678421.2013.778284>.

- [Pinkerton, LE; Hein, MJ; Stayner, LT.](#) (2004). Mortality among a cohort of garment workers exposed to formaldehyde: an update. *Occup Environ Med* 61: 193-200. <http://dx.doi.org/10.1136/oem.2003.007476>.
- [Pira, E; Romano, C; Verga, F; La Vecchia, C.](#) (2014). Mortality from lymphohematopoietic neoplasms and other causes in a cohort of laminated plastic workers exposed to formaldehyde. *Cancer Causes Control* 25: 1343-1349. <http://dx.doi.org/10.1007/s10552-014-0440-0>.
- [Pitarque, M; Vaglenov, A; Nosko, M; Hirvonen, A; Norppa, H; Creus, A; Marcos, R.](#) (1999). Evaluation of DNA damage by the Comet assay in shoe workers exposed to toluene and other organic solvents. *Mutat Res* 441: 115-127. [http://dx.doi.org/10.1016/S1383-5718\(99\)00042-X](http://dx.doi.org/10.1016/S1383-5718(99)00042-X).
- [Pitten, FA; Kramer, A; Herrmann, K; Bremer, J; Koch, S.](#) (2000). Formaldehyde neurotoxicity in animal experiments. *Pathol Res Pract* 196: 193-198. [http://dx.doi.org/10.1016/S0344-0338\(00\)80100-4](http://dx.doi.org/10.1016/S0344-0338(00)80100-4).
- [Plesner, BH; Hansen, K.](#) (1983). Formaldehyde and hexamethylenetetramine in Styles' cell transformation assay. *Carcinogenesis* 4: 457-459. <http://dx.doi.org/10.1093/carcin/4.4.457>.
- [Pontel, LB; Rosado, IV; Burgos-Barragan, G; Garaycochea, JI; Yu, R; Arends, MJ; Chandrasekaran, G; Broecker, V; Wei, W; Liu, L; Swenberg, JA; Crossan, GP; Patel, KJ.](#) (2015). Endogenous Formaldehyde Is a Hematopoietic Stem Cell Genotoxin and Metabolic Carcinogen. *Mol Cell* 60: 177-188. <http://dx.doi.org/10.1016/j.molcel.2015.08.020>.
- [Portier, CJ; Bailer, AJ.](#) (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam Appl Toxicol* 12: 731-737. [http://dx.doi.org/10.1016/0272-0590\(89\)90004-3](http://dx.doi.org/10.1016/0272-0590(89)90004-3).
- [Pottern, LM; Heineman, EF; Olsen, JH; Raffn, E; Blair, A.](#) (1992). Multiple myeloma among Danish women: Employment history and workplace exposures. *Cancer Causes Control* 3: 427-432. <http://dx.doi.org/10.1007/BF00051355>.
- [Pournahabadian, M; Azam, K; Ghasemkhani, M.](#) (2006). Pulmonary function study between formaldehyde exposed and non-exposed staffs at some of the Tehran educational hospitals. *Journal of Medical Sciences* 6: 621-625. <http://dx.doi.org/10.3923/jms.2006.621.625>.
- [Priha, E; Liesivuori, J; Santa, H; Laatikainen, R.](#) (1996). Reactions of hydrated formaldehyde in nasal mucus. *Chemosphere* 32: 1077-1082. [http://dx.doi.org/10.1016/0045-6535\(96\)00015-X](http://dx.doi.org/10.1016/0045-6535(96)00015-X).
- [Priha, E; Pennanen, S; Rantio, T; Uitti, J; Liesivuori, J.](#) (2004). Exposure to and acute effects of medium-density fiber board dust. *J Occup Environ Hyg* 1: 738-744. <http://dx.doi.org/10.1080/15459620490520774>.
- [Pross, HF; Day, JH; Clark, RH; Lees, RE.](#) (1987). Immunologic studies of subjects with asthma exposed to formaldehyde and urea-formaldehyde foam insulation (UFFI) off products. *J Allergy Clin Immunol* 79: 797-810. [http://dx.doi.org/10.1016/0091-6749\(87\)90213-2](http://dx.doi.org/10.1016/0091-6749(87)90213-2).
- [Proud, D; Leigh, R.](#) (2011). Epithelial cells and airway diseases [Review]. *Immunol Rev* 242: 186-204. <http://dx.doi.org/10.1111/j.1600-065X.2011.01033.x>.
- [Pushkina, NN; Gofmekler, VA; Klevtsova, GN.](#) (1968). [Changes in ascorbic and nucleic acid concentration under the influence of benzene and formaldehyde]. *Biull Eksp Biol Med* 66: 51-53.
- [Qiao, Y; Li, B; Yang, G; Yao, H; Yang, J; Liu, D; Yan, Y; Sigsgaard, T; Yang, X.](#) (2009). Irritant and adjuvant effects of gaseous formaldehyde on the ovalbumin-induced hyperresponsiveness and inflammation in a rat model. *Inhal Toxicol* 21: 1200-1207. <http://dx.doi.org/10.3109/08958370902806159>.
- [Quackenboss, JJ; Lebowitz, MD; Hayes, C.](#) (1989a). Epidemiological study of respiratory responses to indoor/outdoor air quality. *Environ Int* 15: 493-502. [http://dx.doi.org/10.1016/0160-4120\(89\)90067-6](http://dx.doi.org/10.1016/0160-4120(89)90067-6).
- [Quackenboss, JJ; Lebowitz, MD; Michaud, JP; Bronnimann, D.](#) (1989b). Formaldehyde exposure and acute health effects study. *Environ Int* 15: 169-176. [http://dx.doi.org/10.1016/0160-4120\(89\)90023-8](http://dx.doi.org/10.1016/0160-4120(89)90023-8).

- Quarcoo, D; Schulte-Herbrüggen, O; Lommatzsch, M; Schierhorn, K; Hoyle, GW; Renz, H; Braun, A. (2004). Nerve growth factor induces increased airway inflammation via a neuropeptide-dependent mechanism in a transgenic animal model of allergic airway inflammation. *Clin Exp Allergy* 34: 1146-1151. <http://dx.doi.org/10.1111/j.1365-2222.2004.01993.x>.
- Que, LG; Liu, L; Yan, Y; Whitehead, GS; Gavett, SH; Schwartz, DA; Stamler, JS. (2005). Protection from experimental asthma by an endogenous bronchodilator. *Science* 308: 1618-1621. <http://dx.doi.org/10.1126/science.1108228>.
- Quievryn, G; Zhitkovich, A. (2000). Loss of DNA-protein crosslinks from formaldehyde-exposed cells occurs through spontaneous hydrolysis and an active repair process linked to proteasome function. *Carcinogenesis* 21: 1573-1580. <http://dx.doi.org/10.1093/carcin/21.8.1573>.
- Raaschou-Nielsen, O; Hermansen, MN; Loland, L; Buchvald, F; Pipper, CB; Sørensen, M; Loft, S; Bisgaard, H. (2010). Long-term exposure to indoor air pollution and wheezing symptoms in infants. *Indoor Air* 20: 159-167. <http://dx.doi.org/10.1111/j.1600-0668.2009.00635.x>.
- Ragan, DL; Boreiko, CJ. (1981). Initiation of C3H/10T1/2 cell transformation by formaldehyde. *Cancer Lett* 13: 325-331. [http://dx.doi.org/10.1016/0304-3835\(81\)90061-6](http://dx.doi.org/10.1016/0304-3835(81)90061-6).
- Rager, JE; Moeller, BC; Doyle-Eisele, M; Kracko, D; Swenberg, JA; Fry, RC. (2013). Formaldehyde and epigenetic alterations: microRNA changes in the nasal epithelium of nonhuman primates. *Environ Health Perspect* 121: 339-344. <http://dx.doi.org/10.1289/ehp.1205582>.
- Rager, JE; Moeller, BC; Miller, SK; Kracko, D; Doyle-Eisele, M; Swenberg, JA; Fry, RC. (2014). Formaldehyde-Associated Changes in microRNAs: Tissue and Temporal Specificity in the Rat Nose, White Blood Cells, and Bone Marrow. *Toxicol Sci* 138: 36-46. <http://dx.doi.org/10.1093/toxsci/kft267>.
- Rager, JE; Smeester, L; Jaspers, I; Sexton, KG; Fry, RC. (2011). Epigenetic changes induced by air toxics: formaldehyde exposure alters miRNA expression profiles in human lung cells. *Environ Health Perspect* 119: 494-500. <http://dx.doi.org/10.1289/ehp.1002614>.
- Rao, GN; Piegorsch, WW; Haseman, JK. (1987). Influence of body weight on the incidence of spontaneous neoplasms in rats and mice of long-term studies. *Am J Clin Nutr* 45: 252-260.
- Ratnayake, WE. (1968). Tests for an effect of the Y-chromosome on the mutagenic action of formaldehyde and x-rays in *Drosophila melanogaster*. *Genet Res* 12: 65-69. <http://dx.doi.org/10.1017/S0016672300011629>.
- Ratnayake, WE. (1970). Studies on the relationship between induced crossing-over and mutation in *Drosophila melanogaster*. *Mutat Res* 9: 71-83. [http://dx.doi.org/10.1016/0027-5107\(70\)90071-0](http://dx.doi.org/10.1016/0027-5107(70)90071-0).
- Ravault, C; Kauffmann, F. (2001). Validity of the IUATLD (1986) questionnaire in the EGEA study. *Int J Tuberc Lung Dis* 5: 191-196.
- Reader, JR; Tepper, JS; Schelegle, ES; Aldrich, MC; Putney, LF; Pfeiffer, JW; Hyde, DM. (2003). Pathogenesis of mucous cell metaplasia in a murine asthma model. *Am J Pathol* 162: 2069-2078. [http://dx.doi.org/10.1016/S0002-9440\(10\)64338-6](http://dx.doi.org/10.1016/S0002-9440(10)64338-6).
- Recio, L; Sisk, S; Pluta, L; Bermudez, E; Gross, EA; Chen, Z; Morgan, K; Walker, C. (1992). p53 mutations in formaldehyde-induced nasal squamous cell carcinomas in rats. *Cancer Res* 52: 6113-6116.
- Reiss, R; Ryan, PB; Koutrakis, P; Tibbetts, SJ. (1995). Ozone reactive chemistry on interior latex paint. *Environ Sci Technol* 29: 1906-1912. <http://dx.doi.org/10.1021/es00008a007>.
- Ren, X; Ji, Z; Mchale, CM; Yuh, J; Bersonda, J; Tang, M; Smith, MT; Zhang, L. (2013). The impact of FANCD2 deficiency on formaldehyde-induced toxicity in human lymphoblastoid cell lines. *Arch Toxicol* 87: 189-196. <http://dx.doi.org/10.1007/s00204-012-0911-6>.
- Reuzel, PGJ; Wilmer, JWG, M; Woutersen, RA; Zwart, A; Rombout, PJA; Feron, VJ. (1990). Interactive effects of ozone and formaldehyde on the nasal respiratory lining epithelium in rats. *J Toxicol Environ Health* 29: 279-292. <http://dx.doi.org/10.1080/15287399009531391>.

- [Ricciardolo, FLM; Nijkamp, F; De Rose, V; Folkerts, G.](#) (2008). The guinea pig as an animal model for asthma. *Curr Drug Targets* 9: 452-465. <http://dx.doi.org/10.2174/138945008784533534>.
- [Riedel, F; Hasenauer, E; Barth, PJ; Kozirowski, A; Rieger, CHL.](#) (1996). Formaldehyde exposure enhances inhalative allergic sensitization in the guinea pig. *Allergy* 51: 94-99. <http://dx.doi.org/10.1111/j.1398-9995.1996.tb00041.x>.
- [Riess, U; Tegtbur, U; Fauck, C; Fuhrmann, F; Markewitz, D; Salthammer, T.](#) (2010). Experimental setup and analytical methods for the non-invasive determination of volatile organic compounds, formaldehyde and NOx in exhaled human breath. *Anal Chim Acta* 669: 53-62. <http://dx.doi.org/10.1016/j.aca.2010.04.049>.
- [Ritchie, IM; Lehnen, RG.](#) (1985). An analysis of formaldehyde concentrations in mobile and conventional homes. *J Environ Health* 47: 300-305.
- [Ritchie, IM; Lehnen, RG.](#) (1987). Formaldehyde-related health complaints of residents living in mobile and conventional homes. *Am J Public Health* 77: 323-328. <http://dx.doi.org/10.2105/ajph.77.3.323>.
- [Roberts, AL; Johnson, NJ; Cudkowicz, ME; Eum, KD; Weisskopf, MG.](#) (2016). Job-related formaldehyde exposure and ALS mortality in the USA [Letter]. *J Neurol Neurosurg Psychiatry* 87: 786-788. <http://dx.doi.org/10.1136/jnnp-2015-310750>.
- [Robinson, CF; Fowler, D; Brown, DP; Lemen, RA.](#) (1987). Plywood mill workers' mortality patterns 1945-1977 (revised March 1987). (NIOSH/00197140). Cincinnati, OH: NIOSH.
- [Roda, C; Kousignian, I; Guihenneuc-Jouyaux, C; Dassonville, C; Nicolis, I; Just, J; Momas, I.](#) (2011). Formaldehyde exposure and lower respiratory infections in infants: findings from the PARIS cohort study. *Environ Health Perspect* 119: 1653-1658. <http://dx.doi.org/10.1289/ehp.1003222>.
- [Rodrigo, R; Prat, H; Passalacqua, W; Araya, J; Guichard, C; Bächler, JP.](#) (2007). Relationship between oxidative stress and essential hypertension. *Hypertens Res* 30: 1159-1167. <http://dx.doi.org/10.1291/hypres.30.1159>.
- [Roemer, E; Anton, HJ; Kindt, R.](#) (1993). Cell proliferation in the respiratory tract of the rat after acute inhalation of formaldehyde or acrolein. *J Appl Toxicol* 13: 103-107. <http://dx.doi.org/10.1002/jat.2550130206>.
- [Roma-Torres, J; Teixeira, JP; Silva, S; Laffon, B; Cunha, LM; Méndez, J; Mayan, O.](#) (2006). Evaluation of genotoxicity in a group of workers from a petroleum refinery aromatics plant. *Mutat Res* 604: 19-27. <http://dx.doi.org/10.1016/j.mrgentox.2005.12.005>.
- [Romanazzi, V; Pirro, V; Bellisario, V; Mengozzi, G; Peluso, M; Pazzi, M; Bugiani, M; Verlato, G; Bono, R.](#) (2013). 15-F2t isoprostane as biomarker of oxidative stress induced by tobacco smoke and occupational exposure to formaldehyde in workers of plastic laminates. *Sci Total Environ* 442: 20-25. <http://dx.doi.org/10.1016/j.scitotenv.2012.10.057>.
- [Ross, WE; Mcmillan, DR; Ross, CF.](#) (1981). Comparison of DNA damage by methylmelamines and formaldehyde. *J Natl Cancer Inst* 67: 217-221.
- [Ross, WE; Shipley, N.](#) (1980). Relationship between DNA damage and survival in formaldehyde-treated mouse cells. *Mutat Res* 79: 277-283. [http://dx.doi.org/10.1016/0165-1218\(80\)90075-0](http://dx.doi.org/10.1016/0165-1218(80)90075-0).
- [Rossant, J; Cross, JC.](#) (2001). Placental development: Lessons from mouse mutants [Review]. *Nat Rev Genet* 2: 538-548. <http://dx.doi.org/10.1038/35080570>.
- [Rothman, KJ; Boice, JD.](#) (1979). Epidemiologic analysis with a programmable calculator. (NIH Publication No. 79-1649). Washington, D.C.: National Institutes of Health.
- [Rothman, N; Lan, Q; Smith, MT; Vermeulen, R; Zhang, L.](#) (2017). Response to letter to the editor of Carcinogenesis by Pira et al., 2017 [Letter]. *Carcinogenesis* 38: 1253-1255. <http://dx.doi.org/10.1093/carcin/bgx111>.
- [Rotstein, J; Sarma, DS; Farber, E.](#) (1986). Sequential alterations in growth control and cell dynamics of rat hepatocytes in early precancerous steps in hepatocarcinogenesis. *Cancer Res* 46: 2377-2385.

- [Roush, GC; Walrath, J; Stayner, LT; Kaplan, SA; Flannery, JT; Blair, A.](#) (1987). Nasopharyngeal cancer sinonasal cancer and occupations related to formaldehyde: A case-control study. *J Natl Cancer Inst* 79: 1221-1224.
- [Rumchev, K; Spickett, J; Bulsara, M; Phillips, M; Stick, S.](#) (2004). Association of domestic exposure to volatile organic compounds with asthma in young children. *Thorax* 59: 746-751. <http://dx.doi.org/10.1136/thx.2003.013680>.
- [Rumchev, KB; Spickett, JT; Bulsara, MK; Phillips, MR; Stick, SM.](#) (2002). Domestic exposure to formaldehyde significantly increases the risk of asthma in young children. *Eur Respir J* 20: 403-408. <http://dx.doi.org/10.1183/09031936.02.00245002>.
- [Rusch, GM; Clary, JJ; Rinehart, WE; Bolte, HF.](#) (1983). A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. *Toxicol Appl Pharmacol* 68: 329-343. [http://dx.doi.org/10.1016/0041-008X\(83\)90276-4](http://dx.doi.org/10.1016/0041-008X(83)90276-4).
- [Rydén, E; Ekström, C; Hellmér, L; Bolcsfoldi, G.](#) (2000). Comparison of the sensitivities of *Salmonella typhimurium* strains TA102 and TA2638A to 16 mutagens. *Mutagenesis* 15: 495-502.
- [Saber Hosnijeh, F; Christopher, Y; Peeters, P; Romieu, J; Xun, W; Riboli, E; Raaschou-Nielsen, O; Tjønneland, A; Becker, N; Nieters, A; Trichopoulou, A; Bamia, C; Orfanos, P; Oddone, E; Luján-Barroso, L; Dorronsoro, M; Navarro, C; Barricarte, A; Molina-Montes, E; Wareham, N; Vineis, P; Vermeulen, R.](#) (2013). Occupation and risk of lymphoid and myeloid leukaemia in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Occup Environ Med* 70: 464-470. <http://dx.doi.org/10.1136/oemed-2012-101135>.
- [Sadakane, K; Takano, H; Ichinose, T; Yanagisawa, R; Shibamoto, T.](#) (2002). Formaldehyde enhances mite allergen-induced eosinophilic inflammation in the murine airway. *J Environ Pathol Toxicol Oncol* 21: 267-276.
- [Sahin-Yilmaz, A; Naclerio, RM.](#) (2011). Anatomy and physiology of the upper airway [Review]. *Proc Am Thorac Soc* 8: 31-39. <http://dx.doi.org/10.1513/pats.201007-050RN>.
- [Saillenfait, AM; Bonnet, P; de Ceaurriz, J.](#) (1989). The effects of maternally inhaled formaldehyde on embryonal and foetal development in rats. *Food Chem Toxicol* 27: 545-548. [http://dx.doi.org/10.1016/0278-6915\(89\)90051-3](http://dx.doi.org/10.1016/0278-6915(89)90051-3).
- [Saito, Y; Nishio, K; Yoshida, Y; Niki, E.](#) (2005). Cytotoxic effect of formaldehyde with free radicals via increment of cellular reactive oxygen species. *Toxicology* 210: 235-245. <http://dx.doi.org/10.1016/j.tox.2005.02.006>.
- [Sakellaris, I; Saraga, D; Mandin, C; de Kluizenaar, Y; Fossati, S; Spinazzè, A; Cattaneo, A; Mihucz, V; Szigeti, T; de Oliveira Fernandes, E; Kalimeri, K; Mabilia, R; Carrer, P; Bartzis, J.](#) (2020). Association of subjective health symptoms with indoor air quality in European office buildings: The OFFICAIR project. *Indoor Air* 31: 426-439. <http://dx.doi.org/10.1111/ina.12749>.
- [Saladino, AJ; Willey, JC; Lechner, JF; Grafstrom, RC; Laveck, M; Harris, CC.](#) (1985). Effects of formaldehyde, acetaldehyde, benzoyl peroxide, and hydrogen peroxide on cultured normal human bronchial epithelial cells. *Cancer Res* 45: 2522-2526.
- [Salas, MM; Hargreaves, KM; Akopian, AN.](#) (2009). TRPA1-mediated responses in trigeminal sensory neurons: Interaction between TRPA1 and TRPV1. *Eur J Neurosci* 29: 1568-1578. <http://dx.doi.org/10.1111/j.1460-9568.2009.06702.x>.
- [Saldiva, PHN; Caldeira, MPR; Massad, E; Calheiros, DF; Cardoso, LMN; Bohm, GM; Saldiva, CD.](#) (1985). Effects of formaldehyde and acetaldehyde inhalation on rat pulmonary mechanics. *J Appl Toxicol* 5: 288-292. <http://dx.doi.org/10.1002/jat.2550050505>.
- [Salonen, H; Pasanen, AL; Lappalainen, S; Riuttala, H; Tuomi, T; Pasanen, P; Back, B; Reijula, K.](#) (2009). Volatile organic compounds and formaldehyde as explaining factors for sensory irritation in office environments. *J Occup Environ Hyg* 6: 239-247. <http://dx.doi.org/10.1080/15459620902735892>.

- [Salthammer, T; Mentese, S; Marutzky, R.](#) (2010). Formaldehyde in the indoor environment. *Chem Rev* 110: 2536-2572. <http://dx.doi.org/10.1021/cr800399g>.
- [Sandel, M; Murphy, JS; Dixon, SL; Adgate, JL; Chew, GL; Dorevitch, S; Jacobs, DE.](#) (2014). A side-by-side comparison of three allergen sampling methods in settled house dust. *J Expo Sci Environ Epidemiol* 24: 650-656. <http://dx.doi.org/10.1038/jes.2014.30>.
- [Sandikci, M; Eren, U; Kum, S.](#) (2007a). Effects of formaldehyde and xylene on alpha-naphthyl acetate esterase positive T-lymphocytes in bronchus associated lymphoid tissue and peripheral blood in rats. *Rev Med Vet* 158: 297-301.
- [Sandikci, M; Eren, U; Kum, S.](#) (2007b). Effects of formaldehyde and xylene on CD4- and CD8-positive T cells in bronchus-associated lymphoid tissue in rats. *Toxicol Ind Health* 23: 471-477. <http://dx.doi.org/10.1177/0748233708089025>.
- [Sandikci, M; Seyrek, K; Aksit, H; Kose, H.](#) (2009). Inhalation of formaldehyde and xylene induces apoptotic cell death in the lung tissue. *Toxicol Ind Health* 25: 455-461. <http://dx.doi.org/10.1177/0748233709106824>.
- [Sanghani, PC; Stone, CL; Ray, BD; Pindel, EV; Hurley, TD; Bosron, WF.](#) (2000). Kinetic mechanism of human glutathione-dependent formaldehyde dehydrogenase. *Biochemistry* 39: 10720-10729. <http://dx.doi.org/10.1021/bi9929711>.
- [Sanotskii, IV; Fomenko, VN; Sheveleva, GA; Sal'nikova, LS; Nakoriakova, MV.](#) (1976). [Study of the effect of pregnancy on animal sensitivity to chemical agents]. *Gig Tr Prof Zabol* (1): 25-28.
- [Santovito, A; Cervella, P; Delpero, M.](#) (2014). Chromosomal damage in peripheral blood lymphocytes from nurses occupationally exposed to chemicals. *Hum Exp Toxicol* 33: 897-903. <http://dx.doi.org/10.1177/0960327113512338>.
- [Santovito, A; Schilirò, T; Castellano, S; Cervella, P; Bigatti, MP; Gilli, G; Bono, R; Delpero, M.](#) (2011). Combined analysis of chromosomal aberrations and glutathione S-transferase M1 and T1 polymorphisms in pathologists occupationally exposed to formaldehyde. *Arch Toxicol* 85: 1295-1302. <http://dx.doi.org/10.1007/s00204-011-0668-3>.
- [Saowakon, N; Ngernsoungnern, P; Watcharavitoon, P; Ngernsoungnern, A; Kosanlavit, R.](#) (2015). Formaldehyde exposure in gross anatomy laboratory of Suranaree University of Technology: a comparison of area and personal sampling. *Environ Sci Pollut Res Int* 22: 19002-19012. <http://dx.doi.org/10.1007/s11356-015-5078-2>.
- [Sapmaz, E; Sapmaz, HI; Vardi, N; Tas, U; Sarsilmaz, M; Toplu, Y; Arici, A; Uysal, M.](#) (2017). Harmful effects of formaldehyde and possible protective effect of *Nigella sativa* on the trachea of rats. *Niger J Clin Pract* 20: 523-529. <http://dx.doi.org/10.4103/1119-3077.183253>.
- [Sapmaz, HI; Sarsilmaz, M; Gödekmerdan, A; Ögetürk, M; Taş, U; Köse, E.](#) (2015). Effects of formaldehyde inhalation on humoral immunity and protective effect of *Nigella sativa* oil: An experimental study. *Toxicol Ind Health* 32: 1564-1569. <http://dx.doi.org/10.1177/0748233714566294>.
- [Sapmaz, HI; Yildiz, A; Polat, A; Vardi, N; Kose, E; Tanbek, K; Cuglan, S.](#) (2018). Protective efficacy of *Nigella sativa* oil against the harmful effects of formaldehyde on rat testicular tissue. *8*: 548-553. <http://dx.doi.org/10.4103/2221-1691.245970>.
- [Sari-Minodier, J; Orsière, T; Bellon, L; Pompili, J; Sapin, C; Botta, A.](#) (2002). Cytogenetic monitoring of industrial radiographers using the micronucleus assay. *Mutat Res* 521: 37-46.
- [Sari, DK; Kuwahara, S; Furuya, M; Tsukamoto, Y; Hori, H; Kunugita, N; Arashidani, K; Fujimaki, H; Sasaki, F.](#) (2005). Hypothalamo-pituitary-adrenal gland axis in mice inhaling toluene prior to low-level long-term exposure to formaldehyde. *J Vet Med Sci* 67: 303-309. <http://dx.doi.org/10.1292/jvms.67.303>.
- [Sari, DK; Kuwahara, S; Tsukamoto, Y; Hori, H; Kunugita, N; Arashidani, K; Fujimaki, H; Sasaki, F.](#) (2004). Effect of prolonged exposure to low concentrations of formaldehyde on the corticotropin releasing hormone neurons in the hypothalamus and adrenocorticotrophic hormone cells in the

- pituitary gland in female mice. *Brain Res* 1013: 107-116.
<http://dx.doi.org/10.1016/j.brainres.2004.03.070>.
- Sarigiannis, DA; Karakitsios, SP; Gotti, A; Liakos, IL; Katsoyiannis, A. (2011). Exposure to major volatile organic compounds and carbonyls in European indoor environments and associated health risk [Review]. *Environ Int* 37: 743-765. <http://dx.doi.org/10.1016/j.envint.2011.01.005>.
- Sarin, S; Undem, B; Sanico, A; Togias, A. (2006). The role of the nervous system in rhinitis. *J Allergy Clin Immunol* 118: 999-1016.
- Sarraf, AM; Krahn, DF; Donovan, SM; O'Neil, RM. (1997). Evaluation of hexamethylphosphoramide for gene mutations in *Salmonella typhimurium* using plate incorporation, preincubation, and suspension assays. *Mutat Res* 380: 167-177. [http://dx.doi.org/10.1016/S0027-5107\(97\)00134-6](http://dx.doi.org/10.1016/S0027-5107(97)00134-6).
- Sarsilmaz, M; Kaplan, S; Songur, A; Colakoglu, S; Aslan, H; Tunc, AT; Ozen, OA; Turgut, M; Baş, O. (2007). Effects of postnatal formaldehyde exposure on pyramidal cell number, volume of cell layer in hippocampus and hemisphere in the rat: A stereological study. *Brain Res* 11: 157-167.
<http://dx.doi.org/10.1016/j.brainres.2007.01.139>.
- Sarsilmaz, M; Ozen, OA; Akpolat, N; Kus, I; Songur, A. (1999). The histopathologic effects of inhaled formaldehyde on leydig cells of the rats in subacute period. *Firat Univ Med Sci J* 13: 37-40.
- Sarto, F; Finotto, S; Giacomelli, L; Mazzotti, D; Tomanin, R; Levis, AG. (1987). The micronucleus assay in exfoliated cells of the human buccal mucosa. *Mutagenesis* 2: 11-17.
<http://dx.doi.org/10.1093/mutage/2.1.11>.
- Sauder, LR; Chatham, MD; Green, DJ; Kulle, TJ. (1986). Acute pulmonary response to formaldehyde exposure in healthy nonsmokers. *J Occup Environ Med* 28: 420-424.
<http://dx.doi.org/10.1097/00043764-198606000-00008>.
- Sauder, LR; Green, DJ; Chatham, MD; Kulle, TJ. (1987). Acute pulmonary response of asthmatics to 3.0 ppm formaldehyde. *Toxicol Ind Health* 3: 569-578.
<http://dx.doi.org/10.1177/074823378700300408>.
- Saurel-Cubizolles, MJ; Hays, M; Estryn-Behar, M. (1994). Work in operating rooms and pregnancy outcome among nurses. *Int Arch Occup Environ Health* 66: 235-241.
<http://dx.doi.org/10.1007/bf00454361>.
- Saurel-Cubizolles, MJ; Job-Spira, N; Estryn-Behar, M. (1993). Ectopic pregnancy and occupational exposure to antineoplastic drugs. *Lancet* 341: 1169-1171. [http://dx.doi.org/10.1016/0140-6736\(93\)91000-C](http://dx.doi.org/10.1016/0140-6736(93)91000-C).
- Savage, JR. (1976). Classification and relationships of induced chromosomal structural changes. *J Med Genet* 13: 103-122. <http://dx.doi.org/10.1136/jmg.13.2.103>.
- Sax, SN; Bennett, DH; Chillrud, SN; Kinney, PL; Spengler, JD. (2004). Differences in source emission rates of volatile organic compounds in inner-city residences of New York City and Los Angeles. *J Expo Anal Environ Epidemiol* 14: S95-S109. <http://dx.doi.org/10.1038/sj.jea.7500364>.
- Schachter, EN; Witek T J, J. R.; Tosun, T; Leaderer, BP; Beck, GJ. (1986a). A STUDY OF RESPIRATORY EFFECTS FROM EXPOSURE TO 2 PARTS-PER-MILLION FORMALDEHYDE IN HEALTHY SUBJECTS. *Arch Environ Health* 41: 229-239.
- Schachter, EN; Witek, TJ, Jr; Brody, DJ; Tosun, T; Beck, GJ; Leaderer, BP. (1987). A study of respiratory effects from exposure to 2.0 ppm formaldehyde in occupationally exposed workers. *Environ Res* 44: 188-205. [http://dx.doi.org/10.1016/S0013-9351\(87\)80227-X](http://dx.doi.org/10.1016/S0013-9351(87)80227-X).
- Schachter, EN; Witek, TJ, Jr; Tosun, T; Leaderer, BP; Beck, GJ. (1986b). A study of respiratory effects from exposure to 2 ppm formaldehyde in healthy subjects. *Arch Environ Occup Health* 41: 229-239.
<http://dx.doi.org/10.1080/00039896.1986.9938338>.
- Schafer, D; Brommer, C; Riechelmann, H; Mann, JW. (1999). In vivo and in vitro effect of ozone and formaldehyde on human nasal mucociliary transport system. *Rhinology* 37: 56-60.

- [Schenker, MB; Weiss, ST; Murawski, BJ.](#) (1982). Health effects of residence in homes with urea formaldehyde foam insulation: A pilot study. *Environ Int* 8: 359-363.
[http://dx.doi.org/10.1016/0160-4120\(82\)90050-2](http://dx.doi.org/10.1016/0160-4120(82)90050-2).
- [Schlink, K; Janssen, K; Nitzsche, S; Gebhard, S; Hengstler, JG; Klein, S; Oesch, F.](#) (1999). Activity of O6-methylguanine DNA methyltransferase in mononuclear blood cells of formaldehyde-exposed medical students. *Arch Toxicol* 73: 15-21. <http://dx.doi.org/10.1007/s002040050581>.
- [Schlosser, PM.](#) (1999). Relative roles of convection and chemical reaction for the disposition of formaldehyde and ozone in nasal mucus. *Inhal Toxicol* 11: 967-980.
<http://dx.doi.org/10.1080/089583799196736>.
- [Schlosser, PM; Lilly, PD; Conolly, RB; Janszen, DB; Kimbell, JS.](#) (2003). Benchmark dose risk assessment for formaldehyde using airflow modeling and a single-compartment, DNA-protein cross-link dosimetry model to estimate human equivalent doses. *Risk Anal* 23: 473-487.
<http://dx.doi.org/10.1111/1539-6924.00328>.
- [Schmid, E; Göggelmann, W; Bauchinger, M.](#) (1986). Formaldehyde-induced cytotoxic, genotoxic and mutagenic response in human lymphocytes and *Salmonella typhimurium*. *Mutagenesis* 1: 427-431. <http://dx.doi.org/10.1093/mutage/1.6.427>.
- [Schmid, O; Speit, G.](#) (2007). Genotoxic effects induced by formaldehyde in human blood and implications for the interpretation of biomonitoring studies. *Mutagenesis* 22: 69-74.
<http://dx.doi.org/10.1093/mutage/gel053>.
- [Schoenberg, JB; Mitchell, CA.](#) (1975). Airway disease caused by phenolic (phenol-formaldehyde) resin exposure. *Arch Environ Health* 30: 574-577.
<http://dx.doi.org/10.1080/00039896.1975.10666782>.
- [Schreiber, H; Bibbo, M; Wied, GL; Saccomanno, G; Nettesheim, P.](#) (1979). Bronchial metaplasia as a benign or premalignant lesion. I. Cytologic and ultrastructural discrimination between acute carcinogen effects and toxin-induced changes. *Acta Cytol* 23: 496-503.
- [Schreider, JP.](#) (1986). Comparative anatomy and function of the nasal passages. In CS Barrow (Ed.), *Toxicology of the nasal passages* (pp. 1-25). Washington, DC: Hemisphere Publishing Corporation.
- [Schroeter, JD; Campbell, J; Kimbell, JS; Conolly, RB; Clewell, HJ; Andersen, ME.](#) (2014). Effects of endogenous formaldehyde in nasal tissues on inhaled formaldehyde dosimetry predictions in the rat, monkey, and human nasal passages. *Toxicol Sci* 138: 412-424.
<http://dx.doi.org/10.1093/toxsci/kft333>.
- [Schuck, EA; Stephens, ER; Middleton, JT.](#) (1966). Eye irritation response at low concentrations of irritants. *Arch Environ Health* 13: 570-575.
- [Schuiling, M; Zuidhof, AB; Zaagsma, J; Meurs, H.](#) (1999). Involvement of tachykinin NK1 receptor in the development of allergen-induced airway hyperreactivity and airway inflammation in conscious, unrestrained guinea pigs. *Am J Respir Crit Care Med* 159: 423-430.
<http://dx.doi.org/10.1164/ajrccm.159.2.9804125>.
- [Schulte-Hermann, R; Bursch, W; Grasl-Kraupp, B; Marian, B; Török, L; Kahl-Rainer, P; Ellinger, A.](#) (1997). Concepts of cell death and application to carcinogenesis. *Toxicol Pathol* 25: 89-93.
<http://dx.doi.org/10.1177/019262339702500117>.
- [Schulte-Hermann, R; Bursch, W; Marian, B; Grasl-Kraupp, B.](#) (1999). Active cell death (apoptosis) and cellular proliferation as indicators of exposure to carcinogens. In D McGregor; JM Rice; S Venitt (Eds.), *The use of short- and medium-term tests for carcinogens and data on genetic effects in carcinogenic hazard evaluation* (pp. 273-285). Lyon, France: International Agency for Research on Cancer. <https://publications.iarc.fr/Book-And-Report-Series/Iarc-Scientific-Publications/The-Use-Of-Short--And-Medium-term-Tests-For-Carcinogens-And-Data-On-Genetic-Effects-In-Carcinogenic-Hazard-Evaluation-1999>.

- [Schwarze, J; Cieslewicz, G; Hamelmann, E; Joetham, A; Shultz, LD; Lamers, MC; Gelfand, EW.](#) (1999). IL-5 and eosinophils are essential for the development of airway hyperresponsiveness following acute respiratory syncytial virus infection. *J Immunol* 162: 2997-3004.
- [SCOEL](#) (Scientific Committee on Occupational Exposure Limits). (2017). SCOEL/REC/125 formaldehyde: recommendation from the scientific committee on occupational exposure limits.
- [Seals, RM; Kioumourtzoglou, MA; Gredal, O; Hansen, J; Weisskopf, MG.](#) (2017). Occupational formaldehyde and amyotrophic lateral sclerosis. *Eur J Epidemiol* 32: 893-899. <http://dx.doi.org/10.1007/s10654-017-0249-8>.
- [Seitz, T; Baron, S.](#) (1990). Health Hazard Evaluation Report HETA87-349-2022, Rockcastle Manufacturing, Mount Vernon, Kentucky (pp. 38). (NTIS/02970481_a). Seitz, T; Baron, S.
- [Sellakumar, AR; Snyder, CA; Solomon, JJ; Albert, RE.](#) (1985). Carcinogenicity of formaldehyde and hydrogen chloride in rats. *Toxicol Appl Pharmacol* 81: 401-406. [http://dx.doi.org/10.1016/0041-008X\(85\)90411-9](http://dx.doi.org/10.1016/0041-008X(85)90411-9).
- [Senichenkova, II.](#) (1991a). Embryotoxic effects of industrial environment pollutants: Formaldehyde and gasoline. *Gig Sanit -*: 35-38.
- [Senichenkova, II.](#) (1991b). [Embryotoxic effects of industrial environment pollutants: formaldehyde and gasoline]. *Gig Sanit -*: 35-38.
- [Senichenkova, IN; Chebotar, NA.](#) (1996a). [The effect of benzene and formaldehyde on the prenatal development of rats with induced iron trace-element disorder]. *Ontogenez* 27: 108-113.
- [Senichenkova, IN; Chebotar, NA.](#) (1996b). Effects of gasoline and formaldehyde on prenatal development of rats with induced iron micronutrient disorder (iron deficiency). *Ontogenez* 27: 108-113.
- [Seow, WJ; Zhang, L; Vermeulen, R; Tang, X; Hu, W; Bassig, BA; Ji, Z; Shiels, MS; Kemp, TJ; Shen, M; Qiu, C; Reiss, B; Beane Freeman, LE; Blair, A; Kim, C; Guo, W; Wen, C; Li, L; Pinto, LA; Huang, H; Smith, MT; Hildesheim, A; Rothman, N; Lan, Q.](#) (2015). Circulating immune/inflammation markers in Chinese workers occupationally exposed to formaldehyde. *Carcinogenesis* 36: 852-857. <http://dx.doi.org/10.1093/carcin/bgv055>.
- [Sernia, S; Di Folco, F; Altrudo, P; Sbriccoli, B; Sestili, C; Colamesta, V; Del Buono, S; Michetti, A; Ortis, M; Dawodu, A; Villari, P; La Torre, G.](#) (2016). [Risk of nasopharyngeal cancer, Leukemia and other tumors in a cohort of employees and students potentially exposed to (FA) formaldehyde in University laboratories]. *Clin Ter* 167: 43-47. <http://dx.doi.org/10.7417/CT.2016.1925>.
- [Serre, K; Mohr, E; Gaspal, F; Lane, PJ; Bird, R; Cunningham, AF; MacLennan, IC.](#) (2010). IL-4 directs both CD4 and CD8 T cells to produce Th2 cytokines in vitro, but only CD4 T cells produce these cytokines in response to alum-precipitated protein in vivo. *Mol Immunol* 47: 1914-1922. <http://dx.doi.org/10.1016/j.molimm.2010.03.010>.
- [Sexton, K; Liu, KS; Petreas, MX.](#) (1986). Formaldehyde concentrations inside private residences: A mail-out approach to indoor air monitoring. *J Air Pollut Control Assoc* 36: 698-704. <http://dx.doi.org/10.1080/00022470.1986.10466104>.
- [Sexton, K; Petreas, MX; Liu, KS.](#) (1989). Formaldehyde exposures inside mobile homes. *Environ Sci Technol* 23: 985-988. <http://dx.doi.org/10.1021/es00066a009>.
- [Shah, JJ; Singh, HB.](#) (1988). Distribution of volatile organic chemicals in outdoor and indoor air: a national VOCs data base. *Environ Sci Technol* 22: 1381-1388. <http://dx.doi.org/10.1021/es00177a001>.
- [Shaham, J; Bomstein, Y; Gurvich, R; Rashkovsky, M; Kaufman, Z.](#) (2003). DNA-protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. *Occup Environ Med* 60: 403-409. <http://dx.doi.org/10.1136/oem.60.6.403>.

- [Shaham, J; Bomstein, Y; Meltzer, A; Kaufman, Z; Palma, E; Ribak, J.](#) (1996). DNA-protein crosslinks, a biomarker of exposure to formaldehyde - in vitro and in vivo studies. *Carcinogenesis* 17: 121-126. <http://dx.doi.org/10.1093/carcin/17.1.121>.
- [Shaham, J; Bomstein, Y; Melzer, A; Ribak, J.](#) (1997). DNA-protein crosslinks and sister chromatid exchanges as biomarkers of exposure to formaldehyde. *Int J Occup Environ Health* 3: 95-104. <http://dx.doi.org/10.1179/107735297800407695>.
- [Shaham, J; Gurvich, R; Kaufman, Z.](#) (2002). Sister chromatid exchange in pathology staff occupationally exposed to formaldehyde. *Mutat Res Genet Toxicol Environ Mutagen* 514: 115-123. [http://dx.doi.org/10.1016/S1383-5718\(01\)00334-5](http://dx.doi.org/10.1016/S1383-5718(01)00334-5).
- [Shangina, O; Brennan, P; Szeszenia-Dabrowska, N; Mates, D; Fabiánová, E; Fletcher, T; T'Mannetje, A; Boffetta, P; Zaridze, D.](#) (2006). Occupational exposure and laryngeal and hypopharyngeal cancer risk in central and eastern Europe. *Am J Epidemiol* 164: 367-375. <http://dx.doi.org/10.1093/aje/kwj208>.
- [She, Y; Li, Y; Liu, Y; Asai, G; Sun, S; He, J; Pan, Z; Cui, Y.](#) (2013). Formaldehyde induces toxic effects and regulates the expression of damage response genes in BM-MSCs. *Acta Biochim Biophys Sin* 45: 1011-1020. <http://dx.doi.org/10.1093/abbs/gmt105>.
- [Sheppard, D; Eschenbacher, W; Epstein, J.](#) (1984). Lack of bronchomotor response to up to 3 ppm formaldehyde in subjects with asthma. *Environ Res* 35: 133-139. [http://dx.doi.org/10.1016/0013-9351\(84\)90120-8](http://dx.doi.org/10.1016/0013-9351(84)90120-8).
- [Sheveleva, G.](#) (1971). Study of the specific effect of formaldehyde on the embryogenesis and progeny of white rats. *Toksikol Nov Prom Khim Veshchestv* 12: 78-86.
- [Shi, L; Lebrun, P; Camu, F; Zizi, M.](#) (2004). Intrathecal catheterization and solvents interfere with cortical somatosensory evoked potentials used in assessing nociception in awake rats. *Anesth Analg* 99: 159-165. <http://dx.doi.org/10.1213/01.ane.0000114552.20268.7f>.
- [Shibamoto, T.](#) (2006). Analytical methods for trace levels of reactive carbonyl compounds formed in lipid peroxidation systems [Review]. *J Pharm Biomed Anal* 41: 12-25. <http://dx.doi.org/10.1016/j.jpba.2006.01.047>.
- [Shin, YS; Takeda, K; Gelfand, EW.](#) (2009). Understanding asthma using animal models. *Allergy Asthma Immunol Res* 1: 10-18. <http://dx.doi.org/10.4168/aaair.2009.1.1.10>.
- [Sholapuri, P; Chintha, V; Matcha, B; Pradeepkiran, J.](#) (2020). Beneficial effects of polyherbal formulation (Bronco-T) on formaldehyde-induced lung toxicity in male Wistar rats. *Toxicology Research* 9: 798-807. <http://dx.doi.org/10.1093/toxres/tfaa083>.
- [Shumilina, AV.](#) (1975). [Menstrual and child-bearing functions of female workers occupationally exposed to the effects of formaldehyde]. *Gig Tr Prof Zabol* 18-21.
- [Shusterman, D.](#) (2007). Trigeminally-mediated health effects of air pollutants: Sources of inter-individual variability [Review]. *Hum Exp Toxicol* 26: 149-157. <http://dx.doi.org/10.1177/0960327107070550>.
- [Siboulet, R; Grinfeld, S; Deparis, P; Jaylet, A.](#) (1984). Micronuclei in red blood cells of the newt *Pleurodeles waltl* Michah: induction with X-rays and chemicals. *Mutat Res* 125: 275-281. [http://dx.doi.org/10.1016/0027-5107\(84\)90077-0](http://dx.doi.org/10.1016/0027-5107(84)90077-0).
- [Siemiatycki, J; Wacholder, S; Richardson, L; Dewar, R; Gérin, M.](#) (1987). Discovering carcinogens in the occupational environment: Methods of data collection and analysis of a large case-referent monitoring system. *Scand J Work Environ Health* 13: 486-492.
- [Siew, C; Deitrich, RA; Erwin, VG.](#) (1976). Localization and characteristics of rat liver mitochondrial aldehyde dehydrogenases. *Arch Biochem Biophys* 176: 638-649. [http://dx.doi.org/10.1016/0003-9861\(76\)90208-3](http://dx.doi.org/10.1016/0003-9861(76)90208-3).

- Siew, SS; Kauppinen, T; Kyrrönen, P; Heikkilä, P; Pukkala, E. (2012). Occupational exposure to wood dust and formaldehyde and risk of nasal, nasopharyngeal, and lung cancer among Finnish men. *Cancer Management and Research* 4: 223-232. <http://dx.doi.org/10.2147/CMAR.S30684>.
- Silva Ibrahim, B; Miranda da Silva, C; Barioni, ED; Correa-Costa, M; Drewes, CC; Saraiva Câmara, NO; Tavares-De-Lima, W; Poliselli Farsky, SH; Lino-Dos-Santos-Franco, A. (2015). Formaldehyde inhalation during pregnancy abolishes the development of acute innate inflammation in offspring. *Toxicol Lett* 235: 147-154. <http://dx.doi.org/10.1016/j.toxlet.2015.04.001>.
- Singh, NP; McCoy, MT; Tice, RR; Schneider, EL. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* 175: 184-191. [http://dx.doi.org/10.1016/0014-4827\(88\)90265-0](http://dx.doi.org/10.1016/0014-4827(88)90265-0).
- Skrzydłowska, E. (2003). Toxicological and metabolic consequences of methanol poisoning. *Toxicol Mech Meth* 13: 277-293. <http://dx.doi.org/10.1080/713857189>.
- Slater, TF. (1984). Free-radical mechanisms in tissue injury [Review]. *Biochem J* 222: 1-15.
- Slikker, W, Jr; Andersen, ME; Bogdanffy, MS; Bus, JS; Cohen, SD; Conolly, RB; David, RM; Doerrner, NG; Dorman, DC; Gaylor, DW; Hattis, D; Rogers, JM; Setzer, RW; Swenberg, JA; Wallace, K. (2004). Dose-dependent transitions in mechanisms of toxicity: Case studies [Review]. *Toxicol Appl Pharmacol* 201: 226-294. <http://dx.doi.org/10.1016/j.taap.2004.06.027>.
- Smedje, G; Norback, D. (2001). Incidence of asthma diagnosis and self-reported allergy in relation to the school environment: A four-year follow-up study in schoolchildren. *Int J Tuberc Lung Dis* 5: 1059-1066.
- Smedje, G; Norbäck, D; Edling, C. (1997). Asthma among secondary schoolchildren in relation to the school environment. *Clin Exp Allergy* 27: 1270-1278. <http://dx.doi.org/10.1046/j.1365-2222.1997.1780977.x>.
- Smith, AE; Coit, DW. (1995). Penalty functions. In T Back; DB Fogel; Z Michalewicz (Eds.), *Environmental computation 2* (pp. 41-48). New York, NY: Institute of Physics Publishing and Oxford University Press. <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.39.4841&rep=rep1&type=pdf>.
- Snedecor, GW; Cochran, WG. (1980). *Statistical methods* (7th ed.). Ames, IA: Iowa State University Press.
- Snyder, RD; van Houten, B. (1986). Genotoxicity of formaldehyde and an evaluation of its effects on the DNA repair process in human diploid fibroblasts. *Mutat Res DNA Repair* 165: 21-30. [http://dx.doi.org/10.1016/0167-8817\(86\)90005-2](http://dx.doi.org/10.1016/0167-8817(86)90005-2).
- Sobels, FH; van Steenis, H. (1957). Chemical induction of crossing-over in *Drosophila* males. *Nature* 179: 29-31. <http://dx.doi.org/10.1038/179029a0>.
- Soffritti, M; Tibaldi, E, va; Padovani, M; Hoel, DG; Giuliani, L; Bua, L; Lauriola, M; Falcioni, L; Manservigi, M; Manservigi, F; Belpoggi, F. (2016). Synergism between sinusoidal-50Hz magnetic field and formaldehyde in triggering carcinogenic effects in male Sprague-Dawley rats. *Am J Ind Med* 59: 509-521. <http://dx.doi.org/10.1002/ajim.22598>.
- Solet, D; Zoloth, SR; Sullivan, C; Jewett, J; Michaels, DM. (1989). Patterns of mortality in pulp and paper workers. *J Occup Med* 31: 627-630.
- Song, J; Kang, J; Lin, B; Li, J; Zhu, Y; Du, J; Yang, X; Xi, Z; Li, R. (2017). Mediating role of TRPV1 ion channels in the co-exposure to PM2.5 and formaldehyde of balb/c mice asthma model. *Sci Rep* 7: 11926. <http://dx.doi.org/10.1038/s41598-017-11833-6>.
- Songur, A; Akpolat, N; Kus, I; Ozen, OA; Zararsiz, I; Sarsilmaz, M. (2003). The effects of the inhaled formaldehyde during the early postnatal period in the hippocampus of rats: A morphological and immunohistochemical study. *Neurosci Res Commun* 33: 168-178. <http://dx.doi.org/10.1002/nrc.10093>.

- [Songur, A; Ozen, OA; Sarsilmaz, M.](#) (2010). The toxic effects of formaldehyde on the nervous system [Review]. *Rev Environ Contam Toxicol* 203: 105-118. http://dx.doi.org/10.1007/978-1-4419-1352-4_3.
- [Songur, A; Sarsilmaz, M; Özen, OA.](#) (2008). The effects of inhaled formaldehyde on oxidant and antioxidant systems of rat cerebellum during the postnatal development process. *Toxicol Mech Meth* 18: 569-574. <http://dx.doi.org/10.1080/15376510701555288>.
- [Sorg, BA; Bailie, TM; Tschirgi, ML; Li, N; Wu, WR.](#) (2001a). Exposure to repeated low-level formaldehyde in rats increases basal corticosterone levels and enhances the corticosterone response to subsequent formaldehyde. *Brain Res* 898: 314-320. [http://dx.doi.org/10.1016/S0006-8993\(01\)02208-9](http://dx.doi.org/10.1016/S0006-8993(01)02208-9).
- [Sorg, BA; Davidson, DL; Hochstatter, T; Sylvester, PW.](#) (2002). Repeated cocaine decreases the avoidance response to a novel aversive stimulus in rats. *Psychopharmacology* 163: 9-19. <http://dx.doi.org/10.1007/s00213-002-1133-z>.
- [Sorg, BA; Hochstatter, T.](#) (1999). Behavioral sensitization after repeated formaldehyde exposure in rats. *Toxicol Ind Health* 15: 346-355. <http://dx.doi.org/10.1177/074823379901500309>.
- [Sorg, BA; Swindell, S; Tschirgi, ML.](#) (2004). Repeated low level formaldehyde exposure produces enhanced fear conditioning to odor in male, but not female, rats. *Brain Res* 1008: 11-19. <http://dx.doi.org/10.1016/j.brainres.2004.02.015>.
- [Sorg, BA; Tschirgi, ML; Swindell, S; Chen, L; Fang, J.](#) (2001b). Repeated formaldehyde effects in an animal model for multiple chemical sensitivity [Review]. *Ann N Y Acad Sci* 933: 57-67. <http://dx.doi.org/10.1111/j.1749-6632.2001.tb05814.x>.
- [Sorg, BA; Willis, JR; Nowatka, TC; Ulibarri, C; See, RE; Westberg, HH.](#) (1996). Proposed animal neurosensitization model for multiple chemical sensitivity in studies with formalin. *Toxicology* 111: 135-145. [http://dx.doi.org/10.1016/0300-483x\(96\)03371-9](http://dx.doi.org/10.1016/0300-483x(96)03371-9).
- [Sorg, BA; Willis, JR; See, RE; Hopkins, B; Westberg, HH.](#) (1998). Repeated low-level formaldehyde exposure produces cross-sensitization to cocaine: Possible relevance to chemical sensitivity in humans. *Neuropsychopharmacology* 18: 385-394. <http://dx.doi.org/10.1038/sj.npp.1395160>.
- [Sorokin, SP.](#) (1988). The respiratory system. In L Weiss (Ed.), *Cell and tissue biology: A textbook of histology* (6th ed., pp. 751-814). Baltimore, MD: Urban & Schwarzenberg.
- [Souza, AD; Devi, R.](#) (2014). Cytokinesis blocked micronucleus assay of peripheral lymphocytes revealing the genotoxic effect of formaldehyde exposure. *Clin Anat* 27: 308-312. <http://dx.doi.org/10.1002/ca.22291>.
- [Speit, G; Kühner, S; Linsenmeyer, R; Schütz, P.](#) (2011a). Does formaldehyde induce aneuploidy. *Mutagenesis* 26: 805-811. <http://dx.doi.org/10.1093/mutage/ger050>.
- [Speit, G; Ladeira, C; Linsenmeyer, R; Schütz, P; Högel, J.](#) (2012). Re-evaluation of a reported increased micronucleus frequency in lymphocytes of workers occupationally exposed to formaldehyde. *Mutat Res* 744: 161-166. <http://dx.doi.org/10.1016/j.mrgentox.2012.02.009>.
- [Speit, G; Merk, O.](#) (2002). Evaluation of mutagenic effects of formaldehyde in vitro: Detection of crosslinks and mutations in mouse lymphoma cells. *Mutagenesis* 17: 183-187. <http://dx.doi.org/10.1093/mutage/17.3.183>.
- [Speit, G; Neuss, S; Schuetz, P; Froehler-Keller, M; Schmid, O.](#) (2008a). The genotoxic potential of glutaraldehyde in mammalian cells in vitro in comparison with formaldehyde. *Mutat Res Genet Toxicol Environ Mutagen* 649: 146-154. <http://dx.doi.org/10.1016/j.mrgentox.2007.08.010>.
- [Speit, G; Schmid, O; Fröhler-Keller, M; Lang, I; G, T.](#) (2007a). Assessment of local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated buccal mucosa cells. *Mutat Res Genet Toxicol Environ Mutagen* 627: 129-135. <http://dx.doi.org/10.1016/j.mrgentox.2006.10.013>.

- [Speit, G; Schmid, O; Neuss, S; Schütz, P.](#) (2008b). Genotoxic effects of formaldehyde in the human lung cell line A549 and in primary human nasal epithelial cells. *Environ Mol Mutagen* 49: 300-307. <http://dx.doi.org/10.1002/em.20386>.
- [Speit, G; Schutz, P; Hogel, J; Schmid, O.](#) (2007b). Characterization of the genotoxic potential of formaldehyde in V79 cells. *Mutagenesis* 22: 387-394. <http://dx.doi.org/10.1093/mutage/gem031>.
- [Speit, G; Schutz, P; Merk, O.](#) (2000). Induction and repair of formaldehyde-induced DNA-protein crosslinks in repair-deficient human cell lines. *Mutagenesis* 15: 85-90. <http://dx.doi.org/10.1093/mutage/15.1.85>.
- [Speit, G; Schütz, P; Weber, I; Ma-Hock, L; Kaufmann, W; Gelbke, HP; Durrer, S.](#) (2011b). Analysis of micronuclei, histopathological changes and cell proliferation in nasal epithelium cells of rats after exposure to formaldehyde by inhalation. *Mutat Res* 721: 127-135. <http://dx.doi.org/10.1016/j.mrgentox.2011.01.008>.
- [Speit, G; Zeller, J; Schmid, O; Elhajouji, A; Ma-Hock, L; Neuss, S.](#) (2009). Inhalation of formaldehyde does not induce systemic genotoxic effects in rats. *Mutat Res Genet Toxicol Environ Mutagen* 677: 76-85. <http://dx.doi.org/10.1016/j.mrgentox.2009.05.020>.
- [Srám, RJ.](#) (1970). The effect of storage on the frequency of dominant lethals in *Drosophila melanogaster*. *MGG Mol gen genet* 106: 286-288.
- [Sripaiboonkij, P; Sripaiboonkij, N; Phanprasit, W; Jaakkola, MS.](#) (2009). Respiratory and skin health among glass microfiber production workers: a cross-sectional study. *Environ Health* 8: 36. <http://dx.doi.org/10.1186/1476-069X-8-36>.
- [Starr, TB; Swenberg, JA.](#) (2013). A novel bottom-up approach to bounding low-dose human cancer risks from chemical exposures. *Regul Toxicol Pharmacol* 65: 311-315. <http://dx.doi.org/10.1016/j.yrtph.2013.01.004>.
- [Starr, TB; Swenberg, JA.](#) (2016). The bottom-up approach to bounding potential low-dose cancer risks from formaldehyde: An update. *Regul Toxicol Pharmacol* 77: 167-174. <http://dx.doi.org/10.1016/j.yrtph.2016.01.021>.
- [Stayner, LT; Elliott, L; Blade, L; Keenlyside, R; Halperin, W.](#) (1988). A retrospective cohort mortality study of workers exposed to formaldehyde in the garment industry. *Am J Ind Med* 13: 667-681. <http://dx.doi.org/10.1002/ajim.4700130606>.
- [Steele, LL; Wilkins, J. R.](#) (1996). Occupational exposures and risks of spontaneous abortion among female veterinarians. *Int J Occup Environ Health* 2: 26-36.
- [Stellman, SD; Demers, PA; Colin, D; Boffetta, P.](#) (1998). Cancer mortality and wood dust exposure among participants in the American Cancer Society Cancer Prevention Study-II (CPS-II). *Am J Ind Med* 34: 229-237. [http://dx.doi.org/10.1002/\(SICI\)1097-0274\(199809\)34:3<229::AID-AJIM4>3.0.CO;2-Q](http://dx.doi.org/10.1002/(SICI)1097-0274(199809)34:3<229::AID-AJIM4>3.0.CO;2-Q).
- [Stern, FB.](#) (2003). Mortality among chrome leather tannery workers: an update. *Am J Ind Med* 44: 197-206. <http://dx.doi.org/10.1002/ajim.10242>.
- [Stewart, PA; Blair, A; Cubit, DA; Bales, RE; Kaplan, SA; Ward, J; Gaffey, W; O'Berg, MT; Walrath, J.](#) (1986). Estimating historical exposures to formaldehyde in a retrospective mortality study. *Appl Ind Hyg* 1: 34-41.
- [Stich, HF; Curtis, JR; Parida, BB.](#) (1982). Application of the micronucleus test to exfoliated cells of high cancer risk groups: Tobacco chewers. *Int J Cancer* 30: 553-559. <http://dx.doi.org/10.1002/ijc.2910300504>.
- [Strait, RT; Morris, SC; Finkelman, FD.](#) (2006). IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIB cross-linking. *J Clin Invest* 116: 833-841. <http://dx.doi.org/10.1172/JCI25575>.

- Stroup, NE; Blair, A; Erikson, GE. (1986). Brain cancer and other causes of death in anatomists. *J Natl Cancer Inst* 77: 1217-1224.
- Stücker, I; Caillard, JF; Collin, R; Gout, M; Poyen, D; Hémon, D. (1990). Risk of spontaneous abortion among nurses handling antineoplastic drugs. *Scand J Work Environ Health* 16: 102-107. <http://dx.doi.org/10.5271/sjweh.1811>.
- Stücker, I; Mandereau, L; Hémon, D. (1993). Relationship between birthweight and occupational exposure to cytostatic drugs during or before pregnancy. *Scand J Work Environ Health* 19: 148-153. <http://dx.doi.org/10.5271/sjweh.1488>.
- Stumm-Tegethoff, BFA. (1969). Formaldehyde-induced mutations in *Drosophila melanogaster* in dependence of the presence of acids. *Theor Appl Genet* 39: 330-334. <http://dx.doi.org/10.1007/BF00281915>.
- Subramaniam, RP; Chen, C; Crump, KS; Devoney, D; Fox, JF; Portier, CJ; Schlosser, PM; Thompson, CM; White, P. (2008). Uncertainties in biologically-based modeling of formaldehyde-induced respiratory cancer risk: Identification of key issues. *Risk Anal* 28: 907-923. <http://dx.doi.org/10.1111/j.1539-6924.2008.01083.x>.
- Subramaniam, RP; Crump, KS; Van Landingham, C; White, P; Chen, C; Schlosser, PM. (2007). Uncertainties in the CIIT model for formaldehyde-induced carcinogenicity in the rat: A limited sensitivity analysis—I. *Risk Anal* 27: 1237-1254. <http://dx.doi.org/10.1111/j.1539-6924.2007.00968.x>.
- Subramaniam, RP; Richardson, RB; Morgan, KT; Kimbell, JS; Guilmette, RA. (1998). Computational fluid dynamics simulations of inspiratory airflow in the human nose and nasopharynx. *Inhal Toxicol* 10: 91-120. <http://dx.doi.org/10.1080/089583798197772>.
- Sul, D; Kim, H; Oh, E; Phark, S; Cho, E; Choi, S; Kang, HS; Kim, EM; Hwang, KW; Jung, WW. (2007). Gene expression profiling in lung tissues from rats exposed to formaldehyde. *Arch Toxicol* 81: 589-597. <http://dx.doi.org/10.1007/s00204-007-0182-9>.
- Suruda, A; Schulte, P; Boeniger, M; Hayes, RB; Livingston, GK; Steenland, K; Stewart, P; Herrick, R; Douthit, D; Fingerhut, MA. (1993). Cytogenetic effects of formaldehyde exposure in students of mortuary science. *Cancer Epidemiol Biomarkers Prev* 2: 453-460.
- Suskov, II; Sazonova, LA. (1982). Cytogenetic effects of epoxy, phenolformaldehyde and polyvinylchloride resins in man. *Mutat Res* 104: 137-140. [http://dx.doi.org/10.1016/0165-7992\(82\)90134-8](http://dx.doi.org/10.1016/0165-7992(82)90134-8).
- Sutton, HC; Downes, TM. (1972). Rate of hydration of formaldehyde in aqueous solution. *J Chem Soc Chem Commun* 1. <http://dx.doi.org/10.1039/C39720000001>.
- Suzuki, H; Miura, S; Liu, YY; Tsuchiya, M; Ishii, H. (1995). Substance P induces degranulation of mast cells and leukocyte adhesion to venular endothelium. *Peptides* 16: 1447-1452. [http://dx.doi.org/10.1016/0196-9781\(95\)02050-0](http://dx.doi.org/10.1016/0196-9781(95)02050-0).
- Svensson, S; Some, M; Lundsjö, A; Helander, A; Cronholm, T; Höög, JO. (1999). Activities of human alcohol dehydrogenases in the metabolic pathways of ethanol and serotonin. *Eur J Biochem* 262: 324-329. <http://dx.doi.org/10.1046/j.1432-1327.1999.00351.x>.
- Swenberg, J; Kerns, W; Pavkov, K; Mitchell, R; Gralla, EJ. (1980a). Carcinogenicity of formaldehyde vapor: interim findings in a long-term bioassay of rats and mice. *Dev Toxicol Environ Sci* 8: 283-286.
- Swenberg, JA; Gross, EA; Martin, J; Popp, JA. (1983a). Mechanisms of formaldehyde toxicity. In JE Gibson (Ed.), *Formaldehyde toxicity* (pp. 132-147). Washington, DC: Hemisphere Publishing.
- Swenberg, JA; Gross, EA; Randall, HW. (1986). Localization and quantitation of cell proliferation following exposure to nasal irritants. In CS Barrow (Ed.), *Toxicology of the nasal passages* (pp. 291-300). New York, NY: Hemisphere Publishing Corp.

- [Swenberg, JA; Gross, EA; Randall, HW; Barrow, CS.](#) (1983b). The effect of formaldehyde exposure on cytotoxicity and cell proliferation. In JJ Clary; JE Gibson; RS Waritz (Eds.), *Formaldehyde, toxicology, epidemiology, mechanisms* (pp. 225-236). New York, NY: Marcel Dekker.
- [Swenberg, JA; Kerns, WD; Mitchell, RI; Gralla, EJ; Pavkov, KL.](#) (1980b). Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapor. *Cancer Res* 40: 3398-3402.
- [Swenberg, JA; Lu, K; Moeller, BC; Gao, L; Upton, PB; Nakamura, J; Starr, TB.](#) (2011). Endogenous versus exogenous DNA adducts: Their role in carcinogenesis, epidemiology, and risk assessment [Review]. *Toxicol Sci* 120: S130-S145. <http://dx.doi.org/10.1093/toxsci/kfq371>.
- [Swiecichowski, AL; Long, KJ; Miller, ML; Leikauf, GD.](#) (1993). Formaldehyde-induced airway hyperreactivity in vivo and ex vivo in guinea pigs. *Environ Res* 61: 185-199. <http://dx.doi.org/10.1006/enrs.1993.1063>.
- [Taffet, GE; Donohue, JF; Altman, PR.](#) (2014). Considerations for managing chronic obstructive pulmonary disease in the elderly [Review]. *Clinical Interventions in Aging* 9: 23-30. <http://dx.doi.org/10.2147/CIA.S52999>.
- [Takahashi, K; Morita, T; Kawazoe, Y.](#) (1985). Mutagenic characteristics of formaldehyde on bacterial systems. *Mutat Res* 156: 153-161. [http://dx.doi.org/10.1016/0165-1218\(85\)90058-8](http://dx.doi.org/10.1016/0165-1218(85)90058-8).
- [Takahashi, S; Tsuji, K; Fujii, K; Okazaki, F; Takigawa, T; Ohtsuka, A; Iwatsuki, K.](#) (2007). Prospective study of clinical symptoms and skin test reactions in medical students exposed to formaldehyde gas. *J Dermatol* 34: 283-289. <http://dx.doi.org/10.1111/j.1346-8138.2007.00274.x>.
- [Takigawa, T; Usami, M; Yamasaki, Y; Wang, B; Sakano, N; Horike, T; Kataoka, H; Ohtsuka, A; Kira, S.](#) (2005). Reduction of indoor formaldehyde concentrations and subjective symptoms in a gross anatomy laboratory. *Bull Environ Contam Toxicol* 74: 1027-1033. <http://dx.doi.org/10.1007/s00128-005-0683-2>.
- [Talibov, M; Lehtinen-Jacks, S; Martinsen, JI; Kjærheim, K; Lynge, E; Sparén, P; Tryggvadottir, L; Weiderpass, E; Kauppinen, T; Kyrrönen, P; Pukkala, E.](#) (2014). Occupational exposure to solvents and acute myeloid leukemia: A population-based, case-control study in four Nordic countries. *Scand J Work Environ Health* 40: 511-517. <http://dx.doi.org/10.5271/sjweh.3436>.
- [Tang, LX; Zhang, YS.](#) (2003). [Health investigation on workers exposed to formaldehyde] (pp. 34-35). Tang, LX; Zhang, YS.
- [Tani, T; Kogi, K; Horiguchi, Y.](#) (1986). Inhibitory effects of formaldehyde inhalation on the cardiovascular and respiratory systems in unanesthetized rabbits. *Jpn J Pharmacol* 40: 551-559. <http://dx.doi.org/10.1254/jjp.40.551>.
- [Tanveer, H; Rastogi, SK; Mathur, N; Gupta, BN.](#) (1995). Respiratory responses of paper mill workers occupationally exposed to formaldehyde. *Indian J Indust Med* 41: 2-8.
- [Tarkowski, M; Gorski, P.](#) (1995). Increased IgE antiovalbumin level in mice exposed to formaldehyde. *Int Arch Allergy Immunol* 106: 422-424. <http://dx.doi.org/10.1159/000236876>.
- [Taskinen, H; Kyrrönen, P; Hemminki, K.](#) (1994). Laboratory work and pregnancy outcome. *J Occup Med* 36: 311-319. <http://dx.doi.org/10.1097/00043764-199403000-00008>.
- [Taskinen, HK; Kyrrönen, P; Sallmen, M; Virtanen, SV; Liukkonen, TA; Huida, O; Lindbohm, ML; Anttila, A.](#) (1999). Reduced fertility among female wood workers exposed to formaldehyde. *Am J Ind Med* 36: 206-212. [http://dx.doi.org/10.1002/\(sici\)1097-0274\(199907\)36:1<206::aid-ajim29>3.0.co;2-d](http://dx.doi.org/10.1002/(sici)1097-0274(199907)36:1<206::aid-ajim29>3.0.co;2-d).
- [Tavernier, G; Fletcher, G; Gee, I; Watson, A; Blacklock, G; Francis, H; Fletcher, A; Frank, T; Frank, P; Pickering, CA; Niven, R.](#) (2006). IPEADAM study: Indoor endotoxin exposure, family status, and some housing characteristics in English children. *J Allergy Clin Immunol* 117: 656-662. <http://dx.doi.org/10.1016/j.jaci.2005.12.1311>.

- Taves, MD; Ashwell, JD. (2020). Glucocorticoids in T cell development, differentiation and function [Review]. *Nat Rev Immunol.* <http://dx.doi.org/10.1038/s41577-020-00464-0>.
- Taylor-Clark, TE; McAlexander, MA; Nassenstein, C; Sheardown, SA; Wilson, S; Thornton, J; Carr, MJ; Undem, BJ. (2008). Relative contributions of TRPA1 and TRPV1 channels in the activation of vagal bronchopulmonary C-fibres by the endogenous autacoid 4-oxononenal. *J Physiol* 586: 3447-3459. <http://dx.doi.org/10.1113/jphysiol.2008.153585>.
- Teixeira, JP; Gaspar, J; Silva, S; Torres, J; Silva, SN; Azevedo, MC; Neves, P; Laffon, B; Méndez, J; Gonçalves, C; Mayan, O; Farmer, PB; Rueff, J. (2004). Occupational exposure to styrene: modulation of cytogenetic damage and levels of urinary metabolites of styrene by polymorphisms in genes CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1. *Toxicology* 195: 231-242. <http://dx.doi.org/10.1016/j.tox.2003.10.010>.
- Temcharoen, P; Thilly, WG. (1983). Toxic and mutagenic effects of formaldehyde in *Salmonella typhimurium*. *Mutat Res* 119: 89-93. [http://dx.doi.org/10.1016/0165-7992\(83\)90115-X](http://dx.doi.org/10.1016/0165-7992(83)90115-X).
- Teng, S; Beard, K; Pourahmad, J; Moridani, M; Easson, E; Poon, R; O'Brien, PJ. (2001). The formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes. *Chem Biol Interact* 130-132: 285-296. [http://dx.doi.org/10.1016/S0009-2797\(00\)00272-6](http://dx.doi.org/10.1016/S0009-2797(00)00272-6).
- Tepper, JS; Moser, VC; Costa, DL; Mason, MA; Roache, N; Guo, Z; Dyer, RS. (1995). Toxicological and chemical evaluation of emissions from carpet samples. *Am Ind Hyg Assoc J* 56: 158-170. <http://dx.doi.org/10.1080/15428119591017196>.
- Teschke, K; Morgan, MS; Checkoway, H; Franklin, G; Spinelli, JJ; van Belle, G; Weiss, NS. (1997). Surveillance of nasal and bladder cancer to locate sources of exposure to occupational carcinogens. *Occup Environ Med* 54: 443-451. <http://dx.doi.org/10.1136/oem.54.6.443>.
- Thetkathuek, A; Yingratanasuk, T; Ekburanawat, W. (2016). Respiratory Symptoms due to Occupational Exposure to Formaldehyde and MDF Dust in a MDF Furniture Factory in Eastern Thailand. 2016: 3705824. <http://dx.doi.org/10.1155/2016/3705824>.
- Thomas, P; Holland, N; Bolognesi, C; Kirsch-Volders, M; Bonassi, S; Zeiger, E; Knasmueller, S; Fenech, M. (2009). Buccal micronucleus cytome assay. *Nat Protoc* 4: 825-837. <http://dx.doi.org/10.1038/nprot.2009.53>.
- Thompson, CM; Ceder, R; Grafström, RC. (2010). Formaldehyde dehydrogenase: beyond phase I metabolism. *Toxicol Lett* 193: 1-3. <http://dx.doi.org/10.1016/j.toxlet.2009.11.023>.
- Thompson, CM; Sonawane, B; Grafstrom, RC. (2009). The ontogeny, distribution, and regulation of alcohol dehydrogenase 3: Implications for pulmonary physiology [Review]. *Drug Metab Dispos* 37: 1565-1571. <http://dx.doi.org/10.1124/dmd.109.027904>.
- Thomson, EJ; Shackleton, S; Harrington, JM. (1984). Chromosome aberrations and sister-chromatid exchange frequencies in pathology staff occupationally exposed to formaldehyde. *Mutat Res* 141: 89-93. [http://dx.doi.org/10.1016/0165-7992\(84\)90016-2](http://dx.doi.org/10.1016/0165-7992(84)90016-2).
- Thrasher, JD; Broughton, A; Madison, R. (1990). Immune activation and autoantibodies in humans with long-term inhalation exposure to formaldehyde. *Arch Environ Health* 45: 217-223. <http://dx.doi.org/10.1080/00039896.1990.9940805>.
- Thrasher, JD; Wojdani, A; Cheung, G; Heuser, G. (1987). Evidence for formaldehyde antibodies and altered cellular immunity in subjects exposed to formaldehyde in mobile homes. *Arch Environ Health* 42: 347-350. <http://dx.doi.org/10.1080/00039896.1987.9934357>.
- Thun, MJ; Lakat, M; Altman, R. (1982). Symptom survey of residents of homes insulated with urea-formaldehyde foam. *Environ Res* 29: 320-334. [http://dx.doi.org/10.1016/0013-9351\(82\)90034-2](http://dx.doi.org/10.1016/0013-9351(82)90034-2).

- Tibbetts, AS; Appling, DR. (2010). Compartmentalization of Mammalian folate-mediated one-carbon metabolism [Review]. *Annu Rev Nutr* 30: 57-81.
<http://dx.doi.org/10.1146/annurev.nutr.012809.104810>.
- Tice, RR; Agurell, E; Anderson, D; Burlinson, B; Hartmann, A; Kobayashi, H; Miyamae, Y; Rojas, E; Ryu, JC; Sasaki, YF. (2000). Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen* 35: 206-221. [http://dx.doi.org/10.1002/\(sici\)1098-2280\(2000\)35:3<206::aid-em8>3.0.co;2-j](http://dx.doi.org/10.1002/(sici)1098-2280(2000)35:3<206::aid-em8>3.0.co;2-j).
- Titenko-Holland, N; Levine, AJ; Smith, MT; Quintana, PJ; Boeniger, M; Hayes, R; Suruda, A; Schulte, P. (1996). Quantification of epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. *Mutat Res* 371: 237-248.
[http://dx.doi.org/10.1016/S0165-1218\(96\)90112-3](http://dx.doi.org/10.1016/S0165-1218(96)90112-3).
- Tobe, M; Kaneko, T; Uchida, Y; Kamata, E; Ogawa, Y; Ikeda, Y; Saito, M. (1985a). Studies on the inhalation toxicity of formaldehyde. 43.
- Tobe, M; Kaneko, Y; Uchida, Y; al., e. (1985b). Studies of the inhalation toxicity of formaldehyde. National Sanitary and Medical Laboratory Service, Toxicity, Tokyo, Japan; T-85-0236. Tobe, M; Kaneko, Y; Uchida, Y; et al.
- Togias, A. (1999). Mechanisms of nose-lung interaction [Review]. *Allergy* 54: 94-105.
<http://dx.doi.org/10.1111/j.1398-9995.1999.tb04410.x>.
- Togias, A. (2004). Systemic effects of local allergic disease [Review]. *J Allergy Clin Immunol* 113: S8-14.
<http://dx.doi.org/10.1016/j.jaci.2003.09.051>.
- Tolbert, PE; Shy, CM; Allen, JW. (1991). Micronuclei and other nuclear anomalies in buccal smears: a field test in snuff users. *Am J Epidemiol* 134: 840-850.
<http://dx.doi.org/10.1093/oxfordjournals.aje.a116159>.
- Tolbert, PE; Shy, CM; Allen, JW. (1992). Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutat Res* 271: 69-77.
- Tong, ZM; Zhu, SX; Shi, J. (2007). [Effect of formaldehyde on blood component and blood biochemistry of exposed workers]. 20: 409-410.
- Torjussen, W; Solberg, LA; Hogetveit, AC. (1979). Histopathological changes of the nasal mucosa in active and retired nickel workers. *Br J Cancer* 40: 568-580.
- Tranah, GJ; Holly, EA; Bracci, PM. (2009). Solvent exposure and non-Hodgkin lymphoma: no risk in a population-based study in the San Francisco Bay Area. *Cancer Epidemiol Biomarkers Prev* 18: 3130-3132. <http://dx.doi.org/10.1158/1055-9965.EPI-09-0821>.
- Trivedi, SG; Lloyd, CM. (2007). Eosinophils in the pathogenesis of allergic airways disease [Review]. *Cell Mol Life Sci* 64: 1269-1289. <http://dx.doi.org/10.1007/s00018-007-6527-y>.
- Tsubone, H; Kawata, M. (1991). Stimulation to the trigeminal afferent nerve of the nose by formaldehyde, acrolein, and acetaldehyde gases. *Inhal Toxicol* 3: 211-222.
<http://dx.doi.org/10.3109/08958379109145285>.
- Tsukahara, S; Yamamoto, S; Shwe, TTW; Ahmed, S; Kunugita, N; Arashidani, K; Fujimaki, H. (2006). Inhalation of low-level formaldehyde increases the Bcl-2/Bax expression ratio in the hippocampus of immunologically sensitized mice. *Neuroimmunomodulation* 13: 63-68.
<http://dx.doi.org/10.1159/000094829>.
- Tuluc, F; Lai, JP; Kilpatrick, LE; Evans, DL; Douglas, SD. (2009). Neurokinin 1 receptor isoforms and the control of innate immunity [Review]. *Trends Immunol* 30: 271-276.
<http://dx.doi.org/10.1016/j.it.2009.03.006>.
- Turner, C; Parekh, B; Walton, C; Spanel, P; Smith, D; Evans, M. (2008). An exploratory comparative study of volatile compounds in exhaled breath and emitted by skin using selected ion flow tube mass spectrometry. *Rapid Commun Mass Spectrom* 22: 526-532. <http://dx.doi.org/10.1002/rcm.3402>.

Supplemental Information—Formaldehyde (Inhalation)

- [U.S. EPA](#) (U.S. Environmental Protection Agency). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry [EPA Report]. (EPA600/890066F). Research Triangle Park, NC.
<https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=71993&CFID=51174829&CFTOKEN=25006317>.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2000). CatReg software documentation [EPA Report]. (EPA/600/R-98/053). Washington, DC.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2002). IRIS Assessment of: 1,3 Butadiene (CASRN 106-99-0). Washington, DC: U.S. Environmental Protection Agency, Integrated Risk Information System. https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=139.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report]. (EPA630P03001F). Washington, DC. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2012). Benchmark dose technical guidance [EPA Report]. (EPA100R12001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. <https://www.epa.gov/risk/benchmark-dose-technical-guidance>.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2015). Peer review handbook [EPA Report] (4th ed.). (EPA/100/B-15/001). Washington, DC: U.S. Environmental Protection Agency, Science Policy Council. <https://www.epa.gov/osa/peer-review-handbook-4th-edition-2015>.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2017). Categorical regression (CATREG) user guide, version 3.1.0.7. BMDs. https://www.epa.gov/sites/production/files/2016-03/documents/catreg_user_guide.pdf.
- [U.S. EPA](#) (U.S. Environmental Protection Agency). (2022). ORD staff handbook for developing IRIS assessments [EPA Report]. (EPA 600/R-22/268). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, Center for Public Health and Environmental Assessment. https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=356370.
- [Uba, G; Pachorek, D; Bernstein, J; Garabrant, DH; Balmes, JR; Wright, WE; Amar, RB.](#) (1989). Prospective study of respiratory effects of formaldehyde among healthy and asthmatic medical students. *Am J Ind Med* 15: 91-101. <http://dx.doi.org/10.1002/ajim.4700150110>.
- [Ulsamer, AG; Gupta, KC; Cohn, MS; Preuss, PW.](#) (1982). Formaldehyde in indoor air: Toxicity and risk. In *Proceedings of the 75th Annual Meeting of the Air Pollution Control Association: Volume 2* (pp. 1-16). Pittsburgh, PA: Air Pollution Control Association.
- [Uotila, L; Koivusalo, M.](#) (1974). Formaldehyde dehydrogenase from human liver: Purification, properties, and evidence for the formation of glutathione thiol esters by the enzyme. *J Biol Chem* 249: 7653-7663.
- [Uotila, L; Koivusalo, M.](#) (1987). Multiple forms of formaldehyde dehydrogenase from human red blood cells. *Hum Hered* 37: 102-106. <http://dx.doi.org/10.1159/000153684>.
- [Uotila, L; Koivusalo, M.](#) (1989). Glutathione-dependent oxidoreductases: Formaldehyde dehydrogenase. In *D Dolphin; R Poulson; O Avramovic (Eds.), Glutathione: Chemical, biochemical, and medical aspects* (pp. 517-551). New York, NY: Wiley-Interscience.
- [Usanmaz, SE; Akarsu, ES; Vural, N.](#) (2002). Neurotoxic effects of acute and subacute formaldehyde exposures in mice. *Environ Toxicol Pharmacol* 11: 93-100. [http://dx.doi.org/10.1016/S1382-6689\(01\)00109-0](http://dx.doi.org/10.1016/S1382-6689(01)00109-0).
- [Van de Perre, P.](#) (2003). Transfer of antibody via mother's milk [Review]. *Vaccine* 21: 3374-3376. [http://dx.doi.org/10.1016/s0264-410x\(03\)00336-0](http://dx.doi.org/10.1016/s0264-410x(03)00336-0).
- [van Helden, YGJ; Keijer, J; Heil, SG; Pico, C; Palou, A; Oliver, P; Munnia, A; Briede, JJ; Peluso, M; Franssen-Van Hal, NL; van Schooten, FJ; Godschalk, RWL.](#) (2009). Beta-carotene affects oxidative

- stress-related DNA damage in lung epithelial cells and in ferret lung. *Carcinogenesis* 30: 2070-2076. <http://dx.doi.org/10.1093/carcin/bgp186>.
- Vardoulakis, S; Giagloglou, E; Steinle, S; Davis, A; Sleguwenhoek, A; Galea, KS; Dixon, K; Crawford, JO. (2020). Indoor Exposure to Selected Air Pollutants in the Home Environment: A Systematic Review [Review]. *Int J Environ Res Public Health* 17: 1-24. <http://dx.doi.org/10.3390/ijerph17238972>.
- Vargová, M; Janota, S; Karellová, J; Barancokova, M; Sulcova, M. (1992). Analysis of the health risk of occupational exposure to formaldehyde using biological markers. *Analisis* 20: 451-454.
- Vasudeva, N; Anand, C. (1996). Cytogenetic evaluation of medical students exposed to formaldehyde vapor in the gross anatomy dissection laboratory. *J Am Coll Health* 44: 177-179. <http://dx.doi.org/10.1080/07448481.1996.9937526>.
- Vaughan, TL. (1989). Occupation and squamous cell cancers of the pharynx and sinonasal cavity. *Am J Ind Med* 16: 493-510. <http://dx.doi.org/10.1002/ajim.4700160503>.
- Vaughan, TL; Davis, S. (1991). Wood dust exposure and squamous cell cancers of the upper respiratory tract. *Am J Epidemiol* 133: 560-564. <http://dx.doi.org/10.1093/oxfordjournals.aje.a115927>.
- Vaughan, TL; Stewart, PA; Teschke, K; Lynch, CF; Swanson, GM; Lyon, JL; Berwick, M. (2000). Occupational exposure to formaldehyde and wood dust and nasopharyngeal carcinoma. *Occup Environ Med* 57: 376-384. <http://dx.doi.org/10.1136/oem.57.6.376>.
- Vaughan, TL; Strader, C; Davis, S; Daling, JR. (1986a). Formaldehyde and cancers of the pharynx, sinus and nasal cavity: I. Occupational exposures. *Int J Cancer* 38: 677-683. <http://dx.doi.org/10.1002/ijc.2910380510>.
- Vaughan, TL; Strader, C; Davis, S; Daling, JR. (1986b). Formaldehyde and cancers of the pharynx, sinus and nasal cavity: II. Residential exposures. *Int J Cancer* 38: 685-688. <http://dx.doi.org/10.1002/ijc.2910380511>.
- Venn, A. (2012). [Email to Glinda Cooper concerning FW: Follow-up question regarding 2003 wheeze study]. Available online at (accessed
- Venn, A; Lewis, S; Cooper, M; Hubbard, R; Hill, I; Boddy, R; Bell, M; Britton, J. (2000). Local road traffic activity and the prevalence, severity and persistence of wheeze in school children: combined cross sectional and longitudinal study. *Occup Environ Med* 57: 152-158. <http://dx.doi.org/10.1136/oem.57.3.152>.
- Venn, AJ; Cooper, M; Antoniak, M; Laughlin, C; Britton, J; Lewis, SA. (2003). Effects of volatile organic compounds, damp, and other environmental exposures in the home on wheezing illness in children. *Thorax* 58: 955-960. <http://dx.doi.org/10.1136/thorax.58.11.955>.
- Verma, A. (1998). Cytogenetic investigations on patients with oral submucous fibrosis. *J Indian Med Assoc* 96: 51-52, 57.
- Viegas, S; Ladeira, C; Gomes, M; Nunes, C; Brito, M; Prista, J. (2013). Exposure and genotoxicity assessment methodologies - the case of formaldehyde occupational exposure. *Current Analytical Chemistry* 9: 476-484. <http://dx.doi.org/10.2174/1573411011309030017>.
- Viegas, S; Ladeira, C; Nunes, C; Malta-Vacas, J; Gomes, M; Brito, M; Mendonca, P; Prista, J. (2010). Genotoxic effects in occupational exposure to formaldehyde: A study in anatomy and pathology laboratories and formaldehyde-resins production. *J Occup Med Toxicol* 5: 25. <http://dx.doi.org/10.1186/1745-6673-5-25>.
- Villeneuve, DL; Crump, D; Garcia-Reyero, N; Hecker, M; Hutchinson, TH; Lalone, CA; Landesmann, B; Lettieri, T; Munn, S; Nepelska, M; Ottinger, MA; Vergauwen, L; Whelan, M. (2014). Adverse outcome pathway (AOP) development I: Strategies and principles. *Toxicol Sci* 142: 312-320. <http://dx.doi.org/10.1093/toxsci/kfu199>.

- Vitoux, MA; Kessal, K; Baudouin, C; Lapr v te, O; Melik Parsadaniantz, S; Achard, S; Brignole-Baudouin, F. (2018). Formaldehyde gas exposure increases inflammation in an in vitro model of dry eye. *Toxicol Sci* 165: 108-117. <http://dx.doi.org/10.1093/toxsci/kfy125>.
- Vock, EH; Lutz, WK; Ilinskaya, O; Vamvakas, S. (1999). Discrimination between genotoxicity and cytotoxicity for the induction of DNA double-strand breaks in cells treated with aldehydes and diepoxides. *Mutat Res* 441: 85-93. [http://dx.doi.org/10.1016/S1383-5718\(99\)00038-8](http://dx.doi.org/10.1016/S1383-5718(99)00038-8).
- Von Hippel, PH; Wong, KY. (1971). Dynamic aspects of native DNA structure: Kinetics of the formaldehyde reaction with calf thymus DNA. *J Mol Biol* 61: 587-613. [http://dx.doi.org/10.1016/0022-2836\(71\)90066-0](http://dx.doi.org/10.1016/0022-2836(71)90066-0).
- Vosoughi, S; Khavanin, A; Salehnia, M; Asilian Mahabadi, H; Shahverdi, A; Esmaeili, V. (2013). Adverse effects of formaldehyde vapor on mouse sperm parameters and testicular tissue. *Int J Fertility Sterility* 6: 250-257.
- Vosoughi, S; Khavanin, A; Salehnia, M; Mahabadi, HA; Soleimanian, A. (2012). Effects of simultaneous exposure to formaldehyde vapor and noise on mouse testicular tissue and sperm parameters. *Health Scope* 1: 110-117. <http://dx.doi.org/10.17795/jhealthscope-7973>.
- Walker, JF. (1975). Formaldehyde (3rd ed.). R.E. Krieger Publishing Company: Huntington, NY.
- Wallner, P; Kundi, M; Moshhammer, H; Piegler, K; Hohenblum, P; Scharf, S; Fr hlich, M; Damberger, B; Tapplere, P; Hutter, HP. (2012). Indoor air in schools and lung function of Austrian school children. *J Environ Monit* 14: 1976-1982. <http://dx.doi.org/10.1039/c2em30059a>.
- Walrath, J; Fraumeni, JF, Jr. (1983). Mortality patterns among embalmers. *Int J Cancer* 31: 407-411. <http://dx.doi.org/10.1002/ijc.2910310403>.
- Walrath, J; Fraumeni, JF, Jr. (1984). Cancer and other causes of death among embalmers. *Cancer Res* 44: 4638-4641.
- Walrath, J, Jr, FJ. (1983). Proportionate mortality among New York embalmers. In JE Gibson (Ed.), *Formaldehyde toxicity* (pp. 227-236). Washington, DC: Hemispheres Publishing Corporation.
- Wang, B; Liu, DD. (2006). [Detection of formaldehyde induced developmental toxicity assessed with single cell gel electrophoresis]. *Fen Zi Xi Bao Sheng Wu Xue Bao* 39: 462-466.
- Wang, F, an; Li, C; Liu, W, ei; Jin, Y. (2014a). Potential mechanisms of neurobehavioral disturbances in mice caused by sub-chronic exposure to low-dose VOCs. *Inhal Toxicol* 26: 250-258. <http://dx.doi.org/10.3109/08958378.2014.882447>.
- Wang, H; Li, H, eC; Lv, M; Zhou, D; Bai, L; Du, L; Xue, X, ia; Lin, P, u; Qiu, S. (2015). Associations between occupation exposure to Formaldehyde and semen quality, a primary study. *Sci Rep* 5: 15874. <http://dx.doi.org/10.1038/srep15874>.
- Wang, HB; Ghiran, I; Matthaei, K; Weller, PF. (2007a). Airway eosinophils: allergic inflammation recruited professional antigen-presenting cells. *J Immunol* 179: 7585-7592.
- Wang, HX; Wang, XY; Zhou, DX; Zheng, LR; Zhang, J; Huo, YW; Tian, H. (2013). Effects of low-dose, long-term formaldehyde exposure on the structure and functions of the ovary in rats. *Toxicol Ind Health* 29: 609-615. <http://dx.doi.org/10.1177/0748233711430983>.
- Wang, HX; Zhou, DX; Zheng, LR; Zhang, J; Huo, YW; Tian, H; Han, SP; Zhang, J; Zhao, WB. (2012). Effects of paternal occupation exposure to formaldehyde on reproductive outcomes. *J Occup Environ Med* 54: 518-524. <http://dx.doi.org/10.1097/JOM.0b013e31824e6937>.
- Wang, K; Wang, TW; Xu, J; Zhu, Y; Jian, L; Au, W; Xia, ZL. (2019). Determination of benchmark dose based on adduct and micronucleus formations in formaldehyde-exposed workers. *Int J Hyg Environ Health* 222: 738-743. <http://dx.doi.org/10.1016/j.ijheh.2019.05.008>.
- Wang, M; Cheng, G; Balbo, S; Carmella, SG; Villalta, PW; Hecht, SS. (2009a). Clear differences in levels of a formaldehyde-DNA adduct in leukocytes of smokers and nonsmokers. *Cancer Res* 69: 7170-7174. <http://dx.doi.org/10.1158/0008-5472.CAN-09-1571>.

- [Wang, M; Cheng, G; Villalta, PW; SS, H.](#) (2007b). Development of liquid chromatography electrospray ionization tandem mass spectrometry methods for analysis of DNA adducts of formaldehyde and their application to rats treated with N-nitrosodimethylamine or 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone. *Chem Res Toxicol* 20: 1141-1148. <http://dx.doi.org/10.1021/tx700189c>.
- [Wang, R; Zhang, Y; Lan, Q; Holford, TR; Leaderer, B; Zahm, SH; Boyle, P; Dosemeci, M; Rothman, N; Zhu, Y; Qin, Q; Zheng, T.](#) (2009b). Occupational exposure to solvents and risk of non-Hodgkin lymphoma in Connecticut women. *Am J Epidemiol* 169: 176-185. <http://dx.doi.org/10.1093/aje/kwn300>.
- [Wang, T; Pysanenko, A; Dryahina, K; Španěl, P; Smith, D.](#) (2008). Analysis of breath, exhaled via the mouth and nose, and the air in the oral cavity. *J Breath Res* 2: 1-13. <http://dx.doi.org/10.1088/1752-7155/2/3/037013>.
- [Wang, W; Yan, Y; Li, CW; Xia, HM; Chao, SS; Wang, d; Wang, ZP.](#) (2014b). Live human nasal epithelial cells (hNECs) on chip for in vitro testing of gaseous formaldehyde toxicity via airway delivery. *Lab Chip* 14: 677-680. <http://dx.doi.org/10.1039/c3lc51208h>.
- [Wangenheim, J; Bolcsfoldi, G.](#) (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. *Mutagenesis* 3: 193-205. <http://dx.doi.org/10.1093/mutage/3.3.193>.
- [Wantke, F; Demmer, CM; Tappler, P; Gotz, M; Jarisch, R.](#) (1996a). Exposure to gaseous formaldehyde induces IgE-mediated sensitization to formaldehyde in school-children. *Clin Exp Allergy* 26: 276-280. <http://dx.doi.org/10.1111/j.1365-2222.1996.tb00092.x>.
- [Wantke, F; Focke, M; Hemmer, W; Bracun, R; Wolf-Abdolvahab, S; Götz, M; Jarisch, R; Götz, M; Tschabitscher, M; Gann, M; Tappler, P.](#) (2000). Exposure to formaldehyde and phenol during an anatomy dissecting course: Sensitizing potency of formaldehyde in medical students. *Allergy* 55: 84-87. <http://dx.doi.org/10.1034/j.1398-9995.2000.00307.x>.
- [Wantke, F; Focke, M; Hemmer, W; Tschabitscher, M; Gann, M; Tappler, P; Götz, M; Jarisch, R.](#) (1996b). Formaldehyde and phenol exposure during an anatomy dissection course: A possible source of IgE-mediated sensitization. *Allergy* 51: 837-841. <http://dx.doi.org/10.1111/j.1398-9995.1996.tb00031.x>.
- [Ward, BJ, Jr; Legator, MS; Pereira, MA; Chang, LW.](#) (1983). Evaluation in man and animals of tests for the detection of population exposures to genotoxic chemicals. In MD Waters; SS Sandhu; J Lewtas (Eds.), *Short-term bioassays in the analysis of complex environmental mixtures* (pp. 461-484). New York, NY: Plenum Press.
- [Ward, JB, Jr; Hokanson, JA; Smith, ER; Chang, LW; Pereira, MA; Whorton, EB, Jr; Legator, MS.](#) (1984). Sperm count, morphology and fluorescent body frequency in autopsy service workers exposed to formaldehyde. *Mutat Res Environ Mutagen Relat Subj* 130: 417-424. [http://dx.doi.org/10.1016/0165-1161\(84\)90014-1](http://dx.doi.org/10.1016/0165-1161(84)90014-1).
- [Watanabe, K; Sakamoto, K; Sasaki, T.](#) (1996). Comparisons on chemically-induced mutagenicity among four bacterial strains, *Salmonella typhimurium* TA102 and TA2638, and *Escherichia coli* WP2/pKM101 and WP2 uvrA/pKM101: Collaborative study I. *Mutat Res* 361: 143-155. [http://dx.doi.org/10.1016/s0165-1161\(96\)90249-6](http://dx.doi.org/10.1016/s0165-1161(96)90249-6).
- [Weber-Tschopp, A; Fischer, T; Grandjean, E.](#) (1977). Reizwirkungen des Formaldehyds (HCHO) auf den Menschen [Irritating effects of formaldehyde on men]. *Int Arch Occup Environ Health* 39: 207-218. <http://dx.doi.org/10.1007/bf00409367>.
- [Wei, C; Chen, M; You, H; Qiu, F; Wen, H; Yuan, J; Xiang, S; Yang, X.](#) (2017a). Formaldehyde and co-exposure with benzene induce compensation of bone marrow and hematopoietic stem/progenitor cells in BALB/c mice during post-exposure period. *Toxicol Appl Pharmacol* 324: 36-44. <http://dx.doi.org/10.1016/j.taap.2017.03.024>.
- [Wei, C; Wen, H; Yuan, L; Mchale, CM; Li, H; Wang, K; Yuan, J; Yang, X; Zhang, L.](#) (2017b). Formaldehyde induces toxicity in mouse bone marrow and hematopoietic stem/progenitor cells and enhances

- benzene-induced adverse effects. *Arch Toxicol* 91: 921-933. <http://dx.doi.org/10.1007/s00204-016-1760-5>.
- [Wei, CN; Harada, K; Ohmori, S; Wei, QJ; Minamoto, K; Ueda, A.](#) (2007). Subjective symptoms of medical students exposed to formaldehyde during a gross anatomy dissection course. *Int J Immunopathol Pharmacol* 20: 23-25. <http://dx.doi.org/10.1177/03946320070200S205>.
- [Wei, H; Tan, K; Sun, R; Yin, L; Zhang, J; Pu, Y.](#) (2014). Aberrant production of Th1/Th2/Th17-related cytokines in serum of C57BL/6 mice after short-term formaldehyde exposure. *Int J Environ Res Public Health* 11: 10036-10050. <http://dx.doi.org/10.3390/ijerph111010036>.
- [Weibel, ER.](#) (1963). *Morphometry of the human lung*. Berlin, Germany: Springer-Verlag. <http://dx.doi.org/10.1007/978-3-642-87553-3>.
- [Weisel, CP; Zhang, J; Turpin, BJ; Morandi, MT; Colome, S; Stock, TH; Spektor, DM; Korn, L; Winer, AM; Kwon, J; Meng, QY; Zhang, L; Harrington, R; Liu, W; Reff, A; Lee, JH; Alimokhtari, S; Mohan, K; Shendell, D; Jones, J; Farrar, L; Maberti, S; Fan, T.](#) (2005). Relationships of indoor, outdoor, and personal air (RIOPA): Part I. Collection methods and descriptive analyses (pp. 1-107; discussion 109-127). (ISSN 1041-5505/EISSN 2688-6855
- HEI Research Report 130-I, NUATRC Research Report 7). Boston, MA: Health Effects Institute. <https://www.proquest.com/scholarly-journals/relationships-indoor-outdoor-personal-air-riopa/docview/70114572/se-2?accountid=171501>.
- [Weisskopf, M; Morozova, N; O'Reilly, EJ; McCullough, ML; Calle, EE; Thun, MJ; Ascherio, A.](#) (2009). Prospective study of chemical exposures and amyotrophic lateral sclerosis mortality. *J Neurol Neurosurg Psychiatry* 80: 558-561. <http://dx.doi.org/10.1136/jnnp.2008.156976>.
- [Wen, H; Yuan, L; Wei, C; Zhao, Y; Qian, Y; Ma, P; Ding, S; Yang, X; Wang, X.](#) (2016). Effects of combined exposure to formaldehyde and benzene on immune cells in the blood and spleen in Balb/c mice. *Environ Toxicol Pharmacol* 45: 265-273. <http://dx.doi.org/10.1016/j.etap.2016.05.007>.
- [Wesseling, C; Ahlbom, A; Antich, D; Rodriguez, AC; Castro, R.](#) (1996). Cancer in banana plantation workers in Costa Rica. *Int J Epidemiol* 25: 1125-1131. <http://dx.doi.org/10.1093/ije/25.6.1125>.
- [West, GB; Brown, JH.](#) (2005). The origin of allometric scaling laws in biology from genomes to ecosystems: Towards a quantitative unifying theory of biological structure and organization [Review]. *J Exp Biol* 208: 1575-1592. <http://dx.doi.org/10.1242/jeb.01589>.
- [West, S; Hildesheim, A; Dosemeci, M.](#) (1993). Non-viral risk factors for nasopharyngeal carcinoma in the Philippines: Results from a case-control study. *Int J Cancer* 55: 722-727. <http://dx.doi.org/10.1002/ijc.2910550504>.
- [Westberg, H; Löfstedt, H; Seldén, A; Lilja, BG; Nayström, P.](#) (2005). Exposure to low molecular weight isocyanates and formaldehyde in foundries using Hot Box core binders. *Ann Occup Hyg* 49: 719-725. <http://dx.doi.org/10.1093/annhyg/mei040>.
- [WHO](#) (World Health Organization). (1967). *Manual of the international statistical classification of diseases, injuries, and causes of death*. Geneva, Switzerland.
- [WHO](#) (World Health Organization). (1977). *Manual of the international statistical classification of diseases, injuries, and causes of death*. Geneva, Switzerland.
- [WHO](#) (World Health Organization). (2010). *Guidelines for indoor air quality: Selected pollutants*. Geneva. http://www.euro.who.int/_data/assets/pdf_file/0009/128169/e94535.pdf.
- [Wilcox, P; Naidoo, A; Wedd, DJ; Gatehouse, DG.](#) (1990). Comparison of *Salmonella typhimurium* TA102 with *Escherichia coli* WP2 tester strains. *Mutagenesis* 5: 285-291. <http://dx.doi.org/10.1093/mutage/5.3.285>.
- [Wilhelmsson, B; Holmstrom, M.](#) (1992). Possible mechanisms of formaldehyde-induced discomfort in the upper airways. *Scand J Work Environ Health* 18: 403-407. <http://dx.doi.org/10.5271/sjweh.1556>.

- Wilkins, RJ; Macleod, HD. (1976). Formaldehyde induced DNA-protein crosslinks in Escherichia Coli. Mutat Res 36: 11-16. [http://dx.doi.org/10.1016/0027-5107\(76\)90016-6](http://dx.doi.org/10.1016/0027-5107(76)90016-6).
- Williams, GM; Mori, H; Mcqueen, CA. (1989a). Structure-activity relationships in the rat hepatocyte DNA-repair test for 300 chemicals [Review]. Mutat Res 221: 263-286. [http://dx.doi.org/10.1016/0165-1110\(89\)90039-0](http://dx.doi.org/10.1016/0165-1110(89)90039-0).
- Williams, JW; Tjota, MY; Sperling, AI. (2012). The contribution of allergen-specific IgG to the development of th2-mediated airway inflammation. J Allergy (Cairo) 2012: 236075. <http://dx.doi.org/10.1155/2012/236075>.
- Williams, RL; Lipari, F; Potter, RA. (1989b). Formaldehyde, methanol, and hydrocarbon emissions from methanol-fueled cars.
- Wilmer, JWG, M; Woutersen, RA; Appelman, LM; Leeman, WR; Feron, VJ. (1987). Subacute (4-week) inhalation toxicity study of formaldehyde in male rats: 8-hour intermittent versus 8-hour continuous exposures. J Appl Toxicol 7: 15-16. <http://dx.doi.org/10.1002/jat.2550070104>.
- Wilmer, JWG, M; Woutersen, RA; Appelman, LM; Leeman, WR; Feron, VJ. (1989). Subchronic (13-week) inhalation toxicity study of formaldehyde in male rats: 8-hour intermittent versus 8-hour continuous exposures. Toxicol Lett 47: 287-293. [http://dx.doi.org/10.1016/0378-4274\(89\)90147-1](http://dx.doi.org/10.1016/0378-4274(89)90147-1).
- Witek, TJ, Jr; Schachter, EN; Tosun, T; Beck, GJ; Leaderer, BP. (1987). An evaluation of respiratory effects following exposure to 2.0 ppm formaldehyde in asthmatics: Lung function, symptoms, and airway reactivity. Arch Environ Health 42: 230-237.
- Witek, TJ, Jr; Schachter, EN; Tosun, T; Leaderer, BP; Beck, GJ. (1986). Controlled human studies on the pulmonary effects of indoor air pollution: Experiences with sulfur dioxide and formaldehyde. Environ Int 12: 129-135. [http://dx.doi.org/10.1016/0160-4120\(86\)90023-1](http://dx.doi.org/10.1016/0160-4120(86)90023-1).
- Wolf, DC; Gross, EA; Lyght, O; Bermudez, E; Recio, L; Morgan, KT. (1995). Immunohistochemical localization of p53, PCNA, and TGF-alpha proteins in formaldehyde-induced rat nasal squamous cell carcinomas. Toxicol Appl Pharmacol 132: 27-35. <http://dx.doi.org/10.1006/taap.1995.1083>.
- Wolff, RK. (1986). Effects of airborne pollutants on mucociliary clearance [Review]. Environ Health Perspect 66: 223-237.
- Wong, MA; Isaza, R; Cuthbert, JK; Brooks, DE; Samuelson, D. (2012). PERIOcular ANTERIOR ADNEXAL ANATOMY AND CLINICAL ADNEXAL EXAMINATION OF THE ADULT ASIAN ELEPHANT (ELEPHAS MAXIMUS). J Zoo Wildl Med 43: 793-801. <http://dx.doi.org/10.1638/2011-0173R2.1>.
- Wood, RW; Coleman, JB. (1995). Behavioral evaluation of the irritant properties of formaldehyde. Toxicol Appl Pharmacol 130: 67-72. <http://dx.doi.org/10.1006/taap.1995.1009>.
- Wortley, P; Vaughan, TL; Davis, S; Morgan, MS; Thomas, DB. (1992). A case-control study of occupational risk factors for laryngeal cancer. Br J Ind Med 49: 837-844. <http://dx.doi.org/10.1136/oem.49.12.837>.
- Woutersen, RA; Appelman, LM; Wilmer, JWG, M; Falke, HE; Feron, VJ. (1987). Subchronic (13-week) inhalation toxicity study of formaldehyde in rats. J Appl Toxicol 7: 43-49. <http://dx.doi.org/10.1002/jat.2550070108>.
- Woutersen, RA; van Garderen-Hoetmer, A; Bruijntjes, JP; Zwart, A; Feron, VJ. (1989). Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. J Appl Toxicol 9: 39-46. <http://dx.doi.org/10.1002/jat.2550090108>.
- Wu, LC; Zarrin, AA. (2014). The production and regulation of IgE by the immune system [Review]. Nat Rev Immunol 14: 247-259. <http://dx.doi.org/10.1038/nri3632>.
- Wu, Y; Duan, J; Li, B; Liu, H; Chen, M. (2020). Exposure to formaldehyde at low temperatures aggravates allergic asthma involved in transient receptor potential ion channel. Environ Toxicol Pharmacol 80: 103469. <http://dx.doi.org/10.1016/j.etap.2020.103469>.

- [Wu, Y; You, H; Ma, P; Li, L; Yuan, Y; Li, J; Liu, X; Yao, H; Chen, R; Lai, K; Yang, X.](#) (2013). Role of transient receptor potential ion channels and evoked levels of neuropeptides in a formaldehyde-induced model of asthma in Balb/c mice. *PLoS ONE* 8: e62827. <http://dx.doi.org/10.1371/journal.pone.0062827>.
- [Xie, SH; Yu, IT; Tse, LA; Au, JS; Lau, JS.](#) (2017). Occupational risk factors for nasopharyngeal carcinoma in Hong Kong Chinese: a case-referent study. *Int Arch Occup Environ Health* 90: 443-449. <http://dx.doi.org/10.1007/s00420-017-1212-4>.
- [Xin, L; Wang, J; Fan, G; Wu, Y; Guo, S.](#) (2015). Activation of HSPA1A promoter by environmental pollutants: An early and rapid response to cellular damage. *Environ Toxicol Pharmacol* 39: 1027-1033. <http://dx.doi.org/10.1016/j.etap.2015.03.011>.
- [Xing Sy, LY.](#) (2007). Toxic effect of formaldehyde on reproduction and heredity in male mice. *Journal of Jilin University - Medicine Edition* 33.
- [Xing, SY; Ye, L; Wang, NN.](#) (2007). Toxic effect of formaldehyde on reproduction and heredity in male mice. *Journal of Jilin University - Medicine Edition* 33: 716-718.
- [Yager, JW; Cohn, KL; Spear, RC; Fisher, JM; Morse, L.](#) (1986). Sister-chromatid exchanges in lymphocytes of anatomy students exposed to formaldehyde-embalming solution. *Mutat Res* 174: 135-139. [http://dx.doi.org/10.1016/0165-7992\(86\)90104-1](http://dx.doi.org/10.1016/0165-7992(86)90104-1).
- [Yan, Y; Ye, Z; Lu, ZS; Qiao, Y; Yang, X; Li, CM.](#) (2005). Nitric oxide level associated with gaseous formaldehyde exposure in lungs of mice. In X Yang; B Zhao; R Zhao (Eds.), *Indoor Air 2005: Proceedings of the 10th International Conference on Indoor Air Quality and Climate*, vol 5 (pp. 3851-3854). Beijing, China: Tsinghua University Press. <https://www.isiaq.org/docs/PDFs/3851.pdf>.
- [Yang, WH.](#) (2007). [Hemogram of workers exposed to low concentration of formaldehyde] (pp. 792-799). Yang, WH.
- [Yang, X; Zhang, YP; Chen, D; Chen, WG; Wang, R.](#) (2001). Eye irritation caused by formaldehyde as an indoor air pollution--a controlled human exposure experiment. *Biomed Environ Sci* 14: 229-236.
- [Yang, XR; Diehl, S; Pfeiffer, R; Chen, CJ; Hsu, WL; Dosemeci, M; Cheng, YJ; Sun, B; Goldstein, AM; Hildesheim, A; Team, CaAGEoNS.](#) (2005). Evaluation of risk factors for nasopharyngeal carcinoma in high-risk nasopharyngeal carcinoma families in Taiwan. *Cancer Epidemiol Biomarkers Prev* 14: 900-905. <http://dx.doi.org/10.1158/1055-9965.EPI-04-0680>.
- [Yang, Y; Luo, H; Liu, R; Li, G; Yu, Y; An, T.](#) (2020). The exposure risk of typical VOCs to the human beings via inhalation based on the respiratory deposition rates by proton transfer reaction-time of flight-mass spectrometer. *Ecotoxicol Environ Saf* 197: 110615. <http://dx.doi.org/10.1016/j.ecoenv.2020.110615>.
- [Ye, X; Ji, Z; Wei, C; Mchale, CM; Ding, S; Thomas, R; Yang, X; Zhang, L.](#) (2013). Inhaled formaldehyde induces DNA-protein crosslinks and oxidative stress in bone marrow and other distant organs of exposed mice. *Environ Mol Mutagen* 54: 705-718. <http://dx.doi.org/10.1002/em.21821>.
- [Ye, X; Yan, W; Xie, H; Zhao, M; Ying, C.](#) (2005). Cytogenetic analysis of nasal mucosa cells and lymphocytes from high-level long-term formaldehyde exposed workers and low-level short-term exposed waiters. *Mutat Res* 588: 22-27. <http://dx.doi.org/10.1016/j.mrgentox.2005.08.005>.
- [Yeatts, KB; El-Sadig, M; Leith, D; Kalsbeek, W; Al-Maskari, F; Couper, D; Funk, WE; Zoubeidi, T; Chan, RL; Trent, CB; Davidson, CA; Boundy, MG; Kassab, MM; Hasan, MY; Rusyn, I; Gibson, JM; Olshan, AF.](#) (2012). Indoor air pollutants and health in the United Arab Emirates. *Environ Health Perspect* 120: 687-694. <http://dx.doi.org/10.1289/ehp.1104090>.
- [Yiamouyiannis, CA; Stengel, PW; Cockerham, SL; Silbaugh, SA.](#) (1995). PULMONARY ACTIONS OF THE NEUROKININ(1)-SPECIFIC AGONIST [SAR(9),MET(O-2)(11)]-SUBSTANCE-P. *Neuropeptides* 28: 35-42.

- [Ying, CJ; Yan, WS; Zhao, MY; Ye, XL; Xie, H; Yin, SY; Zhu, XS.](#) (1997). Micronuclei in nasal mucosa, oral mucosa and lymphocytes in students exposed to formaldehyde vapor in anatomy class. *Biomed Environ Sci* 10: 451-455.
- [Ying, CJ; Ye, XL; Xie, H; Yan, WS; Zhao, MY; Xia, T; Yin, SY.](#) (1999). Lymphocyte subsets and sister-chromatid exchanges in the students exposed to formaldehyde vapor. *Biomed Environ Sci* 12: 88-94.
- [Yokley, KA.](#) (2012). Sensory irritation response in rats II: Recovery and dose-dependence. *Bull Math Biol* 74: 1673-1690. <http://dx.doi.org/10.1007/s11538-012-9730-4>.
- [Yokley, TR.](#) (2009). Ecogeographic variation in human nasal passages. *Am J Phys Anthropol* 138: 11-22. <http://dx.doi.org/10.1002/ajpa.20893>.
- [Yon, DK; Hwang, S; Lee, SW; Jee, HM; Sheen, YH; Kim, JH; Lim, DH; Han, MY.](#) (2019). Indoor Exposure and Sensitization to Formaldehyde among Inner-City Children with Increased Risk for Asthma and Rhinitis. *Am J Respir Crit Care Med* 200: 388-393. <http://dx.doi.org/10.1164/rccm.201810-1980LE>.
- [Yonemitsu, T; Kuroki, C; Takahashi, N; Mori, Y; Kanmura, Y; Kashiwadani, H; Ootsuka, Y; Kuwaki, T.](#) (2013). TRPA1 detects environmental chemicals and induces avoidance behavior and arousal from sleep. *Sci Rep* 3: 3100. <http://dx.doi.org/10.1038/srep03100>.
- [Yoo, SJ; Ito, K.](#) (2018a). Assessment of transient inhalation exposure using in silico human model integrated with PBPK-CFD hybrid analysis. *Sustain Cities Soc* 40: 317-325. <http://dx.doi.org/10.1016/j.scs.2018.04.023>.
- [Yoo, SJ, un; Ito, K.](#) (2018b). Numerical prediction of tissue dosimetry in respiratory tract using computer simulated person integrated with physiologically based pharmacokinetic-computational fluid dynamics hybrid analysis. *Indoor Built Environ* 27: 877-889. <http://dx.doi.org/10.1177/1420326X17694475>.
- [Yorgancilar, E; Deveci, E; Deveci, S.](#) (2012). Effects of formaldehyde on respiratory mucosa in rats. *International Journal of Morphology* 30: 521-523. <http://dx.doi.org/10.4067/S0717-95022012000200026>.
- [Young, JT.](#) (1981). Histopathologic examination of the rat nasal cavity. *Fundam Appl Toxicol* 1: 309-312. [http://dx.doi.org/10.1016/S0272-0590\(81\)80037-1](http://dx.doi.org/10.1016/S0272-0590(81)80037-1).
- [Yu, C; Crump, D.](#) (1998). A review of the emission of VOCs from polymeric materials used in buildings [Review]. *Build Environ* 33: 357-374. [http://dx.doi.org/10.1016/S0360-1323\(97\)00055-3](http://dx.doi.org/10.1016/S0360-1323(97)00055-3).
- [Yu, G; Chen, Q; Liu, X; Guo, C; Du, H; Sun, Z.](#) (2014a). Formaldehyde induces bone marrow toxicity in mice by inhibiting peroxiredoxin 2 expression. *Mol Med Rep* 10: 1915-1920. <http://dx.doi.org/10.3892/mmr.2014.2473>.
- [Yu, GY; Song, XF; Liu, Y; Sun, ZW.](#) (2014b). Inhaled Formaldehyde Induces Bone Marrow Toxicity via Oxidative Stress in Exposed Mice. *Asian Pac J Cancer Prev* 15: 5253-5257. <http://dx.doi.org/10.7314/APJCP.2014.15.13.5253>.
- [Yu, GY; Song, XF; Zhao, SH; Liu, Y; Sun, ZW.](#) (2015a). Formaldehyde induces the bone marrow toxicity in mice by regulating the expression of Prx3 protein. *J Huazhong Univ Sci Technolog Med Sci* 35: 82-86. <http://dx.doi.org/10.1007/s11596-015-1393-6>.
- [Yu, IT; Li, AM; Goggins, W; Leung, JO; Chan, GY; Fung, CK; Chan, CK; Lau, AP.](#) (2017). Association of wheeze during the first 18 months of life with indoor nitrogen dioxide, formaldehyde, and family history of asthma: a prospective cohort study. *Hong Kong Med J* 23 Suppl 2: 19-23.
- [Yu, ITS; Chin, YL; Wong, TW; Tang, JL.](#) (2004). Deaths from nasopharyngeal cancer among waiters and waitresses in Chinese restaurants. *Int Arch Occup Environ Health* 77: 499-504. <http://dx.doi.org/10.1007/s00420-004-0543-0>.
- [Yu, LQ; Jiang, SF; Leng, SG; He, FS; Zheng, YX.](#) (2005). Early genetic effects on workers occupationally exposed to formaldehyde. *Zhonghua Yufang Yixue Zazhi* 39: 392-395.

- Yu, PH; Lai, CT; Zuo, DM. (1997). Formation of formaldehyde from adrenaline in vivo; a potential risk factor for stress-related angiopathy. *Neurochem Res* 22: 615-620. <http://dx.doi.org/10.1023/a:1022478221421>.
- Yu, PH; Zuo, DM. (1996). Formaldehyde produced endogenously via deamination of methylamine. A potential risk factor for initiation of endothelial injury. *Atherosclerosis* 120: 189-197. [http://dx.doi.org/10.1016/0021-9150\(95\)05701-3](http://dx.doi.org/10.1016/0021-9150(95)05701-3).
- Yu, R; Lai, Y; Hartwell, HJ; Moeller, BC; Doyle-Eisele, M; Kracko, D; Bodnar, WM; Starr, TB; Swenberg, JA. (2015b). Formation, Accumulation, and Hydrolysis of Endogenous and Exogenous Formaldehyde-Induced DNA Damage. *Toxicol Sci* 146: 170-182. <http://dx.doi.org/10.1093/toxsci/kfv079>.
- Yu, YH; Blessing, WW. (1997). Cerebral blood flow in rabbits during the nasopharyngeal reflex elicited by inhalation of noxious vapor. *J Auton Nerv Syst* 66: 149-153. [http://dx.doi.org/10.1016/s0165-1838\(97\)00080-5](http://dx.doi.org/10.1016/s0165-1838(97)00080-5).
- Yu, YH; Blessing, WW. (1999). Amygdala co-ordinates sudden falls in ear pinna blood flow in response to unconditioned salient stimuli in conscious rabbits. *Neuroscience* 93: 135-141. [http://dx.doi.org/10.1016/s0306-4522\(99\)00097-4](http://dx.doi.org/10.1016/s0306-4522(99)00097-4).
- Zang, ZJ; Fang, YQ; Ji, SY; Gao, Y; Zhu, YQ; Xia, TT; Jiang, MH; Zhang, YN. (2017). Formaldehyde Inhibits Sexual Behavior and Expression of Steroidogenic Enzymes in the Testes of Mice. *J Sex Med* 14: 1297-1306. <http://dx.doi.org/10.1016/j.jsxm.2017.09.001>.
- Zararsiz, I; Kus, I; Akpolat, N; Songur, A; Ogeturk, M; Sarsilmaz, M. (2006). Protective effects of omega-3 essential fatty acids against formaldehyde-induced neuronal damage in prefrontal cortex of rats. *Cell Biochem Funct* 24: 237-244. <http://dx.doi.org/10.1002/cbf.1204>.
- Zarei, F; Rezazadeh Azari, M; Salehpour, S; Khodakarim, S; Omid, L; Tavakol, E. (2017). Respiratory effects of simultaneous exposure to respirable crystalline silica dust, formaldehyde, and triethylamine of a group of foundry workers. *Journal of Research in Health Sciences* 17: E1-E6.
- Zeller, J; Neuss, S; Mueller, JU; Kühner, S; Holzmann, K; Högel, J; Klingmann, C; Bruckner, T; Triebig, G; Speit, G. (2011). Assessment of genotoxic effects and changes in gene expression in humans exposed to formaldehyde by inhalation under controlled conditions. *Mutagenesis* 26: 555-561. <http://dx.doi.org/10.1093/mutage/ger016>.
- Zendehdel, R; Jouni, FJ; Hajipour, B; Panjali, Z; Kheiri, H; Vahabi, M. (2017). DNA damage in workers exposed to formaldehyde at concentrations below occupational exposure limits. *Toxicol Environ Chem* 99: 1409-1417. <http://dx.doi.org/10.1080/02772248.2017.1343335>.
- Zendehdel, R; Vahabi, M; Sedghi, R. (2018). Estimation of formaldehyde occupational exposure limit based on genetic damage in some Iranian exposed workers using benchmark dose method. *Environ Sci Pollut Res Int* 25: 31183-31189. <http://dx.doi.org/10.1007/s11356-018-3077-9>.
- Zhai, L; Zhao, J; Xu, B; Deng, Y; Xu, Z. (2013). Influence of indoor formaldehyde pollution on respiratory system health in the urban area of Shenyang, China. *Afr Health Sci* 13: 137-143. <http://dx.doi.org/10.4314/ahs.v13i1.19>.
- Zhang, J; He, Q; Li, P. (1994). Characteristics of aldehydes: Concentrations, sources, and exposures for indoor and outdoor residential microenvironments. *Environ Sci Technol* 28: 146-152. <http://dx.doi.org/10.1021/es00050a020>.
- Zhang, J; Sun, R; Chen, Y; Tan, K; Wei, H; Yin, L; Pu, Y. (2014a). Small molecule metabolite biomarker candidates in urine from mice exposed to formaldehyde. *International Journal of Molecular Sciences* 15: 16458-16468. <http://dx.doi.org/10.3390/ijms150916458>.
- Zhang, L; Tang, X; Rothman, N; Vermeulen, R; Ji, Z; Shen, M; Qiu, C; Guo, W; Liu, S; Reiss, B; Freeman, LB; Ge, Y; Hubbard, AE; Hua, M; Blair, A; Galvan, N; Ruan, X; Alter, BP; Xin, KX; Li, S; Moore, LE; Kim, S; Xie, Y; Hayes, RB; Azuma, M; Hauptmann, M; Xiong, J; Stewart, P; Li, L; Rappaport, SM; Huang, H; Fraumeni, JF, Jr; Smith, MT; Lan, Q. (2010). Occupational exposure to formaldehyde,

- hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. *Cancer Epidemiol Biomarkers Prev* 19: 80-88. <http://dx.doi.org/10.1158/1055-9965.EPI-09-0762>.
- Zhang, Q; Yan, W; Bai, Y; Zhu, Y; Ma, J. (2014b). Repeated formaldehyde inhalation impaired olfactory function and changed SNAP25 proteins in olfactory bulb. *Int J Occup Environ Health* 20: 308-312. <http://dx.doi.org/10.1179/2049396714Y.0000000079>.
- Zhang, S; Chen, H; Wang, A; Liu, Y; Hou, H; Hu, Q. (2018a). Combined effects of co-exposure to formaldehyde and acrolein mixtures on cytotoxicity and genotoxicity in vitro. *Environ Sci Pollut Res Int* 25: 25306-25314. <http://dx.doi.org/10.1007/s11356-018-2584-z>.
- Zhang, S; Chen, H; Zhang, J; Li, J, un; Hou, H; Hu, Q. (2020a). The multiplex interactions and molecular mechanism on genotoxicity induced by formaldehyde and acrolein mixtures on human bronchial epithelial BEAS-2B cells. *Environ Int* 143: 105943. <http://dx.doi.org/10.1016/j.envint.2020.105943>.
- Zhang, S; Zhang, J; Chen, H; Wang, A; Liu, Y; Hou, H; Hu, Q. (2019). Combined cytotoxicity of co-exposure to aldehyde mixtures on human bronchial epithelial BEAS-2B cells. *Environ Pollut* 250: 650-661. <http://dx.doi.org/10.1016/j.envpol.2019.03.118>.
- Zhang, S; Zhang, J; Cheng, W; Chen, H; Wang, A; Liu, Y; Hou, H; Hu, Q. (2020b). Combined cell death of co-exposure to aldehyde mixtures on human bronchial epithelial BEAS-2B cells: Molecular insights into the joint action. *Chemosphere* 244: 125482. <http://dx.doi.org/10.1016/j.chemosphere.2019.125482>.
- Zhang, X; Zhao, Y; Song, J; Yang, X; Zhang, J; Zhang, Y; Li, R. (2018b). Differential health effects of constant versus intermittent exposure to formaldehyde in mice: Implications for building ventilation strategies. *Environ Sci Technol* 52: 1551-1560. <http://dx.doi.org/10.1021/acs.est.7b05015>.
- Zhang, Y; Liu, X; Mchale, C; Li, R; Zhang, L; Wu, Y; Ye, X; Yang, X; Ding, S. (2013). Bone marrow injury induced via oxidative stress in mice by inhalation exposure to formaldehyde. *PLoS ONE* 8: e74974. <http://dx.doi.org/10.1371/journal.pone.0074974>.
- Zhao, MY; Ying, CJ; Shao, N; Yang, Y; Yang, CF; Shi, L; Liu, WQ. (1994). The study of health effects of vinyl chloride air pollution on population. *Biomed Environ Sci* 7: 136-143.
- Zhao, W; Guangyin, P; Yang, X. (2009). DNA-protein crosslinks induced by formaldehyde and its repair process. *Int J Environ Pollut* 37: 299-308.
- Zhao, Y, un; Magana, LC; Cui, H; Huang, J; Mchale, CM; Yang, X, u; Looney, MR; Li, R, ui; Zhang, L. (2020). Formaldehyde-induced hematopoietic stem and progenitor cell toxicity in mouse lung and nose. *Arch Toxicol* 95: 693-701. <http://dx.doi.org/10.1007/s00204-020-02932-x>.
- Zheng, W; Blot, WJ; Shu, XO; Diamond, EL; Gao, YT; Ji, BT; Fraumeni, J. R. (1992). A population-based case-control study of cancers of the nasal cavity and paranasal sinuses in Shanghai. *Int J Cancer* 52: 557-561. <http://dx.doi.org/10.1002/ijc.2910520410>.
- Zhitkovich, A; Costa, M. (1992). A simple, sensitive assay to detect DNA-protein crosslinks in intact cells and in vivo. *Carcinogenesis* 13: 1485-1489. <http://dx.doi.org/10.1093/carcin/13.8.1485>.
- Zhong, W; Hee, SQ. (2004). Quantitation of normal and formaldehyde-modified deoxynucleosides by high-performance liquid chromatography/UV detection. *Biomed Chromatogr* 18: 462-469. <http://dx.doi.org/10.1002/bmc.337>.
- Zhong, W; Que Hee, SS. (2004). Formaldehyde-induced DNA adducts as biomarkers of in vitro human nasal epithelial cell exposure to formaldehyde. *Mutat Res* 563: 13-24. <http://dx.doi.org/10.1016/j.mrgentox.2004.05.012>.
- Zhou, D; Zhang, J; Wang, H. (2011a). Assessment of the potential reproductive toxicity of long-term exposure of adult male rats to low-dose formaldehyde. *Toxicol Ind Health* 27: 591-598. <http://dx.doi.org/10.1177/0748233710393401>.

- [Zhou, D; Zhang, J; Wang, H; Xue, Y.](#) (2011b). Effect of formaldehyde exposure on structure and function of epididymis in adult rats: A histological and biochemical study. *Toxicol Environ Chem* 93: 134-144. <http://dx.doi.org/10.1080/02772248.2010.501145>.
- [Zhou, DX; Qiu, SD; Zhang, J; Tian, H; Wang, HX.](#) (2006). The protective effect of vitamin E against oxidative damage caused by formaldehyde in the testes of adult rats. *Asian J Androl* 8: 584-588. <http://dx.doi.org/10.1111/j.1745-7262.2006.00198.x>.
- [Zhou, ES; Kane, YY; Gao, XX; Wu, LF; Lu, ZS; Yan, Y; Qiao, Y; Yang, X.](#) (2005). A pilot investigation on human serum formaldehyde-specific IgE. Paper presented at 10th International Conference on Indoor Air Quality and Climate, September 4-9, 2005, Beijing, China.
- [Zhu, JL; Knudsen, LE; Andersen, AM; Hjollund, NH; Olsen, J.](#) (2005). Time to pregnancy among Danish laboratory technicians who were a part of the National Birth Cohort. *Scand J Work Environ Health* 31: 108-114.
- [Zhu, JL; Knudsen, LE; Andersen, AM; Hjollund, NH; Olsen, J.](#) (2006). Laboratory work and pregnancy outcomes: a study within the National Birth Cohort in Denmark. *Occup Environ Med* 63: 53-58. <http://dx.doi.org/10.1136/oem.2005.021204>.
- [Zimmermann, FK; Mohr, A.](#) (1992). Formaldehyde, glyoxal, urethane, methyl carbamate, 2,3-butanedione, 2,3-hexanedione, ethyl acrylate, dibromoacetonitrile and 2-hydroxypropionitrile induce chromosome loss in *Saccharomyces cerevisiae*. *Mutat Res* 270: 151-166. [http://dx.doi.org/10.1016/0027-5107\(92\)90126-M](http://dx.doi.org/10.1016/0027-5107(92)90126-M).
- [Zitting, A; Savolainen, H; Nickels, J.](#) (1982). Biochemical and toxicological effects of single and repeated exposures to polyacetal thermodegradation products. *Environ Res* 29: 287-296. [http://dx.doi.org/10.1016/0013-9351\(82\)90031-7](http://dx.doi.org/10.1016/0013-9351(82)90031-7).
- [Zwart, A; Woutersen, RA; Wilmer, JWG, M; Spit, BJ; Feron, VJ.](#) (1988). Cytotoxic and adaptive effects in rat nasal epithelium after 3-day and 13-week exposure to low concentrations of formaldehyde vapour. *Toxicology* 51: 87-99. [http://dx.doi.org/10.1016/0300-483X\(88\)90083-2](http://dx.doi.org/10.1016/0300-483X(88)90083-2).
-