

**INTERACTION PROFILE FOR CARBON MONOXIDE,
FORMALDEHYDE, METHYLENE CHLORIDE, NITROGEN DIOXIDE,
AND TETRACHLOROETHYLENE**

**Agency for Toxic Substances and Disease Registry
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PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program, initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found.

To carry out these legislative mandates, ATSDR's Office of Innovation and Analytics, Toxicology Section has developed and coordinated a mixtures program that includes trend analysis to identify the mixtures most often found in environmental media, locate available *in vivo* and *in vitro* toxicological studies evaluating mixtures, perform quantitative modeling of joint action, and develop methods for assessment of joint toxicity. These efforts are interrelated. For example, the trend analysis suggests mixtures of concern for which assessments need to be conducted. If data are not available, further research is recommended. The data thus generated often contribute to the design, calibration, or validation of the methodology. This pragmatic approach allows identification of pertinent issues and their resolution as well as enhancement of our understanding of the mechanisms of joint toxic action. All of the information obtained is thus used to enhance existing or developing methods to assess the joint toxic action of environmental chemicals. Over a number of years, ATSDR scientists, in collaboration with mixtures risk assessors and laboratory scientists, have developed approaches for the assessment of the joint toxic action of chemical mixtures. As part of the mixtures program a series of documents, Interaction Profiles, are being developed for certain priority mixtures that are of special concern to ATSDR.

The purpose of an Interaction Profile is to evaluate data on the toxicology of the "whole" priority mixture (if available) and on the joint toxic action of the chemicals in the mixture in order to recommend approaches for the exposure-based assessment of the potential hazard to public health. Joint toxic action includes additivity and interactions. A weight-of-evidence (WOE) approach is commonly used in these documents to evaluate the influence of interactions in the overall toxicity of the mixture. The WOE evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds.

Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur.

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PEER REVIEW

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These experts collectively have knowledge of toxicology, chemistry, and/or health effects. All reviewers were selected in conformity with Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for the compounds evaluated in this profile.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

SUMMARY

Carbon monoxide, formaldehyde, methylene chloride, nitrogen dioxide, and tetrachloroethylene were chosen as the subject for this interaction profile based on the likelihood of co-exposure to these chemicals in the home. Concentrations of these chemicals commonly are higher in indoor air than in outdoor air. Carbon monoxide is generated as a product of incomplete combustion from sources that include home furnaces and fireplaces. Formaldehyde is found in many products used around the house, such as antiseptics, medicines, cosmetics, dishwashing liquids, fabric softeners, shoe-care agents, carpet cleaners, glues and adhesives, lacquers, paper, plastics, and some types of wood products. Methylene chloride, also known as dichloromethane, is widely used as an industrial solvent and as a paint stripper and can also be found in certain aerosol and pesticide products, some spray paints, automotive cleaners, and other household products. High levels of nitrogen dioxide may be found in the home when unvented combustion appliances are used for cooking or heating (e.g., poorly vented fireplaces or furnaces). Tetrachloroethylene may be found in the home environment as a result of drycleaning operations, or when one or more of the members of the household works in processes involving tetrachloroethylene.

No pertinent health effects data or physiologically based pharmacokinetic (PBPK) models were located for the complete mixture. Therefore, as recommended by ATSDR (2001) guidance, the exposure-based screening assessment of potential health hazards for this mixture depends on an evaluation of the health effects data and mechanistic data for the individual components and on the joint toxic action and mechanistic data for various combinations of the components. This profile discusses and evaluates the evidence for joint toxic action among binary mixtures of these chemicals and recommends how to incorporate concerns regarding possible interactions or additivity into public health assessments of people who may be exposed to mixtures of these chemicals.

There is no single endpoint that is a sensitive target of all components of the mixture. However, several endpoints are common across multiple chemicals within the mixture, including hematological effects, respiratory effects, neurological alterations, hepatic injury, and cancer. With data on the individual components suggesting possible sites of joint toxic action, but no data available on the toxicity or behavior of the complete mixture or the relevant submixtures, a default component-based approach assuming additivity was therefore recommended, using dose addition for noncancer endpoints and response addition for cancer endpoints. The weight-of-evidence (WOE) analysis indicated that data are inadequate to characterize the modes of joint action of many of the components, but the additivity assumption, especially dose additivity, appears to be suitable in the interest of protecting public health

since the components have several shared targets of toxicity (organs or organ systems that are individually affected by the components).

A target-organ toxicity dose (TTD) modification of the hazard index approach is recommended for conducting exposure-based assessments of noncancer health hazards. TTDs for several toxicity targets have been derived for each of the components, including TTDs for hematological, respiratory, neurological, and hepatic effects. If only one or if none of the components has a hazard quotient that is at least 0.1, no further assessment of the *joint toxic action* is needed because dose additivity and/or interactions are unlikely to result in significant health hazard. If the hazard index for any endpoint of concern is ≥ 1 , then further evaluation is needed (ATSDR 2001), using biomedical judgment and community-specific health outcome data and taking into account community health concerns (ATSDR 1992).

For assessment of cancer risks from joint toxic action of the mixture, a similar component-based approach is recommended that involves multiplication of exposure levels for each of the components by U.S. Environmental Protection Agency (EPA) cancer slope factors and summation of the resultant single chemical risk estimates.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ADHD	attention-deficit/hyperactivity disorder
AQG	air quality guideline
ATSDR	Agency for Toxic Substances and Disease Registry
BINWOE	binary weight-of-evidence
CERCLA	Comprehensive Environmental Response, Compensation, and Recovery Act
CFD	computational fluid dynamics
CFK	Coburn-Forster-Kame
CHO	Chinese hamster ovary
CNS	central nervous system
COHb	carboxyhemoglobin
COPD	chronic obstructive pulmonary disease
CYP	cytochrome P450
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
FDH	formaldehyde dehydrogenase
GSH	glutathione
GST- θ	glutathione S-transferase θ
GSTT1-1	theta-glutathione-S-transferase
HHS	Health and Human Services
HEC	human equivalent concentration
HPRT	hypoxanthine-guanine phosphoribosyl transferase
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
MFO	mixed function oxidase
MRL	Minimal Risk Level
NAAQS	National Ambient Air Quality Standard
NOAEL	no-observed-adverse-effect level
PBPK/PD	physiologically-based pharmacokinetic/pharmacodynamic
OR	odds ratio
POD	point of departure
RfC	reference concentration
RNA	ribonucleic acid
TTD	target-organ toxicity dose
TWA	time-weighted average
WHO	World Health Organization
WOE	weight-of-evidence

1. Introduction

The primary purpose of this *Interaction Profile for Carbon Monoxide, Formaldehyde, Methylene Chloride, Nitrogen Dioxide, and Tetrachloroethylene* is to evaluate data on the toxicology of the “whole” mixture and the joint toxic action of the chemicals in the mixture in order to recommend approaches for assessing the potential hazard of this mixture to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern, and adequacy and relevance of physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence (WOE) approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The WOE evaluations are qualitative in nature, although the Agency for Toxic Substances and Disease Registry (ATSDR) recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR Office of Innovation and Analytics, Toxicology Section recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. These approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios. For more information on different approaches to evaluating mixtures and background information on chemical interactions, readers can refer to the [*Framework for Assessing Health Impacts of Multiple Chemical and Other Stressors*](#) (ATSDR 2018).

The carbon monoxide, formaldehyde, methylene chloride, nitrogen dioxide, and tetrachloroethylene mixture was chosen as the subject for this interaction profile based primarily on concerns regarding co-exposure to these chemicals in residential indoor air. All of the components of the mixture are commonly found in the indoor air environment of the home, as described briefly below. Concentrations of these chemicals commonly are higher in indoor air than in outdoor air (Table 1). Since this mixture is common in residential air, understanding potential interactions will be useful for risk characterization in homes, including those near hazardous waste sites. Because they are all highly volatile, the focus of the interaction profile will be on inhalation exposure, with an emphasis on intermediate- and chronic-duration effects.

Table 1. Indoor Air Quality—Levels of Pollutants in Households

Chemical	Exposure level	Exposure scenario
Carbon monoxide	0.5–5 ppm	Homes without gas stoves
	5–15 ppm	Near properly adjusted gas stoves
	>30 ppm	Near poorly adjusted gas stoves
Formaldehyde	<0.1 ppm	Older homes without urea-formaldehyde foam insulation
	>0.3 ppm	Homes with significant amount of new pressed wood products
Nitrogen dioxide	< outdoor levels (by ½)	Homes without combustion appliances
	> outdoor levels	Homes with gas stoves, kerosene heaters, un-vented gas space heaters, etc.
Volatile organic compounds (including methylene chloride and tetrachloroethylene)	2–5 times	Levels inside homes higher compared to outside air regardless of whether the homes are located in rural or highly industrialized area
	1,000 times	During and after certain activities, such as paint stripping, levels higher than background outdoor levels

Source: EPA (2024a, 2024b, 2024c, 2025)

Carbon monoxide is a colorless, odorless gas that is formed as a product of incomplete combustion. Numerous incidents of elevated carbon monoxide levels in the home have been reported, with the primary sources being faulty ventilation of furnaces or fireplaces. Carbon monoxide's toxic effects stem from its binding with the ferrous iron in hemoglobin, resulting in the formation of carboxyhemoglobin (COHb). COHb is unable to bind molecular oxygen, resulting in diminished oxygen-carrying capacity of the blood. Effects of carbon monoxide exposure include headache, nausea, chest pain during exercise, and, at high exposure levels, convulsions, coma, and death. Developmental effects can result from maternal/fetal hypoxia. There is also evidence of cardiovascular disease following repeated exposure to low levels of carbon monoxide. More information on carbon monoxide is found in Appendix A.

Formaldehyde is a colorless gas at room temperature. Sources of formaldehyde exposure within the home include cigarettes and other tobacco products, gas cookers, and open fireplaces. Formaldehyde is found in many products used every day around the house, such as antiseptics, medicines, cosmetics, dishwashing liquids, fabric softeners, shoe-care agents, carpet cleaners, glues and adhesives, lacquers, paper, and plastics, and some types of wood products. It is also used as a preservative in some foods, such as some types of Italian cheeses, dried foods, and fish. It has a pungent, distinct odor and may cause a burning sensation to the eyes, nose, and lungs at high concentrations and damage to the respiratory tissues. There is also some evidence of reproductive or developmental toxicity. The Department of Health and Human Services (HHS) has categorized formaldehyde as a *known to be a human carcinogen*

based on sufficient evidence of carcinogenicity in humans (NTP 2021a). The U.S. Environmental Protection Program (EPA) classifies formaldehyde as *carcinogenic to humans* (EPA 2024d). The International Agency for Research on Cancer (IARC) classifies formaldehyde as *carcinogenic to humans* (Group 1) based on sufficient evidence in humans and experimental animals (IARC 2012). More information on formaldehyde is found in Appendix B and ATSDR (1999).

Methylene chloride, also known as dichloromethane, is a colorless liquid that has a mild sweet odor, evaporates easily, and does not burn easily. It is widely used as an industrial solvent and as a paint stripper. It can also be found in certain aerosol and pesticide products, some spray paints, automotive cleaners, and other household products. Methylene chloride is used in the manufacture of photographic film. Methylene chloride is metabolized in the body to both carbon monoxide and formaldehyde, and may result in COHb formation, compensatory hematopoiesis, damage to respiratory tissues, hepatic effects, and neurological effects, including headache, dizziness, intoxication, and incoordination. HHS (NTP 2021b) has categorized methylene chloride as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals. The EPA (2011a) classifies methylene chloride (dichloromethane) as *likely to be carcinogenic to humans* based on inadequate human data and sufficient evidence of carcinogenicity in animals. IARC (2016) classified methylene chloride (dichloromethane) as *probably carcinogenic to humans* (Group 2A) based on limited evidence in humans and sufficient evidence in experimental animals. More information on methylene chloride can be found in Appendix C and ATSDR (2000).

Nitrogen dioxide is a colorless gas that may be found at high levels in both the indoor and outdoor environment. Within the home, concentrations of nitrogen oxides, including nitrogen dioxide, may be elevated when unvented combustion appliances are used for cooking or heating (e.g., poorly vented fireplaces or furnaces). The primary effects of inhaled nitrogen dioxide involve irritation of the respiratory tract, with high-level exposures also resulting in small deficits to the immune system, particularly in the lungs. Exposure has also been associated with cardiovascular, neurological, and developmental effects. More information on nitrogen dioxide can be found in Appendix D.

Tetrachloroethylene is a synthetic chemical that is widely used for drycleaning of fabrics and for metal-degreasing operations. It is a nonflammable liquid at room temperature but evaporates easily into the air. It may be found in the home environment as a result of drycleaning operations or when one or more of the members of the household work in processes involving tetrachloroethylene. Tetrachloroethylene has a sharp, sweet odor; most people can smell tetrachloroethylene at levels ≥ 1 ppm. The primary effects of

tetrachloroethylene are neurological, including decreased performance, headache, dizziness, and drowsiness. Other effects of tetrachloroethylene include renal and hepatic effects. Tetrachloroethylene is classified as *reasonably anticipated to be a human carcinogen* by HHS (NTP 2021c) based on evidence in experimental animals. Tetrachloroethylene is classified as *likely to be carcinogenic to humans* by the EPA (2012) based on suggestive evidence in humans and conclusive evidence in animals. Based on evidence in experimental animals, IARC (2014) classified tetrachloroethylene as *probably carcinogenic to humans* (Group 2A). More information on tetrachloroethylene can be found in Appendix E and ATSDR (2019).

Before evaluating the relevance of joint toxic action data for these chemicals, some understanding of endpoints of concern for inhalation exposure to this mixture is needed. The endpoints of concern include the critical effects that are the bases for Minimal Risk Levels (MRLs) or other health-based guidance values, and any other endpoints that may become significant because they are shared targets of toxicity or due to interactions (ATSDR 2018).

Carbon monoxide's critical effect is the formation of COHb, which is a hematological effect. ATSDR (2012) has not derived MRLs and EPA has not derived a reference concentration (RfC) for carbon monoxide. Increased blood COHb caused by carbon monoxide exposure may also lead to cardiovascular, neurological, or developmental effects.

The critical effect for formaldehyde inhalation, and the basis for ATSDR's inhalation MRLs and EPA's RfC, is respiratory system toxicity, specifically irritant effects in humans (ATSDR 1999; EPA 2024d). EPA (2024d) derived an inhalation unit risk for cancer of 1.1×10^{-5} per $\mu\text{g}/\text{m}^3$ for formaldehyde.

Several different endpoints are sensitive effects of methylene chloride inhalation. ATSDR's acute-duration inhalation MRL is based on neurological effects, the intermediate-duration inhalation MRL is based on hepatic effects, and the chronic-duration inhalation MRL is based on hematologic effects (ATSDR 2000). EPA's RfC for methylene chloride is based on hepatic effects (EPA 2011a). Methylene chloride exposure may also result in respiratory effects. EPA (2011a) derived an inhalation unit risk for cancer of 1×10^{-8} per $\mu\text{g}/\text{m}^3$ for methylene chloride.

The primary effect of nitrogen dioxide inhalation is injury to the respiratory tract, which is believed to be the result of the reactive nature of nitrogen dioxide. ATSDR has not derived MRLs and EPA has not

derived an RfC for nitrogen dioxide. Nitrogen dioxide may also cause immunological deficits at high doses.

The most sensitive effects of tetrachlorethylene inhalation are neurological, including decreased reaction times, headache, dizziness, and drowsiness. ATSDR's chronic-duration inhalation MRL and EPA's RfC for tetrachloroethylene are based on neurological effects in exposed humans (ATSDR 2019; EPA 2012). Other sensitive endpoints of tetrachloroethylene include hepatic and renal effects. EPA (2011a) derived an inhalation unit risk for cancer of 2.6×10^{-7} per $\mu\text{g}/\text{m}^3$ for tetrachloroethylene.

The basis for the MRLs or other guidance values for these five chemicals, as well as other sensitive effects, are summarized in Table 2. As can be seen, while there is no single endpoint that is a sensitive effect of all components of the mixture, there are several endpoints that are of concern for two or more chemicals in the mixture; those endpoints are the focus of the component-based mixture assessments in Chapters 2 and 3. No pertinent studies of the toxicity or interactions of, or of PBPK models for the complete mixture, or any of the quaternary or tertiary submixtures were located. Only limited toxicological data are available for the individual component binary mixtures. ATSDR toxicological profiles for formaldehyde (ATSDR 2010), methylene chloride (ATSDR 2000), carbon monoxide (ATSDR 2012), and tetrachloroethylene (ATSDR 2019); the EPA Integrated Risk Information System (IRIS) assessment for formaldehyde (EPA 2024d), the ATSDR systematic evidence map (SEM) for methylene chloride (ATSDR 2022), and literature reviews and meta-analyses for carbon monoxide and nitrogen dioxide are the primary source of information presented in the appendices concerning the toxicokinetics, health effects, mechanisms of action, and health-based guidance values for these chemicals.

Table 2. Potential Health Effects of Concern for Intermediate- and Chronic-Duration Inhalation Exposure to the Mixture Carbon Monoxide, Formaldehyde, Methylene Chloride, Nitrogen Dioxide, and Tetrachloroethylene^{a,b}

Endpoint	Carbon monoxide	Formaldehyde	Methylene chloride	Nitrogen dioxide	Tetrachloroethylene
Hematological	X		X		
Cardiovascular	X				
Neurological	X		X		X
Respiratory		X	X	X	
Hepatic			X		X
Renal					X
Developmental	X	X			
Immunological				X	
Cancer		X	X		X

^aSee Appendices A, B, C, D, and E for additional information on health-based guidance values and health effects.

^bThe basis for the MRLs or health assessment approaches are bolded (no MRLs have been set for carbon monoxide or nitrogen dioxide); other sensitive effects are listed in regular typeface.

2. Joint Toxic Action Data for the Mixture of Concern and Binary Mixtures of Components

2.1 Mixture of Concern

Toxicological data or PBPK models were not available for the complete mixture of concern or for any of the three- or four-component submixtures.

2.2 Binary Mixtures of Components

Toxicological data were available only for the binary mixture of carbon monoxide and methylene chloride; toxicological data on the other binary submixtures were not located. PBPK models for methylene chloride generally contain components describing the metabolism of methylene chloride to formaldehyde and carbon monoxide, but to date have not included estimations of co-exposure to either of these compounds.

In the following sections on the binary mixtures, the studies that focus on more relevant toxic endpoints are discussed first, with priority given to those conducted by simultaneous, longer-term inhalation exposure in mammals, followed by studies of less-relevant endpoints (e.g., acute lethal effects), and then studies of chemical interactions and of effects on tissue distribution or metabolism. At the end of each binary mixture section, the experimental results that may be used to support conclusions regarding joint toxic action are summarized in tables. For each listed endpoint and study, the tables present a conclusion regarding the direction of interaction for the influence of each chemical on the toxicity of the other. These conclusions include: additive (dose addition, response addition, or no apparent influence), greater than additive (synergism or potentiation), less than additive (antagonism, inhibition, or masking), or indeterminate (ambiguous, conflicting, or no data).

2.2.1 Carbon Monoxide and Formaldehyde

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of carbon monoxide and formaldehyde. No PBPK models for co-exposure to carbon monoxide and formaldehyde were located. From the available data, carbon monoxide and formaldehyde only share developmental effects as a shared target of toxicity. The present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of carbon monoxide and formaldehyde.

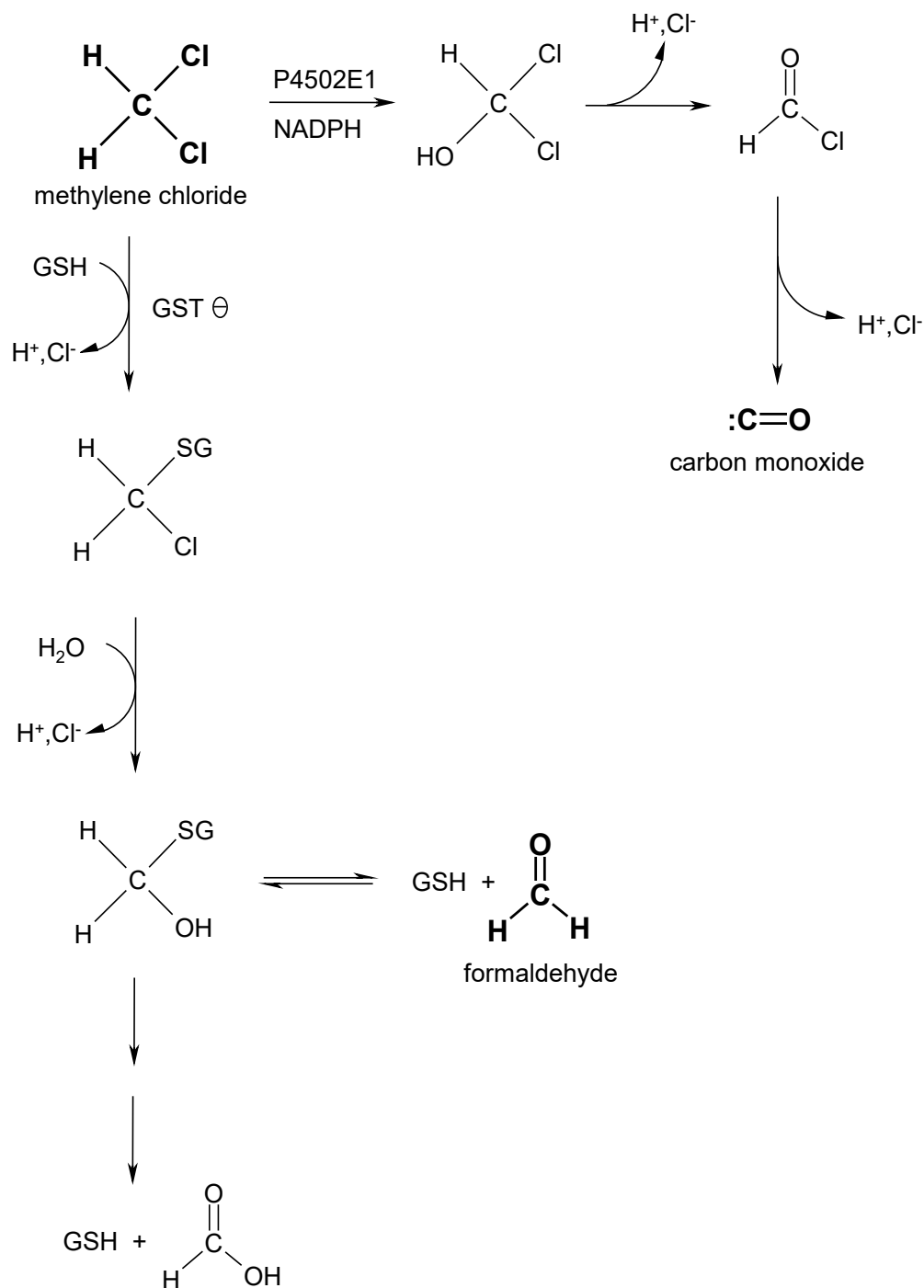
Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.2 Carbon Monoxide and Methylene Chloride

It is well-established that methylene chloride is metabolized by cytochrome P450 (CYP) isozyme 2E1 (CYP2E1) to carbon monoxide, primarily in the liver (see Appendix C and ATSDR 1999). The metabolism of methylene chloride is diagrammed in Figure 1. At low exposure levels, metabolism is primarily via the CYP pathway, resulting in carbon monoxide formation. Numerous studies have demonstrated the formation of COHb following exposure to methylene chloride in humans (Amsel et al. 2001; DiVincenzo and Kaplan 1981; Duenas et al. 2000; Fagin et al. 1980; Stevenson et al. 1978) and animals (DiVincenzo and Hamilton 1975; Kurppa et al. 1981; Rodkey and Collison 1977; Stevens et al. 1980). Available PBPK models for methylene chloride incorporate metabolism to carbon monoxide into the model structure; however, models to date have not incorporated the ability to evaluate co-exposures with carbon monoxide.

No studies examining the joint effects of co-exposure to carbon monoxide and methylene chloride in humans were located. Similarly, no studies of exposure of carbon monoxide prior to methylene chloride, or of methylene chloride exposure prior to carbon monoxide exposure, were located. An acute-duration study in humans comparing the neurological effects of methylene chloride with those of equivalent concentrations of carbon monoxide, in terms of blood COHb levels, found a more pronounced performance deficit for methylene chloride (Winneke 1981). Two distinct toxic actions were identified for methylene chloride neurological effects, nonspecific narcotic action (not associated with COHb) and COHb-induced hypoxia. Thus, the neurological effects of methylene chloride are only partially mediated by carbon monoxide formation, suggesting a potential for response addition for co-exposures of the two compounds for neurological effects mediated via elevated COHb levels. Winneke (1981) also suggested that there may be interaction between the two neurological mechanisms (narcotic, hypoxic) associated with methylene chloride; however, this hypothesis was not directly tested. The Winneke (1981) study is further limited by short duration (≤ 4.0 hours), evaluation of exposure levels much higher than environmental levels (e.g., ≥ 300 ppm for methylene chloride and ≥ 50 ppm for carbon monoxide), an unclear number of subjects per group, and a lack of quantitative presentation of results. These limitations decrease the usefulness of this study to evaluate potential interactions between these two compounds.

Figure 1. Metabolism of Methylene Chloride



GSH = glutathione; NADPH = reduced nicotinamide-adenine dinucleotide phosphate; P4502E1 = cytochrome P450 enzyme involved in xenobiotic metabolism

Source: Ahmed et al. 1980

Kurppa et al. (1981) exposed groups of male Wistar rats to 100 ppm carbon monoxide, 1,000 ppm methylene chloride, or both for 3 hours. Exposure to carbon monoxide alone resulted in a mean 8.8% blood COHb and exposure to methylene chloride alone resulted in a mean 6.2% blood COHb. Combined exposure to 100 ppm carbon monoxide and 1,000 ppm methylene chloride resulted in 14.6% blood COHb; thus, the simultaneous exposure is consistent with response-additive effects of blood COHb levels (see Table 3). No combined effects were noted on induction of CYP or ethoxycoumarin O-deethylase activities; effects on other endpoints were not reported. It is noteworthy that concentrations used in this study are much higher than those that are likely to be found in the environment or in homes. Other animal studies evaluating the effects of co-exposure to methylene chloride and carbon monoxide were not located. The Kurppa et al. (1981) study is limited by short duration (3 hours), evaluation of exposure levels much higher than environmental levels, and inclusion of only single concentrations for each compound either individually or in combination (precluding the evaluation of the dose-response nature of the potential interaction between these two compounds).

Table 3. Summary of Available Data on the Joint Effects of Simultaneous Exposure to Methylene Chloride and Carbon Monoxide

Duration	Endpoint	Results			Conclusions	Reference
		Greater than additive	Additive/no apparent influence	Less than additive		
Acute	Hematological		100 ppm carbon monoxide + 1,000 ppm methylene chloride (rats)		Response addition (rat blood COHb levels)	Kurppa et al. (1981)

COHb = carboxyhemoglobin

The metabolism of methylene chloride to carbon monoxide is complex, particularly when considering the possibility of co-exposure to carbon monoxide itself. Carbon monoxide absorption is driven by a concentration gradient, such that increased levels of blood COHb result in a saturation of absorption (see Appendix A); this would suggest that absorption of carbon monoxide following long-term exposure would be decreased during co-exposure to methylene chloride. However, methylene chloride interacts with the subunits of hemoglobin in a manner not completely understood, shifting both the oxygen and carbon monoxide association curves to the right, representing changes in affinity of these molecules for the heme iron (Harkey et al. 1979). Beyond the Kurppa et al. (1981) study, no studies were located that evaluated the effects of co-exposure to carbon monoxide and methylene chloride, so the potential impact of methylene chloride-derived carbon monoxide on the effects of inhaled carbon monoxide (and vice versa) has not been definitively evaluated.

2.2.3 Carbon Monoxide and Nitrogen Dioxide

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of carbon monoxide and nitrogen dioxide. No PBPK models for co-exposure to carbon monoxide and nitrogen dioxide were located. Several studies have reported simultaneous elevations of carbon monoxide and nitrogen dioxide in the home or in public environments (Cornforth et al. 1998; Lee et al. 1994); however, these studies have not evaluated the effects of exposure to these gases, individually or in combination, on human health. From the available data, carbon monoxide and nitrogen dioxide do not appear to have any sensitive shared targets of toxicity. Similarly, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of carbon monoxide and nitrogen dioxide.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.4 Carbon Monoxide and Tetrachloroethylene

No *in vivo* studies were located regarding possible joint toxic actions of carbon monoxide and tetrachloroethylene. One study demonstrated that oxidation of tetrachloroethylene by hydroxyl radicals (in a photochemical reaction chamber) may result in the formation of carbon monoxide (Itoh et al. 1994), but the extent to which this occurs *in vivo*, and the possible effects that it might have on the toxicities of carbon monoxide and/or tetrachloroethylene, has not been evaluated. The shared targets of toxicity for carbon monoxide and tetrachloroethylene include neurological effects, but studies evaluating the joint effects of the chemicals on either of these endpoints are not available. The present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of carbon monoxide and tetrachloroethylene.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.5 Formaldehyde and Methylene Chloride

As depicted in Figure 1 above and briefly described in Appendix C, a major metabolic pathway for methylene chloride involves conjugation to glutathione (GSH), catalyzed by glutathione S-transferase- θ (GST- θ). The resulting compound, chloromethyl-S-glutathione, can spontaneously react with water to form hydroxymethyl-S-glutathione, which can spontaneously degrade to formaldehyde and GSH or be

further metabolized to formate and GSH (see Figure 1). Numerous studies have suggested that the carcinogenic effects of methylene chloride noted in mice are the result of GST- θ -mediated metabolism to formaldehyde and subsequent interaction with cellular macromolecules, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Support for this hypothesis includes studies demonstrating the formation of DNA-protein crosslinks in mice, but not in hamsters, following acute-duration *in vivo* exposure to methylene chloride (Casanova et al. 1992), and demonstration that mammalian cells with higher levels of GST- θ exposed to methylene chloride generated larger numbers of DNA-protein crosslinks and RNA-formaldehyde adducts (Casanova et al. 1997). In human cells, only those cells that express the theta-glutathione-S-transferase (GSTT1-1) gene (the product of which is the GST- θ enzyme) generate DNA-protein crosslinks and RNA-formaldehyde adducts in response to methylene chloride exposure; levels in cells without the gene are not different from background (Casanova et al. 1997). Similarly, formaldehyde production was not detected in human erythrocytes (Hallier et al. 1994). El-Masri et al. (1999) developed PBPK models in humans and mice to evaluate the influence of the GSTT1-1 polymorphism on the risk of carcinogenesis from methylene chloride exposure.

While the metabolism of methylene chloride to formaldehyde is well-established, studies of the effect of co-exposure to formaldehyde and methylene chloride, either *in vivo* or *in vitro*, were not located. Numerous PBPK models exist that describe the metabolism of methylene chloride to formaldehyde; however, none of these models to date also incorporated simulations of the effects of co-exposure. Given that both formaldehyde and methylene chloride are believed to cause tumors by the reaction of formaldehyde with DNA and/or RNA, an additive association between the carcinogenicity of the two is likely. Since formaldehyde is the proximal toxicant, at the cellular level, this interaction would be dose additive. However, since the primary tissue targets for cancer differ between the compounds, at the organism level, the interaction would be response additive. Available mechanistic data are not sufficient to determine whether joint actions of methylene chloride and formaldehyde on respiratory effects will occur.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.6 Formaldehyde and Nitrogen Dioxide

Maroziene and Grazuleviciene (2002) conducted an epidemiological study of residential air pollution. The only two pollutants measured were formaldehyde and nitrogen dioxide. Multivariate logistic regression was used to estimate the effect each separate pollutant would have on low birth weight and

premature birth. An increased risk of low birth weight was associated with formaldehyde exposure in the first trimester (odds ratio [OR] 2.20, 95% confidence interval [CI] 1.00–4.85) and prematurity was related to first trimester exposure to nitrogen dioxide (OR 1.67, 95% CI 1.28–2.18). When both chemicals were entered into the model together, the estimated effects did not change considerably except that the effect of exposure to nitrogen dioxide in the second trimester presented a risk of prematurity. The Maroziene and Grazuleviciene (2002) study is limited by the use of ecological monitoring data rather than personal exposure monitoring, the exclusion of other routinely monitored air pollutants (sulfur dioxide and total suspended particles) available in the ecological monitoring dataset from the analyses, and the lack of adjustment for other chemical exposures aside from smoking.

No *in vivo* or *in vitro* laboratory studies were located regarding possible joint toxic actions of formaldehyde and nitrogen dioxide. No PBPK models for co-exposure to formaldehyde and nitrogen dioxide were located. Formaldehyde and nitrogen dioxide share the respiratory system as a common site of toxicity, but studies evaluating the effects of exposure to both chemicals on respiratory endpoints are not available.

The present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of formaldehyde and nitrogen dioxide.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.7 Formaldehyde and Tetrachloroethylene

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of formaldehyde and tetrachloroethylene. No PBPK models for co-exposure to formaldehyde and tetrachloroethylene were located. From the available data, formaldehyde and tetrachloroethylene do not appear to have any sensitive shared noncancer targets of toxicity. Both compounds have been shown to be tumorigenic; however, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of formaldehyde and tetrachloroethylene.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.8 Methylene Chloride and Nitrogen Dioxide

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of methylene chloride and nitrogen dioxide. No PBPK models for co-exposure to methylene chloride and nitrogen dioxide were located. The available data indicate that methylene chloride and nitrogen dioxide are both capable of eliciting effects on the respiratory system, but no studies have evaluated the effect of co-exposure on respiratory endpoints. Similarly, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of methylene chloride and nitrogen dioxide.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.9 Methylene Chloride and Tetrachloroethylene

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of methylene chloride and tetrachloroethylene. No PBPK models for co-exposure to methylene chloride and tetrachloroethylene were located. The available data indicate that methylene chloride and tetrachloroethylene are both capable of eliciting neurological and tumorigenic effects, but no studies have evaluated the effect of co-exposure on these endpoints. Similarly, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of methylene chloride and tetrachloroethylene. As both compounds are metabolized by CYP enzymes, it is possible that metabolism will be a possible point of interaction for the two compounds. However, each compound is metabolized primarily by a different CYP isozyme (CYP2E1 for methylene chloride, CYP2B1/2 for tetrachloroethylene), metabolic interactions are unlikely at exposure levels normally found in the environment.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.2.10 Nitrogen Dioxide and Tetrachloroethylene

No *in vivo* or *in vitro* studies were located regarding possible joint toxic actions of nitrogen dioxide and tetrachloroethylene. No PBPK models for co-exposure to nitrogen dioxide and tetrachloroethylene were located. From the available data, nitrogen dioxide and tetrachloroethylene do not appear to have any sensitive shared targets of toxicity. Similarly, the present understanding of the mechanisms of action of these compounds does not suggest any potential joint actions of nitrogen dioxide and tetrachloroethylene.

Because no *in vivo* studies of interactions were located for this pair, there are no summary tables.

2.3 Relevance of the Joint Toxic Action Data and Approaches to Public Health

The carbon monoxide, formaldehyde, methylene chloride, nitrogen dioxide, and tetrachloroethylene mixture is of concern because these chemicals, either alone or in combination, may be found in the home. The exposure route is primarily inhalation, and exposure durations are primarily intermediate to chronic. No epidemiological or toxicological studies of the complete mixture or for any of the three- or four-component submixtures are available. Similarly, no PBPK models are available for the complete mixture or for any of the three- or four-component submixtures. Some information and studies are available for binary mixtures of the components, but they are not adequate to support a quantitative assessment of interactions. Therefore, the WOE approach is appropriate (ATSDR 2001, 2018) to predict the potential impact of interactions (i.e., deviation from additivity). This approach involves determining, for each binary mixture, the WOE for the influence of one component on the toxicity of the other, and vice versa.

The binary weight-of-evidence (BINWOE) classification scheme is summarized in Table 4. This table gives a general idea of the approach, which rates confidence in the predicted direction of interaction according to the quality of the data. The direction of interaction, or lack thereof, is predicted from the available mechanistic and toxicological data. The quality of the data, as it pertains to prediction of direction of interaction, is classified by the main data quality factors for *mechanistic understanding* and *toxicological significance*. If concerns regarding the applicability of the data are not completely addressed under the main data quality factors, they can be addressed by the use of the *modifiers*. More detailed guidance is given in ATSDR guidance documents (ATSDR 2001, 2018). Rationales for the BINWOE determinations are presented in Tables 6–17 at the end of this section. The BINWOE determinations are presented for the binary mixtures in the same order as these mixtures were considered in Section 2.2.

Table 4. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions

Classification	
Direction of Interaction	
=	Additive
>	Greater than additive
<	Less than additive
?	Indeterminate
Quality of the Data	
I.	Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.
II.	Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur has not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.
III.	Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.
A.	The toxicological significance of the interaction has been directly demonstrated.
B.	The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.
C.	The toxicological significance of the interaction is unclear.
1.	Anticipated exposure duration and sequence.
2.	Different exposure duration or sequence.
a.	<i>In vivo</i> data
b.	<i>In vitro</i> data
i.	Anticipated route of exposure
ii.	Different route of exposure

There are 10 unique binary pairs of chemicals in this mixture of 5 chemicals. For the following seven pairs of chemicals, no pertinent interaction data were found, and understanding of mechanisms of action is too incomplete to make projections of joint toxic actions:

- carbon monoxide and formaldehyde
- carbon monoxide and nitrogen dioxide
- carbon monoxide and tetrachloroethylene
- formaldehyde and tetrachloroethylene
- methylene chloride and nitrogen dioxide
- methylene chloride and tetrachloroethylene
- nitrogen dioxide and tetrachloroethylene

Evidence of varying quality and quantity is available supporting projections of additive joint toxic action for the following three pairs of chemicals (summarized in Table 5, with details provided in tables referenced below):

- carbon monoxide and methylene chloride (Tables 6 and 7)
- formaldehyde and methylene chloride (Tables 10 and 11)
- formaldehyde and nitrogen dioxide (Tables 12 and 13)

In summary, there are no data to suggest that non-additive interactions occur for any of the component pairs of the mixture, although it should be emphasized that studies designed to identify and characterize mode of joint toxic action of the components are, for the most part, unavailable.

Table 5. Matrix of BINWOE Determinations for Intermediate- or Chronic-Duration Simultaneous Exposure to Chemicals of Concern^a

		ON THE TOXICITY OF				
		Carbon monoxide	Formaldehyde	Methylene chloride	Nitrogen dioxide	Tetrachloro-ethylene
EFFECT OF	Carbon monoxide		?	IA2 h,n ? r,c	?	?
	Formaldehyde	?		IB1 c IIC1 r ? h,n	IIIC2 r	?
	Methylene chloride	IA2 h IIB2 n,d	IB1 r,c		?	?
	Nitrogen dioxide	?	IIIC2 r ? c	?		?
	Tetrachloroethylene	?	?	?	?	

^aThe BINWOE scheme was explained in Table 4 (ATSDR 2001, 2018). Data supporting the BINWOE determinations are presented in Tables 6–17; some BINWOEs are based on results from high-level, acute-duration exposure studies. Additivity is likely at low-level exposures; dose additivity is assumed for noncancer effects and response additivity is assumed for carcinogenic effects.

BINWOE = binary weight-of-evidence; c = carcinogenic; d = developmental; h = hematological; n = neurological; r = respiratory

Table 6. Effect of Carbon Monoxide on Methylene Chloride

BINWOE: IA2 for hematological effects
BINWOE: IA2 for neurological effects
BINWOE: ? for respiratory effects
BINWOE: ? for hepatic effects
BINWOE: ? for cancer

Direction of Interaction – The metabolism of methylene chloride to carbon monoxide is well-documented. Acute-duration rat data from Kurppa et al. (1981) indicate a response-additive effect of co-exposure on hematological endpoints. Similarly, Winneke (1981) suggested the potential for a response-additive effect of acute neurological effects for the two compounds in humans. Data suggesting possible interactions on respiratory effects, hepatic effects, or cancer are not available.

Mechanistic Understanding – The mechanism by which methylene chloride elicits effects on the hematological and nervous systems is believed to at least partially involve the metabolism of methylene chloride to carbon monoxide by CYP2E1. A human study comparing the acute neurological effects of methylene chloride with those of equivalent concentrations of carbon monoxide, in terms of blood COHb levels, found a more pronounced performance deficit for methylene chloride (Winneke 1981). Therefore, two distinct neurological mechanisms for methylene chloride were identified: nonspecific narcosis and COHb hypoxia. Based on this, the neurological effects of methylene chloride are partially mediated by carbon monoxide formation, suggesting the potential for a response-additive effect for co-exposures of the two compounds. The hematological effects of methylene chloride generally involve the formation of COHb; numerous studies in humans and animals have demonstrated the formation of COHb following methylene chloride exposure (see ATSDR 1999 and Appendix C). COHb-related effects are therefore expected to be additive, based on total blood COHb levels formed by the two compounds. This has been verified for acute-duration exposures by Kurppa et al. (1981), who found additive COHb levels following joint exposures to methylene chloride and carbon monoxide in rats. The mechanisms by which methylene chloride causes respiratory and carcinogenic effects have not been conclusively established, but as these effects have not generally been established as sensitive effects of carbon monoxide exposure, they are unlikely to be the result of metabolism of methylene chloride to carbon monoxide. A rating of I reflects the strong mechanistic understanding of the nature of the interaction.

Toxicological Significance – Only one study has directly evaluated the effects of co-exposure to carbon monoxide and methylene chloride. Kurppa et al. (1981) found that co-exposure to 100 ppm carbon monoxide and 1,000 ppm methylene chloride for a single 3-hour period resulted in response addition of blood COHb formation in rats, based on measurements from single exposure to the chemicals (8.8% for CO, 6.2% for methylene chloride, and 14.6% for combined). Thus, for acute-duration exposures to mixtures of the two, COHb formation appears to be additive. Interaction data are not available for longer combined exposures to carbon monoxide and methylene chloride. A rating of A reflects that the significance of the interaction has been directly demonstrated.

Modifying Factors – The rating of “2” was used to reflect that available data on combined exposure were from an acute-duration exposure, rather than one of longer duration.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 7. Effect of Methylene Chloride on Carbon Monoxide

BINWOE: IA2 for hematological effects
BINWOE: IIB2 for neurological effects
BINWOE: IIB2 for developmental effects

Direction of Interaction – The metabolism of methylene chloride to carbon monoxide is well-documented. Acute-duration data from Kurppa et al. (1981) indicate a response-additive effect of co-exposure on COHb formation in rats, which is the primary mechanism of the toxic effects of carbon monoxide. It is therefore anticipated that for other sensitive effects of carbon monoxide, methylene chloride exposure will result in response addition, based on the formation of endogenous carbon monoxide.

Mechanistic Understanding – Carbon monoxide's toxicity is primarily the result of the formation of COHb and a resulting decrease in the oxygen-carrying capacity of the blood. Metabolism of methylene chloride to carbon monoxide by CYP, and the resulting formation of COHb, is well-documented (see Appendix C and ATSDR 1999). A study by Kurppa et al. (1981) demonstrated a response-additive effect of acute-duration co-exposure to methylene chloride and carbon monoxide on blood COHb formation in rats. While it therefore seems reasonable to assume a response-additive effect of co-exposure based on COHb formation, the effects of combined exposure have received only limited study and available PBPK models for methylene chloride have not been adapted to model co-exposure to the compounds. A rating of I reflects the strong mechanistic understanding of the nature of the interaction for hematological effects, while a rating of II reflects that the mechanism of interaction can be inferred for other effects.

Toxicological Significance – Only one study has directly evaluated the effects of co-exposure to methylene chloride and carbon monoxide. Kurppa et al. (1981) found that co-exposure to 100 ppm carbon monoxide and 1,000 ppm methylene chloride for a single 3-hour period resulted in response addition of blood COHb formation in rats, based on measurements from single exposure to the chemicals (8.8% for CO, 6.2% for methylene chloride, and 14.6% for combined). Thus, for acute-duration exposures to mixtures of the two, COHb formation appears to be additive. Interaction data are not available for longer combined exposures to methylene chloride and carbon monoxide. A rating of A reflects that the significance of the interaction has been directly demonstrated for hematological effects, while a rating of B indicates that such interactions can be inferred but have not been demonstrated.

Modifying Factors – The rating of "2" was used to reflect that available data on combined exposure were from an acute-duration exposure, rather than one of longer duration.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 8. Effect of Carbon Monoxide on Tetrachloroethylene

BINWOE: ? for neurological effects
BINWOE: ? for hepatic effects
BINWOE: ? for cancer

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with carbon monoxide will influence tetrachloroethylene toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between carbon monoxide and tetrachloroethylene.

Mechanistic Understanding – The primary shared target of toxicity for carbon monoxide and tetrachloroethylene is effects on the neurological system. Both compounds have been shown to cause neurological effects following high-dose exposures. However, the mechanisms by which tetrachloroethylene causes neurological effects have not been fully established but likely involve disruption of neuronal membrane structure and function. Therefore, available information is not sufficient to predict whether increased tissue hypoxia, the putative mechanism by which carbon monoxide-induced effects are produced, would have an impact on tetrachloroethylene-induced neurological effects. Similarly, it is not known whether carbon monoxide could influence other endpoints affected by tetrachloroethylene, namely hepatic effects, or cancer.

Toxicological Significance – Relevant interaction data on pertinent health effects with simultaneous inhalation exposure were not located. No studies were located in which pretreatment with carbon monoxide prior to tetrachloroethylene exposure was examined.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 9. Effect of Tetrachloroethylene on Carbon Monoxide

BINWOE: ? for hematological effects
BINWOE: ? for neurological effects
BINWOE: ? for developmental effects

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with tetrachloroethylene will influence carbon monoxide toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between tetrachloroethylene and carbon monoxide.

Mechanistic Understanding – The primary shared target of toxicity for tetrachloroethylene and carbon monoxide is effects on the neurological system. Both compounds have been shown to cause neurotoxicity following high-dose exposures. However, the mechanisms by which tetrachloroethylene causes neurotoxicity has not been fully established but likely involve disruption of neuronal membrane structure and function. Therefore, available information is not sufficient to predict whether increased tissue hypoxia, the putative mechanism by which carbon monoxide-induced effects are produced, would be influenced by tetrachloroethylene-induced neurotoxicity. Similarly, it is not known whether tetrachloroethylene could influence other sensitive targets of toxicity affected by carbon monoxide, namely hematological effects or developmental effects.

Toxicological Significance – Relevant interaction data on pertinent health effects with simultaneous inhalation exposure were not located. No studies were located in which pretreatment with tetrachloroethylene prior to carbon monoxide exposure was examined.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 10. Effect of Formaldehyde on Methylene Chloride

BINWOE: ? for hematological effects
BINWOE: ? for neurological effects
BINWOE: IIC for respiratory effects
BINWOE: ? for hepatic effects
BINWOE: IB for cancer

Direction of Interaction – Studies have strongly implicated the formation of formaldehyde in the carcinogenic effects of methylene chloride (Graves and Green 1996; Graves et al. 1994a, 1994b, 1995, 1996); the direction of interaction is therefore expected to be additive. Similarly, metabolism to formaldehyde could partially explain the respiratory effects seen following high levels of methylene chloride exposure, implicating a dose-additive effect of combined exposure. However, other effects of methylene chloride (e.g., hematological, neurological, and hepatic effects) are not believed to be the result of metabolism to formaldehyde. The direction of interaction for these effects cannot be determined from available data.

Mechanistic Understanding – The metabolism of methylene chloride to formaldehyde has been well-established (see Appendix C and ATSDR 1999). The carcinogenic effects of methylene chloride are believed to be the result of metabolism to formaldehyde and subsequent nucleophilic attack of DNA (Graves and Green 1996; Graves et al. 1994a, 1994b, 1995, 1996). As such, additional exposure to formaldehyde, at the cellular level, is likely to result in additive joint toxicity. This is reflected by a rating of “I” for cancer. High-dose exposures to methylene chloride produce pulmonary effects (see Appendix C). The mechanism(s) for respiratory effects have not been fully elucidated (see Appendix C); however, metabolism to formaldehyde may contribute to observed effects. Since the association between respiratory effects and formaldehyde formation is not as strong as the association between carcinogenesis and formaldehyde formation following methylene chloride exposure, a rating of “II” for respiratory effects was assigned. The hematological and neurological effects of methylene chloride are believed to be at least partially the result of CYP-mediated metabolism to carbon monoxide, and as such are not likely to be appreciably affected by co-exposure to formaldehyde.

Toxicological Significance – Studies evaluating combined exposure to formaldehyde and methylene chloride, or describing pretreatment with formaldehyde prior to methylene chloride exposure, were not located. The inference is stronger for cancer effects, and warranted a “B” rating, while for respiratory effects the significance is less clear, and received a “C.”

Additional Uncertainties – Formaldehyde primarily exerts its effects at the portal of entry (e.g., nasal tissues). Therefore, effects (including any potential interactions with other chemicals) at distant sites may occur only when the capacities for local metabolism and disposition of formaldehyde are exceeded.

Table 11. Effect of Methylene Chloride on Formaldehyde

BINWOE: IB for respiratory effects
BINWOE: IB for developmental effects
BINWOE: IB for cancer

Direction of Interaction – The metabolism of methylene chloride to formaldehyde is well-described in the literature (see Appendix C and ATSDR 1999). The formation of intracellular formaldehyde is expected to result in dose-additive effects when combined with exogenous formaldehyde exposure.

Mechanistic Understanding – The majority of the effects of formaldehyde are due to the reactive nature of the molecule. Respiratory irritation is the primary effect noted following inhalation exposure to formaldehyde; formaldehyde may be interacting with cellular membranes or entering the cells and reacting with intracellular molecules. At high concentrations, formaldehyde may also produce developmental effects. Metabolism of methylene chloride to formaldehyde would result in a higher concentration of intracellular formaldehyde, implying an additive joint toxicity. Similarly, formaldehyde's carcinogenic effects are believed to be the result of the reaction of formaldehyde with DNA and/or RNA (see Appendix B). Methylene chloride-derived formaldehyde has been shown to form similar products with DNA and RNA. Both of these possible interactions received a rating of I.

Toxicological Significance – Studies evaluating combined exposure to methylene chloride and formaldehyde, or describing pretreatment with methylene chloride prior to formaldehyde exposure, were not located. For both endpoints, the association can be strongly inferred but has not been directly demonstrated; ratings of "B" were assigned.

Additional Uncertainties – While methylene chloride is metabolized into formaldehyde, its toxic effects are primarily distal to the portal of entry, suggesting potentially different cellular targets between methylene chloride and formaldehyde. However, nasal metaplasia has been reported in rats following chronic exposure to methylene chloride (see Appendix C), supporting potential for interactions with long-term exposure. Additionally, formaldehyde effects (including any potential interactions with other chemicals) at distant sites may occur when the capacities for local metabolism and disposition of formaldehyde are exceeded.

Table 12. Effect of Formaldehyde on Nitrogen Dioxide**BINWOE: IIC2 for respiratory effects**

Direction of Interaction – Based on a mutually shared mechanism of respiratory irritation, dose-additive effects of co-exposure are predicted.

Mechanistic Understanding – Nitrogen dioxide's effects on the respiratory system are believed to be primarily due to irritation along the portal of entry, owing to the reactive nature of the compound. The respiratory effects of formaldehyde are similarly believed to be due to irritation along the portal of entry. Dose additivity resulting from mutual respiratory irritation therefore seems reasonable. However, data supporting this hypothesis are not available. A confidence rating of "III" was therefore assigned.

Toxicological Significance – Studies evaluating combined exposure to formaldehyde and nitrogen dioxide, or describing pretreatment with formaldehyde prior to nitrogen dioxide exposure, were not located. A rating of "C" reflecting an unclear significance was assigned.

Modifying Factor – Because the available studies have not evaluated longer-term exposures, a rating of "2" was used for different exposure duration.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 13. Effect of Nitrogen Dioxide on Formaldehyde

BINWOE: IIC2 for respiratory effects
BINWOE: ? for developmental effects
BINWOE: ? for cancer

Direction of Interaction – Based on a mutually shared mechanism of respiratory irritation, dose-additive effects of co-exposure to formaldehyde and nitrogen dioxide are predicted. Available data are inadequate to determine what effect, if any, co-exposure to nitrogen dioxide will have on the developmental or carcinogenic effects of formaldehyde.

Mechanistic Understanding – Formaldehyde's effects on the respiratory system are believed to be primarily due to irritation along the portal of entry, owing to the reactive nature of the compound. The respiratory effects of nitrogen dioxide are similarly believed to be due to irritation along the portal of entry. Dose additivity resulting from mutual respiratory irritation therefore seems reasonable. However, data supporting this hypothesis are not available. A confidence rating of "III" was therefore assigned. Current understanding of the mechanisms of nitrogen dioxide and the developmental and carcinogenic effects of formaldehyde is not sufficient to predict the direction or extent of possible interactions on developmental or carcinogenic endpoints.

Toxicological Significance – Studies evaluating combined exposure to nitrogen dioxide and formaldehyde, or describing pretreatment with nitrogen dioxide prior to formaldehyde exposure, were not located. A rating of "C" reflecting an unclear significance was assigned.

Modifying Factor – Because the available studies have not evaluated longer-term exposures, a rating of "2" was used for different exposure duration.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 14. Effect of Methylene Chloride on Nitrogen Dioxide**BINWOE: ? for respiratory effects**

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with methylene chloride will influence nitrogen dioxide toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between methylene chloride and nitrogen dioxide.

Mechanistic Understanding – The primary shared target of toxicity for methylene chloride and nitrogen dioxide is effects on the respiratory system. While the mechanism of nitrogen dioxide's respiratory effects is thought to involve a direct reaction with cells along the respiratory tract, the mechanism of respiratory effects of methylene chloride is more complex. Available data are not sufficient to indicate possible effects of methylene chloride on nitrogen dioxide-induced respiratory effects.

Toxicological Significance – Studies evaluating combined exposure to methylene chloride and nitrogen dioxide, or describing pretreatment with methylene chloride prior to nitrogen dioxide exposure, were not located.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 15. Effect of Nitrogen Dioxide on Methylene Chloride

BINWOE: ? for hematological effects
BINWOE: ? for neurological effects
BINWOE: ? for respiratory effects
BINWOE: ? for hepatic effects
BINWOE: ? for cancer

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with nitrogen dioxide will influence methylene chloride toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between nitrogen dioxide and methylene chloride.

Mechanistic Understanding – The primary shared target of toxicity for methylene chloride and nitrogen dioxide is effects on the respiratory system. While the mechanism of nitrogen dioxide's respiratory effects is thought to involve a direct reaction with cells along the respiratory tract, the mechanism of respiratory effects of methylene chloride is more complex. Available data are not sufficient to indicate possible effects of nitrogen dioxide on methylene chloride-induced respiratory effects. Similarly, mechanistic data are not sufficient to allow for predictions of the possible effects of co-exposure to nitrogen dioxide on the hematological, neurological, hepatic, or carcinogenic effects of methylene chloride.

Toxicological Significance – Studies evaluating combined exposure to nitrogen dioxide and methylene chloride, or describing pretreatment with nitrogen dioxide prior to methylene chloride exposure, were not located.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 16. Effect of Methylene Chloride on Tetrachloroethylene

BINWOE: ? for neurological effects
BINWOE: ? for hepatic effects
BINWOE: ? for cancer

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with methylene chloride will influence tetrachloroethylene toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between methylene chloride and tetrachloroethylene.

Mechanistic Understanding – Sensitive shared targets of toxicity for methylene chloride and tetrachloroethylene include neurological and hepatic effects. However, the mechanisms by which each causes effects on neurological endpoints are not clearly understood and no prediction as to the direction or extent of possible interactions can be made. Similarly, mechanisms by which the two compounds result in hepatic changes are not completely understood but are thought to involve metabolism to reactive intermediates. As both compounds are metabolized by CYP enzymes, it is possible that metabolism will be a possible point of interaction for the two compounds. However, each compound is metabolized primarily by a different CYP isozyme (CYP2E1 for methylene chloride and CYP2B1/2 for tetrachloroethylene), metabolic interactions are unlikely at exposure levels normally found in the environment. Understanding the mechanisms of tetrachloroethylene toxicity is not sufficient to allow for the prediction of possible effects of methylene chloride on other targets of tetrachloroethylene toxicity.

Toxicological Significance – Studies evaluating combined exposure to methylene chloride and tetrachloroethylene, or describing pretreatment with methylene chloride prior to tetrachloroethylene exposure, were not located.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

Table 17. Effect of Tetrachloroethylene on Methylene Chloride

BINWOE: ? for hematological effects
BINWOE: ? for neurological effects
BINWOE: ? for respiratory effects
BINWOE: ? for hepatic effects
BINWOE: ? for cancer

Direction of Interaction – The direction of the interaction cannot be predicted in the absence of: (1) pertinent interaction data; (2) information clearly indicating that pharmacokinetic interactions with tetrachloroethylene will influence methylene chloride toxicity; or (3) mechanistic understanding leading to an unambiguous projection of interactions between tetrachloroethylene and methylene chloride.

Mechanistic Understanding – Sensitive shared targets of toxicity for methylene chloride and tetrachloroethylene include neurological and hepatic effects. However, the mechanisms by which each causes effects on neurological endpoints are not clearly understood and no prediction as to the direction or extent of possible interactions can be made. Similarly, mechanisms by which the two compounds result in hepatic changes are not completely understood but are thought to involve metabolism to reactive intermediates. As both compounds are metabolized by CYP enzymes, it is possible that metabolism will be a possible point of interaction for the two compounds. However, each compound is metabolized primarily by a different CYP isozyme (CYP2E1 for methylene chloride and CYP2B1/2 for tetrachloroethylene), metabolic interactions are unlikely at exposure levels normally found in the environment. Understanding the mechanisms of methylene chloride toxicity is not sufficient to allow for the prediction of possible effects of tetrachloroethylene on other targets of methylene chloride toxicity.

Toxicological Significance – Studies evaluating combined exposure to tetrachloroethylene and methylene chloride, or describing pretreatment with tetrachloroethylene prior to methylene chloride exposure, were not located.

Additional Uncertainties – Uncertainties have been addressed in the above discussion.

2.4 Recommendations for Data Needs

Neither *in vivo* data from human or animal studies nor *in vitro* data examining the toxicity of the five-component mixture, or for four- or three-component submixtures, are available. Similarly, PBPK models describing the behavior of the five-component mixture, or for four- or three-component submixtures, are not available. In the absence of direct interaction data, a component-based approach was utilized. However, data on the joint toxic action of the component pairs of the mixture are generally lacking, with only limited data available for the methylene chloride-carbon monoxide component pair and no adequate joint action data available for the remaining 9 of the 10 component pairs of the mixture. Data on the potential mechanistic interactions between the component pairs are limited but were located for two of the component pairs: formaldehyde with methylene chloride and formaldehyde with nitrogen dioxide.

For the individual components, intermediate- and chronic-duration inhalation MRLs are available for formaldehyde, methylene chloride, and tetrachloroethylene. MRLs for exposures to carbon monoxide or nitrogen dioxide have not been derived.

3. Recommendation for Exposure-Based Assessment of Joint Toxic Action of the Mixture

As discussed above, the mixture of carbon monoxide, formaldehyde, methylene chloride, nitrogen dioxide, and tetrachloroethylene was chosen as the subject for this interaction profile because they are airborne compounds that are commonly found in the home environment. The exposure scenarios of greatest concern are likely to be inhalation exposures of intermediate and chronic durations. Two or more noncancer targets of toxicity have been identified in each of the five chemicals of concern. Three of the chemicals have been identified as likely or known carcinogenic agents (formaldehyde, methylene chloride, and tetrachloroethylene). Separate approaches are recommended for noncancer and carcinogenic effects. All recommendations discussed below are intended to be used in consultation with the [*Framework for Assessing Health Impacts of Multiple Chemical and Other Stressors*](#) (ATSDR 2018).

Because suitable data, joint action models, and PBPK models are lacking for the complete mixture, the recommended approach for the exposure-based assessment of noncancer joint toxic action of this mixture is to use the hazard index method with the target-organ toxicity dose (TTD) modification and qualitative WOE method to assess the potential consequences of dose-additive and interactive joint action of the components of the mixture. These methods are to be applied only under circumstances involving significant exposure to the mixture (i.e., only if hazard quotients for two or more of the compounds are ≥ 0.1) (Figure 1 of ATSDR 2018). Hazard quotients are the ratios of exposure estimates to noncancer health-based guidance values, such as MRLs. If only one compound, or if none of the compounds, has a hazard quotient that is ≥ 0.1 , then no further assessment of the joint toxic action is needed because dose additivity and/or interactions are unlikely to result in a significant noncancer health hazard. As discussed by ATSDR (1992, 2018), the exposure-based assessment of potential health hazard is used in conjunction with biomedical judgment, community-specific health outcome data, and community health concerns to assess the degree of public health hazard.

The TTD modification of the hazard index requires the estimation of endpoint-specific (target-organ-specific) hazard indexes for the endpoints of concern for a particular mixture. The endpoints of concern for this mixture are hematological, neurological, respiratory, hepatic, and developmental effects. Therefore, these endpoints are candidates for TTD development for the components of this mixture. TTDs were not derived for endpoints that are sensitive endpoints for only one component of the mixture. The TTDs were derived as described in the appendices to this document, using the methods recommended by ATSDR (2001, 2018). The derived values are listed in Table 18, which also lists the intermediate- and

chronic-duration inhalation MRLs or other health-based guidance values. BINWOEs have been developed for these endpoints also, as presented in Section 2.3, and summarized later in Section 3.

Table 18. MRLs and TTDs for Inhalation Exposure to Chemicals of Concern^a

Endpoint	Chemical				
	Carbon monoxide	Formaldehyde	Methylene chloride	Nitrogen dioxide	Tetrachloroethylene
Hematological	1 ppm (intermediate- and chronic-duration TTD)	NA	0.3 ppm (intermediate- and chronic-duration TTD)	NA	NA
Neurological	4 ppm (intermediate- and chronic-duration TTD)	NA	0.6 ppm (intermediate- and chronic-duration TTD) ^b	NA	0.006 ppm (intermediate- and chronic-duration MRLs)
Respiratory	NA	0.03 ppm (intermediate-duration MRL) 0.008 ppm (chronic-duration MRL)	2 ppm (intermediate- and chronic-duration TTD)	0.03 ppm (intermediate- and chronic-duration TTD)	NA
Hepatic	NA	NA	0.3 ppm (intermediate- and chronic-duration MRLs)	NA	0.03 ppm (intermediate- and chronic-duration TTD)
Developmental	1 ppm (intermediate- and chronic-duration TTD)	0.2 ppm (intermediate- and chronic-duration TTD)	NA	NA	NA

^aSee Appendices A, B, C, D, and E for details on derivations of TTDs.

^bThe acute-duration MRL for methylene chloride was adopted as the TTD_{NEURO} values for methylene chloride; see Appendix C for details.

MRL = Minimal Risk Level; NA = not applicable; TTD = target-organ toxicity dose

The hazard index is calculated using the health-based guidance values for the effect of concern, shown in Table 18, or newer values as they become available, where the exposure route and duration of the exposure and health-based guidance values should match the route and duration for which the hazard index is developed. The hazard index is unitless so the exposure and guidance values must be in the same units (e.g., ppm). This process is shown, using neurological effects following chronic-duration inhalation exposure as an example, in the following equation:

$$HI_{NEURO} = \frac{E_{CO}}{TTD_{CO,NEURO}} + \frac{E_{MeCl}}{MRL_{MeCl}} + \frac{E_{TCEt}}{MRL_{TCEt}}$$

where HI_{NEURO} is the hazard index for chronic-duration inhalation neurological toxicity, E_{CO} is the exposure to carbon monoxide, $TTD_{CO,NEURO}$ is the chronic-duration TTD_{NEURO} for carbon monoxide (in ppm), E_{MeCl} is the exposure to methylene chloride, MRL_{MeCl} is the acute-duration inhalation MRL for methylene chloride (based on neurological effects, in ppm, which was adopted as the chronic-duration TTD_{NEURO} for methylene chloride), E_{TCEt} is the exposure to tetrachloroethylene (in ppm), and MRL_{TCEt} is the chronic-duration inhalation MRL for tetrachloroethylene (based on neurological effects, in ppm). The same process can be then repeated for each endpoint and duration of concern, using the appropriate exposure concentrations and TTDs/MRLs, resulting in endpoint-specific hazard indices for each effect of concern for the mixture. Components for which data are not available, and therefore no TTD can be derived, or which do not affect the endpoint are not included in the endpoint-specific hazard index calculation.

If the hazard index for effects on a noncancer endpoint of concern for any duration is >1 , it provides preliminary evidence that the mixture may constitute a health hazard due to the joint toxic action of components on that endpoint (ATSDR 2018). The impact of interactions from the WOE analysis was considered; however, since the available data do not indicate non-additive actions for any of the component pairs, the impact of the WOE analysis will be less than in the case of some other mixtures.

The default cancer risk assessment approach for a multi-component mixture for which no data on the carcinogenicity of the mixture are available and no PBPK models have been validated involves summing the component cancer risks, which is a good approximation of response addition for low component risks. The inhalation carcinogenic risk for each component is calculated by multiplying lifetime inhalation exposure estimates for each component by the appropriate EPA cancer inhalation unit risk (an estimate of cancer risk per unit of inhalation exposure). If only one or if none of the component risks is $\geq 1 \times 10^{-6}$, then no further assessment of joint toxic action would be needed due to the low likelihood that additivity and/or interactions would result in a significantly enhanced health hazard. For this particular mixture, formaldehyde, methylene chloride, and tetrachloroethylene have unit risk values, so the focus on carcinogenic risks will lie primarily on those compounds. As noted in Section 2.2.2 (Tables 9 and 10), the available WOE indicates an additive joint action with regard to carcinogenic effects for methylene chloride and formaldehyde.

If this screening procedure indicates preliminary evidence of a mixture exposure health hazard, additional evaluation is needed to assess whether a public health hazard exists (ATSDR 2018). The additional evaluation includes biomedical judgment, assessment of community-specific health outcome data, and consideration of community health concerns (ATSDR 1992).

4. Conclusions

This interaction profile recommends the use of component-based approaches that assume additive joint toxic action in exposure-based assessments of possible noncancer or cancer health hazards from inhalation exposure to mixtures of carbon monoxide, formaldehyde, methylene chloride, nitrogen dioxide, and tetrachloroethylene. This recommendation is based on the following factors: (1) there are no direct data available to characterize health hazards (and dose-response relationships) from mixtures containing all five components; (2) PBPK/PD models have not yet been developed that would predict pertinent target doses of the components under scenarios involving exposure to mixtures of all five components; and (3) available information on toxic actions of the individual components indicates that joint actions of carbon monoxide, formaldehyde, methylene chloride, nitrogen dioxide, and tetrachloroethylene on several toxicity targets are plausible, including hematological effects, respiratory effects, neurological alterations, hepatic injury, developmental effects, and cancer. With data on the individual components suggesting possible sites of joint toxic action, but no data available on the toxicity or behavior of the complete mixture or the relevant submixtures, a default component-based approach assuming additivity was therefore recommended. Data evaluating the complete mixture *in vivo*, *in vitro*, or via computational modeling may provide relevant data to inform future recommendations.

WOE analyses of available data on the joint toxic action of mixtures of these components indicate that scientific evidence for greater-than-additive or less-than-additive interactions among these components is limited, with the majority of limited available interaction data suggesting additive joint toxicity. Data are inadequate to characterize the possible modes of joint action on most of the pertinent toxicity targets. Therefore, it is recommended that dose additivity should generally be assumed as a public health protective measure in exposure-based assessments of noncancer health hazards from exposure to mixtures of these components. The dose additivity approach to screening for potential noncancer health hazards involves the estimation of endpoint-specific hazard indexes using MRLs from the toxicological profiles and TTDs derived in this interaction profile. This approach is appropriate when the hazard quotients of at least two of the components are ≥ 0.1 (ATSDR 2018). Potential cancer risk is estimated by adding the chemical-specific risks for formaldehyde, methylene chloride, and tetrachloroethylene.

Endpoint-specific hazard indices (e.g., hazard indices for hepatic effects) or cancer risks for the same duration (e.g., chronic) can be summed across routes to estimate the aggregate health hazard or risk, if it is likely that the same individual or group of individuals would be exposed by both routes. If an endpoint-specific hazard index is >1 , or the total cancer risk for these chemicals is $\geq 1 \times 10^{-4}$, then further

evaluation is needed (ATSDR 2018), using biomedical judgment and community-specific health outcome data and taking into account community health concerns (ATSDR 1992). For very high exposures, interactions may occur (e.g., ≥ 100 -fold above the MRLs or TTDs), and their potential impact can be determined using the WOE results, as summarized above.

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Appendix A. Background Information for Carbon Monoxide

This appendix was written based primarily on the *Toxicological Profile for Carbon Monoxide* (ATSDR 2012); primary references are cited for the reader's convenience in identifying pertinent studies. Where relevant, additional information was obtained from reviews and meta-analysis published after the profile. For additional information beyond what is presented here, the reader is referred to the toxicological profile.

A.1 Toxicokinetic

Absorption. The absorption of inhaled carbon monoxide is mainly controlled by physical processes, and occurs in two primary steps. The first step is the absorption through the alveolar wall into the alveolar interstitium, which can be affected by the mechanical action of the respiratory system as well as changes to the respiratory tract. From there, the compound moves with a concentration gradient into the red blood cell, similar to molecular oxygen. In both cases, diffusion is very rapid and is driven primarily by the partial pressure differential of carbon monoxide. However, other factors, including oxyhemoglobin and COHb levels, ventilatory pattern, oxygen consumption, blood flow, and functional residual capacity, may affect the rate at which inhaled carbon monoxide enters the blood (Forster 1987). In chronic bronchitics, asthmatics, and other subpopulations at risk (pregnant women, the elderly, etc.), the kinetics of COHb formation will be even more complex because any abnormalities of ventilation and perfusion and gas diffusion will aggravate carbon monoxide exchange between blood and air. Additional factors that may affect carbon monoxide absorption include level of activity (e.g., exercise), altitude, partial pressure of oxygen, and age (ATSDR 2012).

Within the erythrocyte, carbon monoxide binds with hemoglobin to form COHb. The rate of carbon monoxide binding to hemoglobin is approximately 20% that of molecular oxygen, and the dissociation constant is approximately an order of magnitude lower than molecular oxygen (Roughton 1970). However, carbon monoxide has very high affinity for hemoglobin, on the order of 230–270-fold that of molecular oxygen (Kinoshita et al. 2020; Roughton 1970). One part of carbon monoxide and 245 parts of oxygen would form equal parts of oxyhemoglobin and COHb (50% of each), which would be achieved by breathing air containing 21% oxygen and 650 mg carbon monoxide/m³ (570 ppm).

Distribution. Due to its high affinity for hemoglobin, most absorbed carbon monoxide will be found in the blood as COHb, and will therefore be present in all tissues of the body. However, carbon monoxide

can dissociate from COHb and enter other tissues with heme-containing enzymes, including the heart and liver. About 15% of the body's carbon monoxide is found outside of the blood (Coburn and Forman 1987; Longo 1977). A study by Hill et al. (1977) predicted similar levels of blood COHb (%) in a mother and fetus during prolonged exposures of the mother to carbon monoxide (34–340 mg/m³ [30–300 ppm]), suggesting that carbon monoxide can freely dissociate from maternal blood and enter the fetal circulation along a concentration gradient.

Metabolism. The majority of carbon monoxide is removed from the body by exhalation of carbon monoxide. While small amounts are likely converted to carbon dioxide via oxidative metabolism prior to exhalation, this is believed to be a minor pathway.

Elimination. Carbon monoxide is removed from the body by exhalation following dissociation from heme. Both the initial formation and the decline of COHb formation and the decline of COHb levels are best modeled by second-order functions, with an initial rapid decay followed by a more gradual second phase (Landaw 1973; Stewart et al. 1970; Wagner et al. 1975). The half-life of disappearance of carbon monoxide from the blood in humans ranges from 2 to 6.5 hours, although at very high concentrations, this range may be exceeded (Landaw 1973; Peterson and Stewart 1970). The process is diffusion-limited, and breathing of increased levels of oxygen reduces the elimination half-time considerably (Peterson and Stewart 1970).

A.2 Health Effects

The primary toxicological effects of carbon monoxide result from the formation of COHb and subsequent hypoxia of oxygen-sensitive tissues. Background COHb levels are generally <2% in nonsmokers, while current smokers average approximately 4% COHb and heavy smokers may have as high as 14% COHb (ATSDR 2012; Savioli et al. 2024).

The Coburn-Forster-Kane (CFK) equation was developed by Coburn et al. (1965) to describe the dynamics of carbon monoxide uptake and elimination and the formation of COHb as a function of concentration of carbon monoxide in air, duration of exposure, and alveolar ventilation. This equation has been used by several investigators to predict blood COHb formation resulting from carbon monoxide exposure, with generally acceptable results (Benignus et al. 1994; Peterson and Stewart 1970, 1975; Tikuisis et al. 1992). The nonlinear form of the equation is as follows:

$$\frac{d[COHb]_t}{d_t} = \frac{V_{CO}}{V_b} + \frac{1}{V_b * \beta} * (P_I CO - \frac{[COHb]_{t-1} * P_c O_2}{[O_2Hb]_{t-1} * M})$$

$$\beta = \frac{1}{D_L CO} + \frac{P_B - 47}{V_A}$$

where:

$[COHb]$ = COHb concentration in mL carbon monoxide per mL blood under standard temperature and pressure and dry conditions (STPD)

t = time at the end of the integration time step

t-1 = time at the beginning of the time step

V_{CO} = endogenous carbon monoxide production rate in mL/minute (STPD)

V_b = blood volume in mL

$P_I CO$ is the carbon monoxide partial pressure in inhaled air in Torr

$P_c O_2$ = average partial pressure of O_2 in lung capillaries in Torr

$[O_2Hb]$ = oxyhemoglobin concentration in mL O_2 per mL blood (STPD)

M = Haldane constant

$D_L CO$ = lung diffusing capacity of carbon monoxide in mL/minute-Torr (STPD)

P_B = the barometric pressure (Torr)

47 = the partial pressure of water in water saturated air (Torr)

V_A = alveolar ventilation in mL/minute (STPD)

Cardiovascular Effects. Exposure to carbon monoxide, and the resulting increase in COHb levels, has been shown to have cardiovascular effects in humans. Significant decreases in short-term maximal exercise duration have been noted in healthy men with blood COHb levels ranging from 2 to 7% (Drinkwater et al. 1974; Ekblom and Huot 1972; Horvath et al. 1975), while decreases in maximal oxygen consumption have been observed in groups of healthy men with COHb levels ranging from 5 to 20% (Ekblom and Huot 1972; Pirnay et al. 1971; Vogel and Gleser 1972; Weiser et al. 1978). The lowest observed COHb blood levels associated with significant decreases in exercise time producing chest pain (angina) were 2–5.9% (Adams et al. 1988; Allred et al. 1989; Anderson et al. 1973; Aronow et al. 1984; Kleinman et al. 1989); the chest pain in these cases is thought to be a response to myocardial ischemia.

Available studies have suggested that humans with coronary artery disease are susceptible to carbon monoxide-induced ventricular arrhythmias. An early study by Hinderliter et al. (1989) did not report an increase in ventricular arrhythmia following exposure of patients with coronary artery disease to carbon monoxide sufficient to result in 4 or 6% COHb, while a later study by the same group (Sheps et al. 1990) examining a group of 46 coronary artery disease patients found an increase in arrhythmia frequency at 6% COHb, but not at 4%. Dahms et al. (1993) did not find a significant association between carbon monoxide exposures resulting in 3 or 5% COHb and the frequency of arrhythmias during rest or exercise.

It therefore appears that at $\leq 5\%$ COHb, humans are not at increased risk for ventricular arrhythmias, but that there may be a risk in sensitive populations at higher COHb levels.

Epidemiology studies of workers exposed to atmospheres containing carbon monoxide provide support for the development of carbon monoxide-induced ischemic cardiovascular disease. Hernberg et al. (1976) found a significant correlation between carbon monoxide exposure and angina pectoris, but not between carbon monoxide exposure and electrocardiographic findings, in a group of Finnish foundry workers. In a follow-up study of the same population, no significant differences between carbon monoxide exposure and mortality rates from cardiovascular disease or ischemic heart disease were reported (Koskela 1994). A series of studies by Stern et al. (1981, 1988) did not find a significant increase in mortality from cardiovascular disease in New Jersey motor vehicle examiners during a period between 1944 and 1973 or in New York City bridge officers between 1951 and 1985, but did report an increased mortality rate among New York City tunnel officers, who were generally exposed to higher carbon monoxide levels than the other two exposure populations.

A large number of reviews have evaluated the association between chronic carbon monoxide exposure and the development of ischemic cardiovascular disease (ATSDR 2012). The reviews generally agree that acute-duration carbon monoxide exposures can aggravate symptoms of cardiovascular disease, primarily by generating tissue hypoxia, but that available evidence is not sufficient to establish a causative link between low-level (<100 ppm) carbon monoxide exposure and the development of cardiovascular disease. Meta-analyses of epidemiological data identified associations between carbon monoxide exposure and risks of major adverse cardiovascular events (Du et al. 2024), heart failure hospitalization or mortality (Shah et al. 2013), and cardiovascular disease (Chen et al. 2022). An additional meta-analysis did not identify an association between long-term carbon monoxide exposure and risk of heart failure (Jia et al. 2023). Although Mustafic et al. (2012) reported an increased risk of myocardial infarction with carbon monoxide via meta-analysis, Stanley Young and Kindzierski (2019) have called the results into question based on possible manipulation of p-values in the base publications. No associations were identified for carbon monoxide exposure and stroke incidence, mortality, or hospital admission (Niu et al. 2021).

In experimental animal studies, carbon monoxide inhalation exposures resulted in cardiac hypertrophy, cardiac arrhythmias, compensatory alterations in hemodynamics, and remodeling of perivascular and interstitial cardiac tissue (ATSDR 2012). Conflicting evidence is available regarding whether carbon

monoxide induces atherosclerotic lesions, and no evidence is available to indicate that it causes hypertension.

Neurological Effects. Effects on the central nervous system (CNS) are well-documented at high blood COHb levels, while at lower levels, many COHb-related effects have been noted, but have been difficult to consistently demonstrate and quantify. The first neurological effects from carbon monoxide exposure begin to appear at 5–9% COHb in the blood, manifesting mainly as a transient alteration of visual thresholds (Crystal and Ginsberg 2000). At higher levels (16–20% COHb), headache is common. As COHb levels continue to increase, other symptoms include loss of manual dexterity, nausea and vomiting, convulsions, coma, and death (Crystal and Ginsberg 2000). However, there is considerable variability between studies, and within individual studies, concerning the COHb levels at which neurological symptoms begin to appear, making it difficult to draw conclusions.

At moderate (10–50%) COHb levels, however, studies have shown a consistent trend of neurological effects, including severe headache, dizziness, nausea, fatigue, and dimness of vision (Benignus et al. 1987; Dolan 1985; Fawcett et al. 1992; Olson 1984). Extremely high blood COHb levels (50–80%) result in severe neurological effects, including disorientation, seizures, coma, respiratory failure, and death (Dolan 1985; Olson 1984). Meta-analyses of epidemiological studies have identified increased risks of dementia (Jones et al. 2025; Tang et al. 2023; Zhang et al. 2024), depression (Borroni et al. 2022), and autism spectrum disorders (Duque-Cartagena et al. 2024) with carbon monoxide exposure. Mixed results were found for Parkinson’s disease (Chen et al. 2022; Hu et al. 2019; Xie et al. 2025).

Developmental Effects. The developing fetus is believed to be particularly sensitive to the effects of hypoxia, and therefore to the effects of carbon monoxide. The developmental effects of low levels of carbon monoxide in humans are not known, but higher exposures have been demonstrated to result in malformations, functional changes, or fetal death (Norman and Halton 1990); these effects have only been noted in cases where noted maternal toxicity (i.e., maternal hypoxia) was present. Additionally, the specific pregnancy outcome is likely dependent on fetal age, with sensitivity increasing with fetal age (ATSDR 2012). The majority of epidemiological meta-analyses have not identified associations between carbon monoxide exposure and birth defects (Chen et al. 2014; Huang et al. 2023b; Vrijheid et al. 2011). A meta-analysis by Hu et al. (2020) reported an association between carbon monoxide exposure and development of tetralogy of Fallot, a congenital heart defect that was not evaluated in the other meta-analyses. An association was identified for carbon monoxide exposure and low birth weight or preterm birth in one meta-analysis (Stieb et al. 2012).

Animal studies provide strong evidence that exposure to carbon monoxide can result in effects on the developing fetus. The available data indicate that carbon monoxide exposures producing 15–25% COHb in the mother produce reductions in birth weight, cardiomegaly, delays in behavioral development, and deficits in cognitive function (for review, see EPA 2011b). Higher exposure levels, producing COHb levels $\geq 48\%$, resulted in maternal and fetal death. Adverse neurodevelopmental effects, including altered brain neurotransmitter levels, altered behavior (impaired righting reflexes, motor activity, and response to stimuli), and impaired auditory system development, have been documented in experimental rodent studies; the lowest maternal exposure lowest-observed-adverse-effect level (LOAEL) of 12 ppm produced a blood COHb level of 1.8% (ATSDR 2012). The human equivalent concentration (HEC) resulting in the same blood COHb level is approximately 10 ppm.

A.3 Mechanisms of Action

The primary mechanism of action of carbon monoxide toxicity is the formation of COHb. This reduces the amount of hemoglobin available to carry oxygen to the tissues as well as interferes with oxygen release at the tissue level (Stucki and Stahl 2020). These two factors combine to diminish cellular respiration, resulting in tissue hypoxia (Abramov et al. 2024; Wang and Zhang 2024). Tissues sensitive to hypoxia, such as the lung, heart, and CNS, are particularly sensitive to the effects of carbon monoxide poisoning (Barn et al. 2018). Carbon monoxide additionally binds to and inhibits other heme-containing proteins including cytochrome C, resulting in disrupted mitochondrial electron transport chains and impaired cellular energy production (Abramov et al. 2024; Savioli et al. 2024; Wang and Zhang 2024). Other mechanisms of carbon monoxide-induced toxicity have been hypothesized and assessed, such as hydroxyl radical production (Piantadosi et al. 1997) and lipid peroxidation (Thom 1990, 1992, 1993) in the brain of carbon monoxide-poisoned rats; however, these are likely high-dose phenomena and have not been demonstrated at low carbon monoxide exposure levels. Individuals with cardiovascular disease have greater susceptibility for carbon monoxide-induced effects due to pre-existing deficits in oxygen transport (Barn et al. 2018; Kinoshita et al. 2020).

A.4 Health Guidelines

ATSDR (2012) has not derived MRLs for carbon monoxide for any exposure duration or route.

EPA does not list an RfC or cancer classification for carbon monoxide on IRIS (EPA 2024f). The National Ambient Air Quality Standard (NAAQS) values for carbon monoxide are 9 ppm with an 8-hour averaging time and 35 ppm with a 1-hour averaging time; neither standard is to be exceeded more than once per year (EPA 2011b).

The World Health Organization (WHO) recommends that carbon monoxide exposures be kept to levels below which a COHb level of 2.5% would be generated (WHO 1999). These correspond to 100 mg/m³ (87 ppm) for 15 minutes, 60 mg/m³ (52 ppm) for 30 minutes, 30 mg/m³ (26 ppm) for 1 hour, or 10 mg/m³ (9 ppm) for 8 hours. For indoor air quality, WHO (2010a) established a 15-minute guideline of 100 mg/m³ (87 ppm), a 1-hour guideline of 35 mg/m³ (31 ppm), an 8-hour guideline of 10 mg/m³ (9 ppm), and a 24-hour guideline of 7 mg/m³ (6 ppm). WHO (2021) has established a recommended short-term (24-hour) air quality guideline (AQG) of 4 mg/m³ (3.48 ppm) for carbon monoxide based on increased risks of myocardial infarction admission or mortality (Lee et al. 2020).

HHS, EPA, and IARC have not categorized the carcinogenicity of carbon monoxide.

A.5 Derivation of Target-Organ Toxicity Dose(s)

The endpoints of concern for carbon monoxide in this mixture are hematological, neurological, and developmental. These endpoints have sufficient animal data to allow derivation of TTDs. Additional endpoints of concern may be relevant for human health (see Section A.2) but insufficient quantitative data are available for these endpoints. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2018).

Hematological Effects, Intermediate and Chronic Inhalation. The primary effects of exposure of healthy individuals to carbon monoxide involve the formation of COHb, the result of binding of carbon monoxide to the ferrous iron in hemoglobin. Carbon monoxide has a significantly higher affinity for binding hemoglobin, compared to oxygen. COHb formation is responsible for the effects of carbon monoxide on other organs and tissues due to a significant reduction in the blood's capacity to carry oxygen, leading to oxygen deprivation in tissues. The lowest observed COHb blood levels associated with significant decreases in exercise time producing chest pain (angina) were 2–5.9% (Adams et al. 1988; Allred et al. 1989; Anderson et al. 1973; Aronow et al. 1984; Kleinman et al. 1989). Therefore, the 2% COHb level, which has been shown to cause decreases in maximal exercise duration as well as decreased time to chest pain during exercise, was selected as the critical effect level for deriving the TTDs

for hematological effects. According to Raub et al. (2000), and based on the CFK equation, the equilibrium carbon monoxide concentration required to achieve 2% blood COHb is 10 ppm; this value was therefore used as the point of departure (POD) for the TTDs. To the value of 10 ppm, an uncertainty factor of 9 (3 for human variability and 3 for use of a minimal LOAEL) resulting in TTDs of 1 ppm. A full factor of 10 was not used for human variability because variability in COHb formation is expected to be small. Since the TTD is based on a biological threshold not dependent on exposure duration, the TTD of 1 ppm is used for intermediate and chronic durations.

Neurological Effects, Intermediate and Chronic Inhalation. Effects on the CNS are well documented at high blood COHb levels, while at lower levels, many COHb-related effects have been noted but have been difficult to consistently demonstrate and quantify. The first neurological effects from carbon monoxide exposure begin to appear at 5–9% COHb in the blood, manifesting mainly as a transient alteration of visual thresholds (Crystal and Ginsberg 2000). At higher levels (16–20% COHb), headache is common. As COHb levels continue to increase, other symptoms include loss of manual dexterity, nausea and vomiting, convulsions, coma, and death (Crystal and Ginsberg 2000). However, there is considerable variability between studies, and within individual studies, concerning the COHb levels at which neurological symptoms begin to appear, making it difficult to draw conclusions.

Using the estimates presented in Raub et al. (2000) for equilibrium COHb levels in humans as a guide, 5% blood COHb will be reached at 33 ppm; 33 ppm was therefore selected as the POD. To the LOAEL of 33 ppm, an uncertainty factor of 9 (3 for human variability and 3 for use of a minimal LOAEL) was applied to resulting in TTDs of 4 ppm. Since the TTD is based on a biological threshold not dependent on exposure duration, the TTD of 4 ppm is used for intermediate and chronic durations.

Developmental Effects, Intermediate and Chronic Inhalation. Available data indicate carbon monoxide can adversely affect the developing organism. Based on animal data, these effects are mediated by blood COHb levels. Therefore, the TTD_{HEMATO} of 1 ppm is adopted as the TTD_{DEV}.

Summary (TTD for Carbon Monoxide)

Intermediate-Duration Inhalation TTDs:

TTD_{HEMATO} = 1 ppm

TTD_{NEURO} = 4 ppm

TTD_{DEV} = 1 ppm

Chronic-Duration Inhalation TTDs:

TTD_{HEMATO} = 1 ppm

TTD_{NEURO} = 4 ppm

TTD_{DEV} = 1 ppm

A.6 References

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Appendix B. Background Information for Formaldehyde

This appendix consists primarily of excerpts from the *Toxicological Profile for Formaldehyde* (ATSDR 1999); primary references are cited for the reader's convenience in identifying pertinent studies. Where relevant, additional information was obtained from EPA (2024e), reviews, and meta-analysis published after the profile. For additional information beyond what is presented here, the reader is referred to the toxicological profile or relevant secondary source.

B.1 Toxicokinetics

Absorption. Formaldehyde is absorbed by the tissues of the respiratory tract during inhalation exposure in several species. Heck et al. (1985) determined the fate of inhaled formaldehyde in humans exposed to a 1.9 ppm air concentration of formaldehyde for 40 minutes. Shortly before and shortly after the exposure, venous blood samples were taken from each person (each person served as his/her own control) and the blood was analyzed for formaldehyde content. Mean venous blood formaldehyde concentrations in humans prior to exposure showed a blood concentration of 2.61 ± 0.41 $\mu\text{g/g}$ of blood. Individual variability was markedly present. Immediately after a 40-minute exposure, mean blood concentration of formaldehyde was 2.77 ± 0.28 $\mu\text{g/g}$ of blood. There was no significant difference between pre- and post-exposure blood concentrations of formaldehyde at the formaldehyde air concentrations tested in this study. This result suggests that formaldehyde was absorbed only into the tissues of the respiratory tract. The absence of increased formaldehyde concentrations in the blood is likely due to its rapid metabolism in these tissues and/or fast reaction with cellular macromolecules. However, a recent review concluded that there is insufficient evidence for systemic delivery of exogenous formaldehyde at environmentally relevant exposure levels (Vincent et al. 2024).

During a nose-only inhalation exposure of rats to 14.4 ± 2.4 ppm of formaldehyde for 2 hours, no changes in the quantities of formaldehyde were detected in the blood, relative to unexposed animals (Heck et al. 1985). In a similar study by Heck et al. (1983), Fischer 344 rats were exposed by inhalation to ^{14}C -formaldehyde at 8 ppm for 6 hours. Concentrations of total ^{14}C radioactivity (most likely as ^{14}C -formate) in the whole blood and plasma were monitored for an additional 8 days. Plasma concentrations of ^{14}C increased over the exposure period, reaching a maximum at the termination of exposure. Plasma ^{14}C concentrations then declined slowly over the next few days. In a later study, Heck et al. (1985) determined the fate of inhaled formaldehyde in the rat. Male Fischer 344 rats were placed in a nose-only inhalation chamber and exposed to a 14.4 ± 2.4 ppm air concentration of formaldehyde for

2 hours, were sacrificed, and a venous blood sample was collected and analyzed for formaldehyde content. Unexposed control rats had a mean formaldehyde blood level of 2.24 ± 0.07 $\mu\text{g/g}$ of blood. Rats exposed to the 14.4 ppm air concentration of formaldehyde had blood concentrations of 2.25 ± 0.07 $\mu\text{g/g}$.

Egle (1972) measured the retention of formaldehyde along the entire respiratory tract, both upper and lower portions, and measured the effects of ventilation rate, tidal volume, and concentration of inhaled formaldehyde on these parameters. Mongrel dogs of both sexes (at least four dogs per experiment) were anesthetized and exposed to 0.15–0.35 μg (122–235 ppm) of formaldehyde vapor produced from formalin. Retention of formaldehyde when the entire upper and lower respiratory tract was exposed was near 100% and seemed to be independent of the airborne concentration of formaldehyde or variations in the tidal volume. When the upper respiratory tract was isolated from the lungs, the two-way exposures showed a 100% uptake of formaldehyde, while one-way exposures of formaldehyde showed that retention was slightly lower than in the two-way exposure, but still exceeded 95% at all respiratory rates. When the lower respiratory tract was isolated and examined, the uptake of formaldehyde still exceeded 95%; however, it appeared to decrease slightly as the ventilation rates increased. This study concluded that when formaldehyde is inhaled at the concentrations studied, very little formaldehyde vapor would actually reach the lower respiratory tract. Further experiments in dogs, rats, and monkeys confirmed that, except when exposed to high formaldehyde concentrations or under exercise conditions, the majority of inhaled formaldehyde is deposited in the upper respiratory tract (EPA 2024e). Studies in exposed human volunteers indicate that 90–95% of inhaled formaldehyde is deposited in the upper respiratory tract.

In another study by Casanova et al. (1988), blood levels of formaldehyde were determined in Rhesus monkeys after exposure to 6 ppm formaldehyde for 6 hours/day, 5 days/week for 4 weeks. Immediately after the last exposure, the monkeys were sedated and blood samples were collected within 7 minutes and at 45 hours after exposure. Blood concentrations of formaldehyde in the three nonexposed monkeys (2.42 $\mu\text{g/g}$) were not significantly different from those of the exposed group. The study authors concluded that exposure to moderately high levels of formaldehyde had no effect on blood concentrations due to rapid local metabolism.

Distribution. No studies were located that described the distribution of formaldehyde or its metabolites in humans after inhalation exposure.

Several studies are available that describe the distribution of formaldehyde in laboratory animals. Heck et al. (1983) examined the fate of ^{14}C -formaldehyde in Fischer 344 rats exposed by inhalation to

^{14}C -formaldehyde at 8 ppm for 6 hours. Concentrations of total radioactivity in the whole blood and plasma were monitored for 8 days. The terminal half-life of the ^{14}C was approximately 55 hours, which was considerably longer than the known half-life of formaldehyde (about 1.5 minutes in monkeys), indicating both the metabolism of ^{14}C -formaldehyde to other molecules (i.e., formate) and incorporation into other molecules. Radioactivity in the packed blood cell fraction was multiphasic; it initially increased during exposure, declined during the first hour postexposure, then began to increase again, reaching a maximum at approximately 35 hours postexposure. The terminal phase of the packed red blood cell fraction had a very slow decline in radioactivity, which would likely continue for several weeks after exposure ended (half-life >55 hours).

Heck et al. (1983) also examined distribution of ^{14}C -formaldehyde in formaldehyde-naive and formaldehyde-pretreated male Fischer 344 rats. Pretreated rats were exposed whole-body to 15 ppm formaldehyde 6 hours/day for 9 days. On the 10th day, these rats and the formaldehyde-naive rats (never exposed to formaldehyde vapors) were then exposed head-only to ^{14}C -formaldehyde at concentrations of 14.9 ppm for 6 hours. All rats were sacrificed immediately after completion of the ^{14}C -formaldehyde exposure. Immediately after completion of the inhalation exposure, ^{14}C concentrations were greatest in the mucosal tissues. At 15 ppm, ^{14}C concentrations were as follows: nasal mucosa, 2 μmole equivalents/g tissue; trachea, 0.3 μmole equivalents/g tissue; and plasma, 0.1 μmole equivalents/g tissue. Radioactive concentrations were relatively equivalent in all of the mucosal linings monitored. Tissue concentrations of ^{14}C in naive and pretreated rats did not differ from each other. Tissue concentrations of ^{14}C were low, resembling plasma concentrations; the ratio of ^{14}C in internal organs to that in plasma were: esophagus, 4.94 ± 1.23 ; kidney, 3.12 ± 0.47 ; liver, 2.77 ± 0.25 ; intestine, 2.64 ± 0.48 ; lung, 2.05 ± 0.36 ; spleen, 1.59 ± 0.50 ; heart, 1.09 ± 0.09 ; brain, 0.37 ± 0.06 ; testes, 0.31 ± 0.05 ; and erythrocytes, 0.30 ± 0.08 . A similar study by Chang et al. (1983) found that the amounts of radioactivity deposited in the nasal cavities of naive and pretreated rats were similar, but that pretreated rats had less visceral radioactivity compared to naive animals while more radioactivity was found in the nasal cavity of naive mice. The decreased visceral radioactivity seen in the pretreated mice was thought to be due to decreased grooming and mucociliary clearance.

Metabolism. Formaldehyde is rapidly metabolized and storage is not a factor in its toxicity (ATSDR 1999; EPA 2024e). The metabolism of formaldehyde to formate (via formaldehyde dehydrogenase [FDH]/class III alcohol dehydrogenase) takes place in all of the tissues of the body as a consequence of endogenous formation of formaldehyde and the formate is quickly removed by the supporting blood supply (Heck et al. 1982). FDH is the major metabolic enzyme involved in the metabolism of

formaldehyde in all of the tissues studied; it is widely distributed in animal tissues, particularly in the rat nasal mucosa, and is specific for the GSH adduct of formaldehyde. If formaldehyde is not metabolized by FDH, it can form cross linkages between proteins or between protein and single-stranded DNA, or enter the one-carbon intermediary metabolic pool by initially binding to tetrahydrofolate (Bolt 1987). Several enzymes can catalyze the reaction that oxidizes formaldehyde to formic acid (i.e., nonspecific aldehyde dehydrogenase and catalase); however, FDH is the primary enzyme that performs this function and is specific for formaldehyde; other aldehydes are left intact in the presence of FDH. Endogenous or exogenous formaldehyde enters the FDH metabolic pathway and is eliminated from the body as metabolites, primarily as formate or carbon dioxide. FDH activity does not increase (i.e., is not inducible) in response to formaldehyde exposure (Casanova-Schmitz et al. 1984); thus, no increase in metabolism occurs with increasing concentrations of formaldehyde.

Elimination. Heck et al. (1983) examined the fate of ^{14}C -formaldehyde in male Fischer 344 rats. Rats were exposed to 0.63 or 13.1 ppm formaldehyde for 6 hours. Upon completion of the exposure, the rats were placed in metabolic cages, which allowed the continuous collection of urine, feces, and expired air; they remained in the cages for 70 hours and were then sacrificed. The average $^{14}\text{CO}_2$ excretion was biphasic, with a rapid decline over the first 12 hours followed by a more gradual decline in excretion over the remainder of time. Changing the concentration of formaldehyde did not affect the proportion of dose recovered in each type of excreta. Radioactivity in urine accounted for 17.6 and 17.3% of the total radioactivity detected for low- and high-dose rats, respectively; radioactivity in feces accounted for 4.2 and 5.3% of the total respective amounts of recovered radioactivity. Exhalation was the major route of excretion, accounting for 39.4% of the low dose and 41.9% of the high dose. The amount of ^{14}C remaining in the carcass after 70 hours was roughly equivalent (38.9% of low dose; 35.2% of high dose) to the amount expired over the same period. At 15 ppm, ^{14}C concentrations were as follows: nasal mucosa, 2 $\mu\text{mole equivalents/g}$ tissue; trachea, 0.3 $\mu\text{mole equivalents/g}$ tissue; and plasma, 0.1 $\mu\text{mole equivalents/g}$ tissue.

PBPK Models. Pharmacokinetic models describing the rate of formation of formaldehyde-induced DNA-protein crosslinks in different regions of the nasal cavity as a function of formaldehyde air concentration have been developed for rats and monkeys (Casanova et al. 1991; Heck and Casanova 1994; Campbell et al. 2020). Rates of formation of DNA-protein crosslinks have been used as a dose surrogate for formaldehyde tissue concentrations in extrapolating exposure-response relationships for nasal tumors in rats to estimate cancer risks for humans (ATSDR 1999; EPA 2024e). The models assume that rates of crosslink formation are proportional to tissue concentration of formaldehyde and include saturable and

non-saturable elimination pathways, and that regional and species differences in crosslink formation are primarily dependent on anatomical and physiological parameters (e.g., minute volume and quantity of nasal mucosa) rather than biochemical parameters. The models were developed with data from studies in which concentrations of DNA-protein crosslinks were measured in different regions of the nasal cavities of rats (Casanova et al. 1989) and Rhesus monkeys (Casanova et al. 1991; Heck et al. 1989) exposed by inhalation to radiolabeled formaldehyde. In agreement with the observed data, the models predict that overall rates of DNA-protein crosslink formation in rat respiratory mucosa are higher than rates in Rhesus monkeys, and that there is a nonlinear, convex relationship between this dose surrogate in nasal tissues and increasing air concentrations of formaldehyde (Casanova et al. 1991). Similar nonlinear, convex exposure-response relationships have also been observed in formaldehyde-exposed rats for nasal tumor incidence (Kerns et al. 1983; Monticello et al. 1996) and cell proliferation indices in regions of the rat nasal epithelium where tumors develop (Monticello et al. 1996).

Computational fluid dynamics (CFD) models of airflow in the nasal passages of rats, monkeys, and humans have been developed to determine the degree to which interspecies and interregional differences in uptake patterns along airway passages may account for differing distributions of formaldehyde-induced upper respiratory tract lesions in rats and primates. These models enable extrapolation of exposures associated with upper respiratory tract tissue damage in rats or monkeys to human exposures (Cohen Hubal et al. 1997; Kepler et al. 1998; Kimbell et al. 1993, 1997a, 1997b; Morgan 1997; Morgan et al. 1991; Subramaniam et al. 1998). Airflow pattern is expected to be one of three important determinants of upper respiratory tract tissue uptake, along with interactions at the airway/tissue interface such as off-gassing and tissue properties influencing absorption rates (e.g., mucociliary clearance or rate of metabolism).

Driving forces behind the development of these airflow models include: (1) differences in nasal anatomy and breathing patterns between rats and primates; (2) observations that nonneoplastic respiratory tract lesions in rats exposed to 6 ppm formaldehyde are confined to epithelial tissue in specific anterior regions of the nose posterior to the vestibule (Chang et al. 1983; Morgan et al. 1986), whereas monkeys exposed to 6 ppm formaldehyde show a wider distribution of similar epithelial lesions in the nose posterior to the vestibule with some extension of the lesions into the tracheal and bronchial regions (Monticello et al. 1989); (3) histochemical localization observations suggesting that regional differences in FDH, a key enzyme in formaldehyde detoxification, were insufficient to account for localized toxicity in the rat nose (Keller et al. 1990); and (4) observations of correlations between sites of formaldehyde-induced lesions in the nasal epithelium of rats and Rhesus monkeys and site-specific rates of DNA-protein crosslink

formation (a putative internal dosimeter for formaldehyde as discussed earlier; Casanova et al. 1989, 1991, 1994) or site-specific rates of cellular proliferation (Monticello et al. 1989, 1996).

B.2 Health Effects

Although formaldehyde is a normal intermediary cellular metabolite involved in the biosynthesis of purines, thymidine, and several amino acids, it is a highly reactive molecule that can be directly irritating to tissues with which it comes into contact (ATSDR 1999; EPA 2024e). Human and animal studies indicate that formaldehyde, at appropriate exposure levels, can be irritating to the upper respiratory tract and eyes with inhalation exposure, to the skin with dermal exposure, and to the gastrointestinal tract with oral exposure. Reports of allergic dermal sensitization to formaldehyde are widespread and supported by results from animal studies, but the evidence that formaldehyde sensitizes the respiratory tract is less convincing.

Studies of volunteers exposed to airborne formaldehyde for short periods of time (≤ 8 hours) indicate that eye, nose, and throat irritation occurs at concentrations in the range of 0.4–3 ppm (ATSDR 1999; EPA 2024e). At the lower end of this range, the irritation is typically described as mild and noted by a lower percentage of exposed subjects than at the upper end of the range. Results of residential population epidemiology studies corroborated those of volunteer exposure studies; the prevalence of eye irritation increased with formaldehyde air concentrations ≥ 0.4 mg/m³ (EPA 2024e). Studies of monkeys, rats, and mice exposed to higher concentrations in the range of 3–9 ppm for acute to intermediate periods of time demonstrate that formaldehyde nonneoplastic toxic effects are largely restricted to lesions (squamous metaplasia and hyperplasia) in the epithelium of the upper respiratory tract; the incidence and/or severity of these effects was also dependent on the exposure duration, with an increasing magnitude of alterations observed with increasing durations (ATSDR 1999; EPA 2024e).

Studies of animals exposed for life to formaldehyde in air or drinking water show that formaldehyde primarily damages tissue at portals of entry (i.e., the upper respiratory tract and gastrointestinal tract); evidence for toxic effects at distant sites is less consistent (ATSDR 1999; EPA 2024e). Replicated inhalation studies have shown that formaldehyde induced malignant nasal tumors in rats at high exposure concentrations (10–15 ppm) that also induced nasal epithelial necrosis and cellular proliferation, but not at lower concentrations (0.3–2 ppm) that did not markedly damage nasal epithelial tissue (Albert et al. 1982; Kamata et al. 1997; Kerns et al. 1983b; Monticello et al. 1996; Woutersen et al. 1989). Exposure-related cancer or noncancer lesions at other sites were not found in these studies. Statistically significant

increased incidences of nasal tumors, however, were not found in mice exposed by inhalation for two years (Kerns et al. 1983) or in hamsters exposed for 18 months (Dalbey 1982) at concentrations similar to those producing nasal tumors in rats. Nonneoplastic nasal epithelial damage was found in mice exposed to 14 ppm, but not in mice exposed to 2 ppm (Kerns et al. 1983b). Three lifetime drinking-water exposure studies in rats that found no consistent, exposure-related cancer or noncancer effects at sites distant from the gastrointestinal tract (Soffritti et al. 1989; Til et al. 1989; Tobe et al. 1989) provide support for the expectation that formaldehyde-induced health effects are restricted to portals of entry except at high concentrations that saturate metabolic and/or binding capacities at the portals of entry.

Occupational and residential exposure to formaldehyde has been associated with reports of symptoms of eye, nose, and throat irritation from exposure to airborne formaldehyde (Garry et al. 1980; Holness and Nethercott 1989; Horvath et al. 1988; Ritchie and Lehnen 1987), and there are numerous reports of skin irritation and contact dermatitis most likely resulting from dermal exposure to formaldehyde in liquids (Fischer et al. 1995; Kiec-Swierczynska 1996; Maibach 1983; Meding and Swanbeck 1990; Menné et al. 1991). Several cross-sectional studies of nasal epithelial tissue specimens from workers exposed to airborne formaldehyde in the approximate average concentration range of 0.2–1 ppm found evidence in some of the workers for mild lesions (stratified squamous epithelium and mild dysplasia) that are indicative of the irritant and reactive properties of formaldehyde (Ballarin et al. 1992; Boysen et al. 1990; Edling et al. 1988; Holmstrom et al. 1989).

Formaldehyde-induced noncancer and cancer effects dominate at portals of entry, which is consistent with the highly reactive nature of formaldehyde and the existence of physiological mechanisms of protection, such as the nasal mucosal barrier and the detoxifying metabolism of formaldehyde in most, if not all, cells. The available WOE indicates that distant site effects from formaldehyde may occur only when the capacities for local metabolism and disposition of formaldehyde are exceeded (Vincent et al. 2024).

Developmental Effects. The EPA concluded the available evidence indicates that formaldehyde likely causes an increased risk of developmental or female reproductive effects in humans given sufficient exposure conditions (EPA 2024e). It is noted that this conclusion is based primarily on results of occupational studies reporting increased spontaneous abortion risk and increased time-to-pregnancy, both endpoints categorized as reproductive effects by ATSDR. Reviews also provide limited evidence of potential associations between maternal exposure to formaldehyde and altered birth outcomes, including intrauterine growth retardation, decreased birth weight, and congenital malformations (Duong et al. 2011; EPA 2024e). A meta-analysis of 12 epidemiological studies identified an association between maternal

formaldehyde exposure and reproductive and developmental effects combined (spontaneous abortion, low birth weight, and birth defects or malformations) when considering all exposure reporting; this association remained when the meta-analysis was restricted to the seven studies that did not rely on self-reported exposure data (Duong et al. 2011). Only one study evaluated the effects of paternal formaldehyde exposure; the association remained if it was included in the meta-analysis (Duong et al. 2011).

Prenatal developmental toxicity studies in animals identified decreased fetal survival and growth and increased incidences of structural abnormalities (reduced ossification) (ATSDR 1999; Duong et al. 2011; EPA 2024e). EPA (2024e) judged the animal developmental toxicity studies to be of low quality due to methodological deficiencies, including lack of test substance characterization and the use of formalin (37% aqueous formaldehyde solution stabilized with 10% (v/v) methanol) rather than formaldehyde. No suitable 1- or 2-generation animal studies are available to evaluate formaldehyde's developmental effects (EPA 2024e).

Reproductive Effects. A meta-analysis of seven epidemiological studies identified an association between maternal formaldehyde exposure and spontaneous abortion (miscarriage); however, when the meta-analysis was limited to the three studies with exposure data that were not self-reported, the association was no longer observed (Duong et al. 2011). Only one study evaluated the effects of paternal formaldehyde exposure; the association remained if it was included in the meta-analysis (Duong et al. 2011). In addition to spontaneous abortion, reviews of epidemiological data also suggested potential relationships between inhalation exposure to formaldehyde and altered menstrual irregularities or disorders, reduced fecundity, increased time-to-pregnancy, endometriosis, and preterm birth (Duong et al. 2011; EPA 2024e). EPA (2024e) noted that animal studies for female reproductive data were limited to an intermediate-duration inhalation study in mice reporting hypoplasia of the ovaries and uterus. Based primarily on occupational studies in women, the EPA (2024e) concluded that “inhalation of formaldehyde likely causes increased risk of developmental or female reproductive toxicity in humans, given sufficient exposure conditions. This conclusion is based on *moderate* evidence in observational studies finding increases in time-to-pregnancy and spontaneous abortion risk among women occupationally exposed to formaldehyde; the evidence in animals is *indeterminate*.” However, effects are only expected to occur under conditions that would result in systemic distribution of formaldehyde.

In males, decreased sperm motility was observed in male woodworkers occupationally exposed to formaldehyde (EPA 2024e). Reviews of reproductive toxicity studies in experimental animals exposed to formaldehyde via inhalation identified decreased testosterone levels, altered sperm parameters, and

histopathological changes to the testes in male rats and mice (Duong et al. 2011; EPA 2024e). No suitable 1- or 2-generation animal studies are available to evaluate formaldehyde's effects on fertility or reproductive performance (EPA 2024e). The final hazard conclusion by EPA (2024e) for male reproductive effects is that "inhalation of formaldehyde likely causes increased risk of reproductive toxicity in men, given sufficient exposure conditions, based on *robust* evidence in animals that presents a coherent array of adverse effects in two species, and *slight* evidence from observational studies of occupational formaldehyde exposure." However, effects are only expected to occur under conditions that would result in systemic distribution of formaldehyde.

Cancer. A large number of epidemiology studies (cohort studies of industrial workers, cohort studies of medical specialists and embalmers, and case-control studies) examining the potential for occupational formaldehyde exposure to induce cancer have provided evidence of a relationship between formaldehyde exposure and nasopharyngeal cancer, sinonasal cancer, and myeloid leukemia in humans, with less convincing evidence for oropharyngeal/hypopharyngeal cancer, lymphatic leukemia, multiple myeloma, and Hodgkin's lymphoma (EPA 2024e; IARC 2012;). Recent meta-analyses did not identify statistically significant associations between formaldehyde exposure and Hodgkin's lymphoma, myeloid leukemia, or multiple myeloma (Vincent et al. 2024).

B.3 Mechanisms of Action

The toxicity of formaldehyde is route-dependent; irritation at the point of contact is seen by inhalation, oral, and dermal routes. There is evidence that formaldehyde-induced upper respiratory tract irritation and pulmonary function impacts involve sensory nerve activation (ATSDR 1999; EPA 2024e). High doses are cytotoxic and result in degeneration and necrosis of mucosal and epithelial cell layers. These observations are consistent with the hypothesis that toxic effects are mediated by formaldehyde itself and not by metabolites. No specific target molecule has been identified, although DNA-protein crosslinks have been identified (Casanova and Heck 1987). Aldehydes as a group are reactive chemicals with a highly electronegative oxygen atom and less electronegative atoms of carbon(s), and hence have a substantial dipole moment. The carbonyl atom is the electrophilic site of these types of molecules, making it react easily with nucleophilic sites on cell membranes and in body tissues and fluids such as the amino groups in protein and DNA (Feron et al. 1991). It is known that formaldehyde readily combines with free, unprotonated amino groups of amino acids to yield hydroxymethyl amino acid derivatives and a proton (H⁺), which is believed to be related to its germicidal properties. Higher concentrations will

precipitate protein (Loomis 1979). Either one of these mechanistic properties or perhaps other unknown properties may be responsible for the irritation effects seen with formaldehyde exposure.

Oral and inhalation toxicity studies with animals generally have found that toxic effects from formaldehyde are largely restricted to portal-of-entry tissue, but there are scattered reports of toxic effects at sites distant from portals of entry. It is probable that formaldehyde toxicity occurs when intracellular levels saturate FDH activity, overwhelming the natural protection against formaldehyde, and allowing the unmetabolized intact molecule to exert its effects locally (ATSDR 1999; EPA 2024e). Regarding developmental toxicity, the EPA considered plausible mechanisms to include indirect oxidative stress or inflammation and possible disruption of neuroendocrine signaling (EPA 2024e). The primary metabolite of formaldehyde, formate, is not expected to be as reactive as formaldehyde itself and is subject to excretion as a salt in the urine, entrance into the one-carbon metabolic pool for incorporation into other cellular components, or further metabolism to carbon dioxide. The mechanism whereby distant site toxicity may be expressed is unclear, but given the highly reactive nature of formaldehyde and the ubiquitous metabolic capability of cells to metabolize formaldehyde, it is plausible that distant site effects may occur only when the capacities for local metabolism and disposition of formaldehyde are exceeded.

It has been demonstrated that formaldehyde can form crosslinks between protein and DNA *in vivo*. Casanova-Schmitz et al. (1984) reported that the predominant route of formaldehyde metabolism was metabolic incorporation into macromolecules (DNA, RNA, and proteins) in the respiratory and olfactory mucosa and bone marrow of male Fischer 344 rats. Later studies by Casanova et al. (1991) described the formation of DNA-protein crosslinks in the respiratory tract measured in male Fischer 344 rats as well as in Rhesus monkeys; concentrations of DNA-protein crosslinks were greatest in the middle turbinate tissues and lowest in the nasopharyngeal tissues, with some evidence of crosslink formation observed in the larynx/trachea/carina and major intrapulmonary airway tissues.

The relationship between formaldehyde concentration and total dose has been studied in experiments where rats were exposed to a range of concentrations for various lengths of time so that the total inhaled dose was constant (Wilmer et al. 1987, 1989). Studies have shown that formaldehyde concentration in the inspired air may be more important than exposure duration in determining the extent of nasal damage (Wilmer et al. 1987, 1989), assuming a constant value for concentration times time.

Although there is evidence to suggest that exposure concentration is more important than exposure duration in determining the extent of formaldehyde-induced nasal epithelial damage, the development of

formaldehyde-induced nasal squamous cell carcinomas is likely to require repeated and prolonged damage to the nasal epithelium. Several key points or events determine the mechanism by which formaldehyde induces cancer in rats. First, a single high dose (≤ 40 ppm) for acute durations is not likely sufficient to induce squamous cell carcinoma cancer (Bhalla et al. 1991; Monteiro-Riviere and Popp 1986; Wilmer et al. 1987); repeated exposures for protracted durations are required to induce nasal cancer in rats. Second, the data indicate that a sequence of cellular events must occur in order to induce nasal carcinomas. The induction of nasal cancer in rats by formaldehyde requires repeated exposure for prolonged periods of time to high concentrations that are both irritating and that cause cell damage to a population of the nasal mucosa cells lining the nose. Exposure to high concentrations for prolonged periods during inhalation exposure overwhelms or otherwise exhausts the inherent defense mechanisms to formaldehyde (e.g., mucociliary clearance, metabolism by FDH, DNA repair). This cellular and tissue damage inflicted by unmetabolized formaldehyde is then followed by a regenerative hyperplasia and metaplasia phase (Chang et al. 1983; Feron et al. 1988; Rusch et al. 1983; Wilmer et al. 1987; Woutersen et al. 1987, 1989), which results in increased cell-turnover rates within the mucosa.

Formaldehyde has been demonstrated to be genotoxic in some (but not all) cell lines and test systems (ATSDR 2010; IARC 2012). In occupational studies, formaldehyde exposure has consistently produced evidence of genotoxicity in peripheral blood lymphocytes (EPA 2024e). DNA-protein crosslinks have been demonstrated in experimental animals after inhalation exposure to formaldehyde and can cause mutation or chromosomal aberrations if not repaired prior to cell replication. The DNA damage that occurs in these altered cells is carried into subsequent cell populations and thereby greatly enhances the progression of preneoplastic cells to cancer. In this manner, formaldehyde likely can act as a complete carcinogen (providing initiation, promotion, and progression) with repeated and prolonged duration of exposure at cytotoxic concentrations.

B.4 Health Guidelines

ATSDR (1999) derived an acute-duration inhalation MRL of 0.04 ppm for formaldehyde. The MRL was calculated from a minimal LOAEL of 0.4 ppm for symptoms of increased itching, sneezing, mucosal congestion, and transient burning sensation of the eyes and of the nasal passages, and elevated eosinophil counts and a transient increase in albumin content of nasal lavage fluid in volunteers exposed to formaldehyde for 2 hours (Pazdrak et al. 1993). The LOAEL was divided by an uncertainty factor of 9 (3 for the use of a minimal LOAEL and 3 for human variability).

ATSDR (1999) derived an intermediate-duration inhalation MRL of 0.03 ppm for formaldehyde. The MRL is based on a no-observed-adverse-effect level (NOAEL) of 0.98 ppm for clinical signs of nasopharyngeal irritation (hoarseness and nasal congestion and discharge) and lesions in the nasal epithelium (squamous metaplasia and hyperplasia) observed in Cynomolgus monkeys exposed to formaldehyde for 22 hours/day, 5 days/week for 26 weeks (Rusch et al. 1983). The LOAEL was 2.95 ppm. The NOAEL was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability) to derive the MRL.

ATSDR (1999) derived a chronic-duration inhalation MRL of 0.008 ppm for formaldehyde. The MRL is based on a minimal LOAEL of 0.24 ppm for histological changes (loss of cilia, goblet cell hyperplasia, and cuboidal and squamous cell metaplasia replacing the columnar epithelium) in nasal tissue specimens from a group of 70 workers employed for an average 10.4 years (range 1–36 years) in a chemical plant that produced formaldehyde and formaldehyde resins for impregnating paper (Holmstrom et al. 1989). The MRL was derived by dividing the LOAEL by an uncertainty factor of 30 (3 for the use of a minimal LOAEL and 10 for human variability).

EPA (2024d) reported a chronic RfC of 0.007 mg/m³ (0.006 ppm) for formaldehyde based on decreased pulmonary function and asthma/allergic conditions effects in humans (Annesi-Maesano et al. 2012; Krzyzanowski et al. 1990; Venn et al. 2003).

HHS has categorized formaldehyde as *known to be a human carcinogen* based on sufficient evidence of carcinogenicity in humans (NTP 2021a). EPA (2024d, 2024e) concluded formaldehyde is *carcinogenic to humans* via the inhalation route of exposure (EPA 2005). The inhalation unit risk for formaldehyde is 1.1x10⁻⁵ per µg/m³. IARC (2012) concluded that formaldehyde is *carcinogenic to humans* (Group 1) on the basis of sufficient evidence in humans and sufficient evidence in experimental animals.

B.5 Derivation of Target-Organ Toxicity Dose(s)

The noncancer endpoints of concern for formaldehyde in this mixture are respiratory and developmental toxicity. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2018). The derivations are based primarily on data provided in ATSDR (1999) and, in particular, the Levels of Significant Exposure (LSE) tables.

Respiratory Effects, Intermediate. ATSDR (1999) derived an intermediate-duration inhalation MRL of 0.03 ppm. The MRL is based on a NOAEL of 0.98 ppm for clinical signs of nasopharyngeal irritation (hoarseness and nasal congestion and discharge) and lesions in the nasal epithelium (squamous metaplasia and hyperplasia) observed in Cynomolgus monkeys exposed to formaldehyde for 22 hours/day, 5 days/week for 26 weeks (Rusch et al. 1983). The LOAEL was 2.95 ppm. The MRL was derived by dividing the NOAEL by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability). Note that the NOAEL was not adjusted to a continuous exposure concentration due to evidence that concentration rather than exposure time is the dominant determining factor for formaldehyde-induced respiratory irritation.

Developmental and Reproductive Effects, Intermediate and Chronic. As discussed above, formaldehyde is expected to produce systemic toxicity, including developmental and reproductive toxicity, only at concentrations greater than those producing respiratory effects. The most reliable animal developmental toxicity study is the Saillenfait et al. (1989) prenatal rat developmental toxicity study. In this study, fetal body weight per litter decreased 21% at the nominal concentration of 40 ppm; a 4.6% decrease was observed at 20 ppm, which is not considered adverse. The equivalent measured analytical concentrations were 20.04 and 38.96 ppm. Therefore, the NOAEL and LOAEL for this study are 20.04 and 38.96 ppm, respectively. The NOAEL was adjusted for continuous exposure (6 hours/day) resulting in a NOAEL_{ADJ} of 5.01 ppm. A NOAEL_{HEC} was calculated by multiplying the NOAEL_{ADJ} by the ratio of the blood:gas partition coefficients in animals and humans. Blood:gas partition coefficients for formaldehyde were not provided in ATSDR (1999). Thus, the default ratio of 1 is assumed; the NOAEL_{HEC} is 5.01 ppm. Application of an uncertainty factor of 30 (3 for animal to human extrapolations with dosimetric adjustments and 10 for human variability) yields intermediate- and chronic-duration TTD_{DEVELOP} values of 0.2 ppm.

Respiratory Effects, Chronic. ATSDR (1999) derived a chronic-duration inhalation MRL of 0.008 ppm for formaldehyde. The MRL is based on a minimal LOAEL of 0.24 ppm for histological changes (loss of cilia, goblet cell hyperplasia, and cuboidal and squamous cell metaplasia replacing the columnar epithelium) in nasal tissue specimens from a group of 70 workers employed for an average 10.4 years (range 1–36 years) in a chemical plant that produced formaldehyde and formaldehyde resins for impregnating paper (Holmstrom et al. 1989c). The MRL was derived by dividing the LOAEL by an uncertainty factor of 30 (3 for the use of a minimal LOAEL and 10 for human variability). Note that the LOAEL was not adjusted to a continuous exposure concentration as there is evidence that concentration

rather than exposure time is the dominant determining factor for formaldehyde-induced respiratory effects.

Summary (TTD for Formaldehyde)

Intermediate-Duration Inhalation TTDs:

$MRL_{RESP} = 0.03$ ppm

$TTD_{DEV} = 0.2$ ppm

Chronic-Duration Inhalation TTDs:

$MRL_{RESP} = 0.008$ ppm

$TTD_{DEV} = 0.2$ ppm

B.6 References

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Appendix C. Background Information for Methylene Chloride

This appendix consists primarily of excerpts from the *Toxicological Profile for Methylene Chloride* (ATSDR 2000); primary references are cited for the reader's convenience in identifying pertinent studies. Where relevant, additional information was obtained from reviews and meta-analysis published after the profile. For additional information beyond what is presented here, the reader is referred to the toxicological profile or secondary source.

C.1 Toxicokinetics

Inhalation is the main route of exposure to methylene chloride for humans. Within the first few minutes of exposure, approximately 70–75% of inhaled vapor is absorbed (DiVincenzo and Kaplan 1981). However, as the concentration of methylene chloride in the blood increases, the net uptake is greatly reduced until at steady-state, it is equal to metabolic clearance, which has a maximum (determined by the fraction of blood flowing to the liver) of 25% (EPA 1994). Under conditions of continuous exposure to air concentrations of up to approximately 300 ppm, blood steady-state concentrations of methylene chloride are reached in about 4 hours (DiVincenzo and Kaplan 1981; McKenna et al. 1980). Pulmonary absorption is influenced by exercise and body fat (Astrand et al. 1975; DiVincenzo et al. 1972; Engstrom and Bjurstrom 1977). In animals, pulmonary absorption is proportional to magnitude and duration of exposure over a concentration range of 100–8,000 ppm (AMRL 1972; DiVincenzo et al. 1972; McKenna et al. 1982). An increase of the steady-state blood/air concentration ratio at high exposure levels reflects saturation of metabolic pathways rather than an increased absorption coefficient. There is only qualitative evidence of oral absorption in humans. In animals, methylene chloride is easily absorbed from the gastrointestinal tract, particularly from aqueous media. Seventy-five to 98% of an administered dose may be absorbed in 10–20 minutes (Angelo et al. 1986). There are no quantitative data on dermal absorption of methylene chloride, although it is known to occur.

Distribution data in humans are lacking, but methylene chloride has been found in human breast milk and blood. Methylene chloride is widely distributed in animal tissues after inhalation exposure. The highest concentrations are found in adipose tissue and liver (Carlsson and Hultengren 1975; McKenna et al. 1982). Methylene chloride has been found in blood from rat fetuses. After acute-duration exposure, methylene chloride disappears rapidly from fat. Distribution of methylene chloride does not seem to be route-dependent and it does not bioaccumulate in tissues.

There are two main competing metabolic pathways for methylene chloride; one initially catalyzed by CYP enzymes (CYP2E1) and the other by GSTT1-1. The CYP2E1 pathway (mixed function oxidase [MFO]) produces carbon monoxide (leading to COHb formation) and carbon dioxide via formyl chloride (Gargas et al. 1986; Stewart et al. 1972) and the GSH pathway (GST) produces carbon dioxide via a postulated GSH conjugate (S-chloromethyl glutathione) and formaldehyde. The MFO pathway is a high-affinity, low-capacity pathway with a metabolic rate of 47 $\mu\text{mol/kg/hour}$, while the GST pathway has a lower affinity than the MFO pathway but a higher capacity (Gargas et al. 1986). The oxidative pathway is preferred at lower exposure concentrations and becomes saturated as exposure levels increase. Oxidative biotransformation of methylene chloride is similar in rats and humans. The GST pathway is more active in mice than in rats and less active in hamsters and humans than in rats. In humans, a polymorphism exists in the GSTT1-1 gene, with a percentage of the population unable to metabolize methylene chloride to formaldehyde; the distribution of this polymorphism appears to vary somewhat with ethnic background (for review, see Haber et al. 2002).

After inhalation exposure, humans rapidly eliminate methylene chloride primarily in expired air, although small amounts are eliminated more slowly in the urine (DiVincenzo et al. 1972). In rats, following a single exposure to radioactive methylene chloride, exhaled air had the most radioactivity, but radioactivity was also found in urine and feces (McKenna et al. 1982). In exhaled air, the radiolabel was mostly as carbon monoxide and carbon dioxide.

PBPK models have been developed to describe disposition of methylene chloride in humans and animals. These models were designed to distinguish contributions of the two metabolic pathways in lung and liver tissue, to look for correlations between tumor incidence and various measures of target tissue dose predicted by the models, and to extrapolate cancer risks from mice to humans. For a more detailed discussion of available PBPK models for methylene chloride, see ATSDR (2000).

C.2 Health Effects

The hematological, neurological, respiratory, and hepatic systems are the major target organs of toxicity associated with exposure to methylene chloride.

Hematological Effects. In humans, average blood COHb levels measure <1% in an atmosphere free of carbon monoxide and <4% in a normal atmosphere. Blood COHb concentrations were about 30% higher than normal in two cases of lethal poisoning following acute-duration inhalation of extremely high

concentrations of methylene chloride in air (estimated ~168,000 ppm) in workers who were burying barrels containing mixed solvents and solid chemical waste in a well about 2 m below ground level (Manno et al. 1992). Employees monitored at the end of 1 work day following exposure to methylene chloride at 7–90 ppm (8-hour time-weighted average [TWA]) had average COHb concentrations between 1.7 and 4.0% for nonsmokers, and between 4.95 and 6.35% for smokers (Soden et al. 1996). Additional daily cumulative exposure to methylene chloride did not produce increased levels of COHb. In volunteers who were exposed to methylene chloride at 200 ppm for 4 hours, blood COHb levels rose to approximately 5% (Putz et al. 1979); this was equivalent to the levels seen in volunteers after inhaling 70 ppm of carbon monoxide for 4 hours. In nonsmoking volunteers exposed to 50, 100, 150, or 200 ppm of methylene chloride for 7.5 hours, blood COHb levels rose to 1.9, 3.4, 5.3, and 6.8%, respectively, and blood COHb levels declined immediately following exposure (DiVincenzo and Kaplan 1981).

Other studies in humans reported increases in the red cell count, hemoglobin, and hematocrit in women occupationally exposed to concentrations up to 475 ppm during an 8-hour workday, but no effects were found in men. These effects were judged by the study authors to be suggestive of compensatory hematopoiesis (Ott et al. 1983a). It may be anticipated that stress polycythemia will occur in the majority of individuals, especially cigarette smokers, who are chronically exposed to methylene chloride vapor concentrations in the 500 ppm range.

In animals, no significant hematologic or clinical chemistry alterations were reported in dogs and monkeys exposed continuously to up to 100 ppm methylene chloride for 100 days (Haun et al. 1972). In dogs, COHb increased from 0.5 to about 2% during exposure to 100 ppm methylene chloride, but no significant increase was seen at 25 ppm. In monkeys, COHb levels were approximately 0.5, 1.7, and 4.5% in controls, 25 ppm, and 100 ppm exposed groups, respectively. No treatment-related effects on common hematologic parameters (cell counts, hemoglobin concentration differentials, white cell counts, etc.) were observed among rats chronically exposed to methylene chloride at concentrations up to 3,500 ppm (Burek et al. 1984; Nitschke et al. 1988).

Neurological Effects. A number of human studies reveal that the nervous system is perhaps the most important target of acute methylene chloride toxicity. All 33 cases of acute-duration inhalation exposure to methylene chloride that were reported to occupational health authorities in the United Kingdom between 1961 and 1980 involved depression of the CNS (Bakinson and Jones 1985). Unconsciousness occurred in 13 of these cases and other common effects included headache and dizziness; a few instances of confusion, intoxication, incoordination, and paresthesia were also reported. Acute-duration inhalation

exposure to methylene chloride-based paint strippers in rooms with inadequate ventilation led to unconsciousness in four cases and to generalized seizures in one of these (Hall and Rumack 1990); 10/21 respondents to an occupational health questionnaire reported experiencing dizziness and headache while working in these conditions, but the symptoms abated when they moved to fresh air. In volunteers, a single 4-hour exposure to 200 ppm methylene chloride significantly decreased visual and psychomotor performance and auditory function (Putz et al. 1979). Auditory monitoring, eye-hand coordination, and high-difficulty peripheral brightness test performances were not degraded until the final hour of exposure, by which time, the level of carbon monoxide in exhaled breath had risen to 50 ppm and the level of COHb in blood had risen to 5%. A single 3–4-hour exposure to methylene chloride at 300 ppm caused decreased visual and auditory functions in volunteers, but the adverse effects were reversible once exposure ceased (Fodor and Winneke 1971; Winneke 1974). Winneke (1974) attributed these effects to methylene chloride rather than its metabolite COHb, since exposure to carbon monoxide at concentrations up to 100 ppm did not cause similar effects. At the lowest exposure level (300 ppm of methylene chloride), critical flicker fusion frequency (visual) and auditory vigilance tasks were impaired (Fodor and Winneke 1971). Similarly, psychomotor performance (reaction time, hand precision, steadiness) was impaired, but this occurred at higher exposure levels (800 ppm for 4 hours) (Winneke 1974). Alterations in visual evoked response were observed in humans exposed to methylene chloride at 515–986 ppm for 1–2 hours (Stewart et al. 1972). In another study, there were no effects on electroencephalogram, visual evoked response, or a battery of cognitive effects in humans exposed to concentrations of methylene chloride up to 500 ppm (NIOSH 1974). While some changes in tests related to mood have been reported in humans after acute-duration combined exposure to methylene chloride (28–173 ppm) and methanol (Cherry et al. 1983), no evidence of neurological or behavioral impairment was observed at exposure levels of 75–100 ppm methylene chloride (Cherry et al. 1981). Dementia and gait impairment were reported in one case of a person exposed to methylene chloride (500–1,000 ppm) for 3 years (Barrowcliff and Knell 1979).

No acute CNS effects were observed among 12 Swedish male graffiti removers employed to clean underground stations using methylene-chloride-based solvent compared to the general population (Anundi et al. 1993). The 8-hour TWA to which these workers were exposed ranged from 5 to 340 ppm. No neurological effects, as measured by responses to questions relating to neurotoxicity (e.g., recurring severe headaches, numbness/tingling in hands or feet, loss of memory, dizziness) were reported in a group of 150 employees in a fiber plant occupationally exposed to methylene chloride (mean 8-hour TWA 475 ppm) for >10 years, when compared to a similar, nonexposed cohort (Soden 1993). In a retrospective epidemiology study, there were no significant associations between potential solvent exposure and self-

reported neurological symptoms (based on a standard battery of medical surveillance questions) among workers exposed to a variety of solvents, including methylene chloride, at a pharmaceutical company (Bukowski et al. 1992). However, Bukowski et al. (1992) concluded that questionnaires were not the most appropriate tool to investigate potential neurobehavioral changes caused by low-level exposure to solvents, and recommended the use of neurological test batteries. This caveat would also apply to the study of Soden (1993). The neurotoxicity of occupational exposure to methylene chloride was examined in a cohort study of retired airline mechanics who had been chronically exposed to methylene chloride at concentrations ranging from a mean 8-hour TWA of 105–336 ppm, with short-term, high exposures ranging from 395 to 660 ppm (Lash et al. 1991). None of the measured variables (three tests of physiological characteristics, four tests of psychophysical variables, and six psychological variables) were statistically different between the exposed and control groups. Lack of precision, sampling biases, and random measurement errors might also have affected the results. However, the study authors concluded that overall no effects on the CNS were attributable to chronic, low-level exposures to methylene chloride, a finding they reported as being consistent with that of Cherry et al. (1981).

More recently, Berr et al. (2010) identified increased risks of poor cognitive performance (<25th percentile in the Mini Mental State Examination or the Digit Symbol Substitution Test) among utility company workers with greater than median methylene chloride exposure. It should be noted that these workers were exposed to multiple chlorinated, aromatic, and petroleum solvents and all three classes of solvents were inversely associated with cognitive performance.

Acute-duration studies in animals are consistent with findings in humans that methylene chloride affects the CNS. Narcotic effects of methylene chloride (incoordination, reduced activity, somnolence) were observed in monkeys, rabbits, rats, and guinea pigs exposed to 10,000 ppm for up to 4 hours (Heppel et al. 1944); reduced activity was measured in rats exposed to 5,000 ppm (Heppel and Neal 1944). Dogs exposed to 10,000 ppm for 4 hours, first became uncoordinated, then excited and hyperactive to the extent of bruising themselves, but rapidly recovered afterwards (Heppel et al. 1944). Somatosensory-evoked potentials were altered in rats after 1 hour of exposure to methylene chloride at concentration levels $\geq 5,000$ ppm (Rebert et al. 1989). Decreased levels of succinate dehydrogenase were measured in the cerebellum of rats exposed to 500 ppm of methylene chloride for 2 weeks (Savolainen et al. 1981).

Changes in neurotransmitter amino acids and brain enzymes were observed in gerbils after continuous exposure to 210 ppm for 3 months (Briving et al. 1986; Karlsson et al. 1987; Rosengren et al. 1986). The DNA concentration decreased in the hippocampus and cerebellum in gerbils exposed to ≥ 210 ppm of

methylene chloride, indicating decreased cell density in these brain regions, probably due to cell loss (Karlsson et al. 1987; Rosengren et al. 1986). Methylene chloride (4,500 ppm) did not affect wheel running activity and avoidance learning in rats born to dams exposed prior to and/or during gestation (Bornschein et al. 1980). No treatment-related alterations in sensory evoked potentials, reflexes, posture, or locomotion were observed in rats exposed at 2,000 ppm (Mattsson et al. 1990). Dogs exposed to 5,000 ppm 6 hours/day, 5 days/week for 12 weeks exhibited stupor, incoordination, drowsiness, and loss of bowel and bladder control (DuPont 1949).

Respiratory Effects. Respiratory symptoms (cough, breathlessness, chest tightness) were reported in only 4 of 33 cases of acute-duration inhalation exposure to methylene chloride that were reported to occupational health authorities in the United Kingdom between 1961 and 1980 (Bakinson and Jones 1985); no exposure levels were provided in this study. No pulmonary function abnormalities were found in humans exposed to methylene chloride vapors (50–500 ppm) for 6 weeks (NIOSH 1974). Irritative symptoms of the respiratory tract were more prevalent among 12 Swedish male graffiti removers, employed to clean underground stations by using methylene chloride-based solvent, than those of the general population (Anundi et al. 1993). The 8-hour TWA to which these workers were exposed ranged from 18 to 1,200 mg/m³.

Two clinical case studies (Snyder et al. 1992a, 1992b) were reported in which two men who had been working in confined spaces with a nationally advertised brand of paint remover (consisting of >80% w/w methylene chloride) presented to the hospital emergency department complaining of dyspnea, cough, and discomfort in the midchest. In chest x-rays, each of the patients showed alveolar and interstitial infiltrates. One patient was treated with oxygen and albuterol and his symptoms improved over 48 hours; a repeat chest x-ray showed complete clearing of the infiltrates. During the next year, the patient continued to have episodic cough with wheeze and breathlessness, which improved with albuterol therapy. The patient had no prior history of asthma or cough. A methacholine challenge test verified that he had hyperactive airways. The second patient was treated with oxygen and his symptoms improved during the next 48–72 hours; a repeat chest x-ray taken 3 days later revealed marked, but not complete, resolution of previously-noted lung infiltrates. Ten days later, he was asymptomatic and his chest x-ray was normal. Methylene chloride exposure was not associated with asthma symptoms in Hispanic children (Delfino et al. 2003a, 2003b).

Pulmonary effects were observed in animals that died following exposure to high concentrations of methylene chloride (Heppel et al. 1944). Extreme pneumonia was found in 3/14 guinea pigs exposed to

5,000 ppm for up to 6 months, and pulmonary congestion and edema with focal necrosis was found in 3/5 rabbits and 2/16 rats exposed to 10,000 ppm for up to 8 weeks (Heppel et al. 1944). A high incidence of foreign body pneumonia, involving focal accumulation of mononuclear and multinucleate inflammatory cells, was observed in 10/20 rats exposed to methylene chloride at 8,400 ppm for 13 weeks (NTP 1986a). The significance of this finding is uncertain since the effect was observed only at the highest concentration tested. Male B6C3F1 mice exposed to 4,000 ppm methylene chloride for 6 hours/day, 5 days/week for 13 weeks showed acute club cell (formerly called Clara cell) damage in the lung after a 1-day exposure to methylene chloride, which appeared to resolve after 5 consecutive daily exposures (Foster et al. 1992). The appearance and disappearance of the lesion in club cells correlated well with the activity of CYP monooxygenase in club cells, as assessed immunocytochemically in the whole lung, and biochemically in freshly isolated club cells. Nasal cavity squamous metaplasia was observed in rats exposed intermittently to 1,000 ppm methylene chloride in the NTP (1986a) bioassay.

Hepatic Effects. In animals, inhalation exposures to methylene chloride increased plasma levels of hepatic enzymes and liver weight and produced histopathological changes in the liver characterized by hepatocellular vacuolization and centrilobular fatty or hydropic degeneration in intermediate- and chronic-duration studies (ATSDR 2000). These effects were generally reversible following exposure cessation. The lowest LOAELs for histopathological alterations in the liver are 25 ppm from an intermediate-duration study in rats (Haun et al. 1972) and 200 ppm from a chronic-duration study in rats (Nitschke et al. 1988).

Cancer. No excess risk of death from malignant neoplasms has been detected in workers exposed to methylene chloride at levels up to 475 ppm (Friedlander et al. 1978; Hearne et al. 1987, 1990; Hearne and Pifer 1999; Lanes et al. 1993; Ott et al. 1983b; Tomenson 2011). Some occupational studies (Cantor et al. 1995; Cocco et al. 1999; Gibbs et al. 1996; Heineman et al. 1994; Kumagai et al. 2013) have suggested a correlation between methylene chloride exposure and cancer mortality; dose-response analysis has been minimal or absent and the studies have had considerable limitations, including lack of exposure characterization and co-exposure to other airborne chemicals.

More recently, Gold et al. (2011) identified an increased risk of multiple myeloma with occupational methylene chloride exposure; statistical significance was only achieved when jobs with low confidence in methylene chloride exposure were considered as unexposed. In the Sister Study prospective cohort, Niehoff et al. 2019 identified a potential increased risk of overall and estrogen receptor positive (ER+) breast cancer with methylene chloride exposure based on hazard ratios for the fourth quintile of

methylene chloride air concentrations. A cluster of brain and CNS cancers was identified in the region immediately surrounding a factory that emitted methylene chloride (Makris and Voniatis 2018). Additional epidemiological studies did not identify statistically-significantly increased risks of head and neck cancer (Barul et al. 2017), colorectal cancer (El-Zaemey et al. 2018), lymphoma (Seidler et al. 2007), breast cancer (Garcia et al. 2015), adult chronic lymphocytic leukemia (Talibov et al. 2017), or brain cancer (Neta et al. 2012; Ruder et al. 2013) with methylene chloride exposure. A meta-analysis failed to find a statistically significant association between occupational exposure to methylene chloride and risk of pancreatic cancer (Ojajarvi et al. 2001).

In mice and rats, inhalation of very high levels of methylene chloride significantly increased the incidence of liver and lung cancer (Aiso et al. 2014; Mennear et al. 1988; NTP 1986a) and benign mammary gland tumors (fibroadenomas or adenomas) (Aiso et al. 2014; Mennear et al. 1988; Nitschke et al. 1988a; NTP 1986a, 1994). In rats exposed to low levels of methylene chloride (100 ppm) for 2 years, there was a nonsignificant increase in the total incidence of malignant tumors (Maltoni et al. 1988).

In rats, statistically significant increases in the incidence of mammary gland adenoma or fibroadenoma were identified in males at 4,000 ppm and in females in a dose-responsive manner at $\geq 1,000$ ppm (NTP 1986a). In mice, statistically significant and dose-related increased incidences of alveolar/bronchiolar adenoma or carcinoma and hepatocellular adenoma or carcinoma were observed in males and females at $\geq 2,000$ ppm (NTP 1986a). NTP (1986a) concluded that there was “some evidence of carcinogenicity” in male rats and “clear evidence of carcinogenicity” in female rats based on the increased incidences of benign mammary neoplasms, and that there was “clear evidence for carcinogenicity” for methylene chloride chronic-duration inhalation exposure, based on the increased incidence of alveolar/bronchiolar neoplasms and of hepatocellular neoplasms in male and female mice.

In two related studies, Kari et al. (1993) and Maronpot et al. (1995) examined the progressive development of lung and liver tumors in B6C3F1 mice exposed via chamber inhalation to 2,000 ppm methylene chloride for 6 hours/day, 5 days/week, for 104 weeks. In addition, a series of stop exposure experiments were performed to evaluate the effects of differing exposure durations on tumor development. Kari et al. (1993) examined histology and histopathology of lung and liver tumors, whereas Maronpot et al. (1995) evaluated DNA synthesis and oncogene expression during tumor development. Chronic, high-concentration exposure to methylene chloride resulted in: (1) an 8-fold increase in the incidence of animals having lung adenomas or carcinomas as compared to controls; (2) a 13-fold increase in the total number of lung tumors in each animal at risk; (3) a 2.5-fold increase in the incidence of mice

having liver adenomas or carcinomas compared to controls; and (4) a 3-fold increase in the number of liver tumors in each animal at risk. The development of the first lung tumors in methylene chloride exposed mice occurred 1 year earlier than in control animals. In contrast, there was no difference in the latency to first liver tumor period between exposed and control animals. The incidences of tumors in lungs, but not liver, continued to increase after cessation of exposure. Maronpot et al. (1995) found that 26 weeks of exposure was sufficient to significantly and irreversibly increase the incidence of lung tumors at 2 years, whereas the incidence of hepatic tumors increased with 78 weeks of exposure, but not with 25 or 52 weeks of exposure. Furthermore, vulnerability to methylene chloride may have been age-related, since no lung tumor increase was observed in mice that were kept under control conditions for 52 weeks prior to methylene chloride exposure for 52 weeks. Based on these results, Kari et al. (1993) and Maronpot et al. (1995) concluded that methylene chloride is a more potent lung than liver carcinogen in female B6C3F1 mice; the differing incidence of lung and liver tumors under various exposure regimes suggests that the mechanisms of tumorigenesis in these target organs may be different.

C.3 Mechanisms of Action

Non-neoplastic Mechanisms. In humans, Snyder et al. (1992a, 1992b) reported headache, chest discomfort, cough, and the presence of alveolar and interstitial infiltrates in the lung as a result of short-term high-concentration vapor exposure to methylene chloride in confined, unventilated rooms or basements. In B6C3F1 mice exposed to 4,000 ppm of methylene chloride vapors for 6 hours (Foster et al. 1992), the major initial morphological effect observed in mouse lung was acute club cell damage. However, the damage appeared to resolve after five consecutive daily exposures to methylene chloride. The appearance and disappearance of the lesion in the club cell correlated well with the activity of CYP monooxygenase in the club cell, as assessed immunocytochemically in the whole lung and biochemically in the freshly isolated club cell (as determined by ethoxycoumarin O-dealkylation and aldrin epoxidation).

Over 13 weeks (5 days/week) of exposure, the acute club cell damage, which developed after a 1-day exposure but resolved after 5 consecutive exposures, reappeared on re-exposure after a 2-day weekly break. The severity of the lesion diminished as the study progressed. The study authors suggested that the reason for the decrease or disappearance of the lesion was due to an adaptation/tolerance in the club cell to methylene chloride that was linked to a marked decrease of methylene chloride metabolism by CYP pathways. GST activity in the club cell either remained unchanged or increased following methylene chloride exposure.

Inhalation and ingestion exposures to methylene chloride result in the production of carbon monoxide associated mainly with metabolism via the MFO pathway. Carbon monoxide binds to hemoglobin and can cause carboxyhemoglobinemia. In two fatal human cases of methylene chloride poisoning, COHb was elevated to approximately 30% (Manno et al. 1992). Other reports on human and animals show that COHb increases from baselines from 0–2 to 4–15%, under varying regimes of methylene chloride inhalation exposure.

Neurotoxicity resulting from exposure to methylene chloride is believed to be associated with the lipophilic properties of methylene chloride; however, the precise mechanisms of neurotoxicity are not known. Presumably, the methylene chloride enters cell membranes, which in the case of neurons interferes with signal transmission in a manner similar to general anesthetics (DeJongh et al. 1998; Sikkema et al. 1995). Neurotoxicity is also assumed to be caused by the hypoxia that results from the formation of COHb.

Neoplastic Mechanisms. With regard to tumor induction in the rodent lung and liver, methylene chloride is postulated to be activated to an unknown reactive intermediate via metabolism. There are two major metabolic pathways: the MFO pathway, specifically CYP2E1 and GST-mediated pathway. The MFO pathway is oxidative and appears to yield carbon monoxide as well as considerable amounts of carbon dioxide. The GSH-dependent pathway produces formaldehyde and carbon dioxide, but no carbon monoxide. Potentially reactive intermediates are formed in each of the metabolic pathways for methylene chloride: formyl chloride in the oxidative pathway, and formaldehyde and chloromethyl GSH in the conjugative pathway. Neither formyl chloride nor the GSH conjugate of methylene chloride has been isolated or characterized, although Green (1997) reported that their formation is entirely consistent with available information on GSH-mediated metabolism. Distribution of methylene chloride metabolism between these pathways is dose-dependent. The MFO pathway is a high affinity, limited-capacity pathway that saturates at relatively low atmospheric concentrations (approximately 200–500 ppm). The GST pathway, in contrast, has a lower affinity for methylene chloride, but does not appear to saturate at experimentally produced concentrations (<5,000 ppm). Thus, the MFO pathway accounts for most of the metabolized methylene chloride at concentrations <500 ppm, but as exposure concentrations increase above the MFO saturation level, increases in the amount of methylene chloride metabolized by the secondary GSH pathway are seen (Reitz 1990).

There is no evidence to suggest that methylene chloride is a direct acting carcinogen; the marked species differences in carcinogenicity induced by methylene chloride are not typical behavior of direct-acting

compounds. Methylene chloride also does not exhibit the chemical reactivity towards nucleophiles normally associated with direct action (Green 1997). Therefore, metabolic activation is required, which interacts in some way with mouse tissues to cause tumors.

A series of bacterial mutagenicity tests demonstrated that methylene chloride induction of bacterial mutagenicity is expressed more strongly in *Salmonella typhimurium* TA1535 modified to express a mammalian GST- θ class enzyme (NM5004 strain) than in the original strain (Oda et al. 1996); methylene chloride induction of bacterial mutagenicity *S. typhimurium* strain TA100 is unaffected by the presence of GST- α or - π classes (Simula et al. 1993); methylene chloride is less mutagenic in a *S. typhimurium* GSH-deficient strain (TA100/NG11) as compared to TA100 (Graves et al. 1994a); and bacterial testing with three K12 strains of *Escherichia coli* showed that methylene chloride (activated by S9 mouse liver fraction) and formaldehyde were mutagenic only in the wild-type *E. coli*, a characteristic shared with crosslinking agents; these data initially suggested a mutagenic role for metabolically-derived formaldehyde in *E. coli* (Graves et al. 1994a). These bacterial assays demonstrated that, in *in vitro* tests, methylene chloride was activated by a θ class GST enzyme to a bacterial mutagen in *S. typhimurium* and behaved similarly to formaldehyde in *E. coli* tester strains.

However, in the Chinese Hamster ovary (CHO) assay involving the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene assay, studies of DNA single strand breaks and DNA-protein crosslinks at mutagenic concentrations of methylene chloride and formaldehyde showed that both of these compounds induced DNA single-strand breaks; only formaldehyde induced significant DNA-protein crosslinking (Graves et al. 1996). Similar findings were observed in cultured, freshly isolated mouse hepatocytes (Graves and Green 1996), but not in rat hepatocytes (Graves et al. 1994b, 1995). The study authors concluded that, although formaldehyde might play a role in methylene chloride genotoxicity, its weak mutagenicity and the absence of methylene chloride-induced DNA-protein crosslinking in the CHO/HPRT assay suggested that methylene chloride-induced DNA damage and resulting mutations are likely produced by its GSH conjugate, putatively chloromethylglutathione. Graves and Green (1996) also concluded that these results suggested that the mechanism for methylene chloride tumorigenicity in the mouse liver was likely to be genotoxic and mediated by the GSH pathway. Observed species differences in liver tumorigenicity between the mouse and the rat might result from species differences in the amount of GSH-mediated metabolism induced by methylene chloride exposure. However, the precise mode of action of methylene chloride-induced mouse tumorigenicity has not yet been confirmed (Maronpot et al. 1995).

C.4 Health Guidelines

ATSDR (2000) derived an acute-duration inhalation MRL of 0.6 ppm for methylene chloride, based on a LOAEL of 300 ppm for neurological effects (reduced flicker fusion frequency) in exposed human volunteers (Winneke 1974). A PBPK model for this experiment was used to adjust the dosage to a 24-hour exposure period, thus resulting in a LOAEL of 60 ppm for the same endpoint (Reitz et al. 1997). The MRL was derived by dividing the LOAEL of 60 ppm by an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability).

ATSDR (2000) derived an intermediate-duration inhalation MRL of 0.3 ppm for methylene chloride, based on a LOAEL of 25 ppm for hepatic effects (changes in liver histopathology) in rats exposed to methylene chloride for 14 weeks (Haun et al. 1972). This resulted in an MRL of 0.3 ppm by dividing the LOAEL of 25 ppm by an uncertainty factor of 90 (3 for use of a minimal LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

ATSDR (2000) derived a chronic-duration inhalation MRL of 0.3 ppm for methylene chloride, based on a NOAEL of 50 ppm for hepatic effects (changes in liver histopathology) in rats exposed to methylene chloride for 2 years (Nitschke et al. 1988). The NOAEL of 50 ppm was adjusted for continuous exposure (6 hours/day, 5 days/week) resulting in an adjusted NOAEL (NOAEL_{ADJ}) of 8.92 ppm. The NOAEL_{ADJ} was multiplied by the default blood:air partition coefficient ratio of 1 resulting in a NOAEL_{HEC} of 8.92 ppm; the default ratio was used since the ratio of the blood:air partition coefficient in the rat to the blood:air partition coefficient in the human was >1. This resulted in an MRL of 0.3 ppm by dividing the NOAEL_{HEC} of 8.92 ppm by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

EPA (2011a) derived an RfC of 0.6 mg/m³ (0.2 ppm) for methylene chloride (dichloromethane) based on hepatic effects (hepatic vacuolation) identified in rats exposed via inhalation for two years (Nitschke et al. 1988). EPA utilized a PBPK model to estimate a rat internal dose using a dose metric of mg methylene chloride metabolized via the CYP pathway/liter of liver tissue/day. Benchmark dose analysis was conducted using the rat internal doses. The BMDL₁₀ was multiplied by a pharmacokinetic allometric scaling factor of body weight (BW^{0.75}). The 1st percentile of human equivalent concentration was 17.2 mg/m³. An uncertainty factor of 30 (3 for extrapolation from animals to humans after use a PBPK model to extrapolate internal doses from rats to humans, 3 for human variability after application of a

PBPK model accounting for toxicodynamic differences across human populations, and 3 for database deficiencies due to missing neurodevelopmental toxicity data) was applied to derive the RfC.

HHS (NTP 2021b) has categorized methylene chloride (dichloromethane) as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals. EPA (2011a) classifies methylene chloride (dichloromethane) as *likely to be carcinogenic to humans* according (EPA 2005) based on inadequate human data and sufficient evidence of carcinogenicity in animals; results from available studies include increased incidence of hepatocellular neoplasms and alveolar/bronchiolar neoplasms in male and female mice, and increased incidence of benign mammary tumors in both sexes of rats, salivary gland sarcomas in male rats, and leukemia in female rats. This classification is supported by some positive genotoxicity data, although results in mammalian systems are generally negative. EPA derived an inhalation unit risk of 1×10^{-8} per $\mu\text{g}/\text{m}^3$ for methylene chloride. IARC (2016) classified methylene chloride (dichloromethane) to Group 2A (*probably carcinogenic to humans*) based on sufficient evidence in experimental animals and limited evidence in humans.

C.5 Derivation of Target-Organ Toxicity Dose(s)

The noncancer endpoints of concern for methylene chloride in this mixture are hematological, neurological, respiratory, and hepatic. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2018). The derivations are based primarily on data provided in ATSDR (2000) and, in particular, the LSE tables.

The POD_{HEC} for an extra-respiratory effect is calculated by multiplying the duration-adjusted POD by the ratio of the blood:gas partition coefficients in animals and humans $[(\text{Hb}/\text{g})_{\text{A}} / (\text{Hb}/\text{g})_{\text{H}}]$. Since the partition coefficient for methylene chloride in rodents is greater than in humans (see ATSDR 2018), a default value of 1 is used for the ratio.

Hematological Effects, Intermediate and Chronic Inhalation. In a chronic-duration study in rats exposed to methylene chloride at 0, 50, 200, or 500 ppm for 2 years, blood COHb levels were >10% consistently in animals exposed to 200 ppm (Nitschke et al. 1988). Therefore, a NOAEL of 50 ppm is identified for hematological effects (increased COHb levels) in rats exposed to methylene chloride for 2 years. The NOAEL of 50 ppm was adjusted for continuous exposure (6 hours/day, 5 days/week) and converted to a $\text{NOAEL}_{\text{HEC}}$ of 8.92 ppm as described above. The $\text{TTD}_{\text{HEMATO}}$ of 0.3 ppm was derived by dividing the $\text{NOAEL}_{\text{HEC}}$ of 8.92 ppm by an uncertainty factor of 30 (3 for extrapolation from animals to

humans with dosimetric adjustment and 10 for human variability). The chronic-duration TTD of 0.3 ppm is conservatively adopted as the intermediate TTD for methylene chloride.

Neurological Effects, Intermediate and Chronic Inhalation. ATSDR (2000) derived an acute-duration inhalation MRL of 0.6 ppm for methylene chloride based on a LOAEL of 300 ppm for neurological effects (reduced cranial flicker frequency) in exposed volunteers (Winneke 1974). A PBPK model was used to adjust the dosage to a 24-hour exposure period, thus resulting in a LOAEL of 60 ppm for the same endpoint (Reitz et al. 1997). The MRL was derived by dividing the LOAEL of 60 ppm by an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability). Despite being of shorter duration, this value was used as the TTD for neurological effects, because neurological effects of methylene chloride are generally acute effects that occur at slightly greater levels than other sensitive endpoints of methylene chloride toxicity.

Respiratory Effects, Intermediate and Chronic Inhalation. No effects on pulmonary function have been noted in humans acutely exposed to up to 500 ppm for 6 weeks (NIOSH 1974). In animals, the lowest effects noted following inhalation exposure were nasal squamous cell metaplasia in female rats chronically exposed to 4,000 ppm (mean analytical concentration of 3,982 ppm) methylene chloride for 2 years (NTP 1986a); no statistically significant increase in the incidence of nasal squamous cell metaplasia was identified at the next lowest dose of 2,000 ppm (mean analytical concentration of 2,009 ppm). Rats, guinea pigs, and rabbits exposed to methylene chloride for intermediate durations exhibited pneumonia or pulmonary edema and congestion with focal necrosis at concentrations $\geq 5,000$ ppm (ATSDR 2000). Therefore, the 2,009 ppm NOAEL value from rats was used to derive the TTDs for intermediate and chronic respiratory effects. The NOAEL was adjusted for continuous exposure (6 hours/day, 5 days/week), resulting in a $NOAEL_{ADJ}$ of 359 ppm. For extrathoracic effects, the $NOAEL_{HEC}$ is calculated as the product of the $NOAEL_{ADJ}$ and the regional gas dose ratio (rat:human) for the extrathoracic region of the respiratory tract ($RGDR_{ET}$) (EPA 1994). $RGDR_{ET}$ is calculated as:

$$RGDR_{ET} = \left(\frac{V_E \text{ rat}}{SA_{ET} \text{ rat}} \right) / \left(\frac{V_E \text{ human}}{SA_{ET} \text{ human}} \right)$$

where:

$SA_{ET} \text{ human}$ = surface area of the human extrathoracic region = 200 cm² (EPA 1994)

$SA_{ET} \text{ rat}$ = surface area of the rat extrathoracic region = 15 cm² (EPA 1994)

$V_E \text{ rat}$ = minute volume for female F334/N rats in a chronic-duration study = 0.24 m³ per day (167 mL per minute) (EPA 1988)

$V_E \text{ human}$ = minute volume for humans = 13.8 L per minute = 13,800 mL per minute (EPA 1994)

$$RGDR_{ET} = \left(\frac{V_E \text{ rat}}{SA_{ET} \text{ rat}} \right) / \left(\frac{V_E \text{ human}}{SA_{ET} \text{ human}} \right) = \frac{\frac{167 \text{ mL per min}}{15 \text{ cm}^2}}{\frac{13,800 \text{ mL per min}}{200 \text{ cm}^2}} = 0.161$$

The $NOAEL_{HEC}$ is therefore $359 \text{ ppm} \times 0.161 = 58 \text{ ppm}$. The $NOAEL_{HEC}$ was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability), resulting in a TTD of 2 ppm.

Hepatic Effects, Intermediate Inhalation. ATSDR (2000) derived an intermediate-duration MRL of 0.3 ppm for methylene chloride based on a LOAEL of 25 ppm for hepatic effects (changes in liver histopathology) in rats continuously exposed to methylene chloride for 14 weeks (Haun et al. 1972). The LOAEL of 25 ppm was converted to a $LOAEL_{HEC}$ of 25 ppm as described above. The $LOAEL_{HEC}$ of 25 ppm was divided by an uncertainty factor of 90 (3 for use of a minimal LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, and 10 for human variability) resulting in the MRL of 0.3 ppm.

Hepatic Effects, Chronic Inhalation. ATSDR (2000) derived a chronic-duration MRL of 0.3 ppm for methylene chloride based on a NOAEL of 50 ppm identified for liver histopathology (hepatocellular cytoplasmic vacuolization and multinucleate hepatocytes) in a 2-year study in rats (Nitschke et al. 1988). The NOAEL of 50 ppm was adjusted for continuous exposure (6 hours/day, 5 days/week), resulting in a $NOAEL_{ADJ}$ of 8.92 ppm. The $NOAEL_{ADJ}$ was converted to a $NOAEL_{HEC}$ of 8.92 ppm as described above. This resulted in an MRL of 0.3 ppm by dividing the $NOAEL_{HEC}$ of 8.92 ppm by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability).

Summary (TTD for Methylene Chloride)

Intermediate-Duration Inhalation TTDs:

$$TTD_{HEMATO} = 0.3 \text{ ppm}$$

$$TTD_{NEURO} = 0.6 \text{ ppm}$$

$$TTD_{RESP} = 2 \text{ ppm}$$

$$MRL_{HEPATIC} = 0.3 \text{ ppm}$$

Chronic-Duration Inhalation TTDs:

TTD_{HEMATO} = 0.3 ppm

TTDL_{NEURO} = 0.6 ppm

TTD_{RESP} = 2 ppm

MRL_{HEPATIC} = 0.3 ppm

C.6 References

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Appendix D. Background Information for Nitrogen Dioxide

D.1 Toxicokinetics

The uptake of nitrogen dioxide has been assessed following inhalation exposure in humans. Absorption was between 81 and 90% of the total nitrogen dioxide exposure in healthy volunteers briefly exposed to a nitrogen oxide/nitrogen dioxide mixture (exposure duration not specified) with normal breathing (Wagner 1970). When the subjects were at maximal ventilation, the absorption increased to 91–92% (Wagner 1970). In asthmatic subjects exposed to 0.3 ppm for 30 minutes, the deposition was slightly less, with average uptakes of 72% at rest and 87% during exercise (Bauer et al. 1986). The uptake of nitrogen dioxide in animal studies is similarly extensive, with near-complete absorption in acute-duration studies. For example, Kleinman and Mautz (1987) determined that total respiratory absorption in dogs exposed to up to 5 ppm of nitrogen dioxide was 85% at rest and nearly 100% with high ventilation rates. In animal studies, the uptake of nitrogen dioxide in the upper respiratory tract was 42% for dogs and 28% for rats (Cavanagh and Morris 1987; Yokoyama 1968), indicating considerable absorption in both the upper and lower respiratory tract.

Once deposited, nitrogen dioxide tends to react quickly with respiratory tissues, and the products are rapidly taken up into the bloodstream. The primary products found in the blood are NO_2^- and NO_3^- , created by the reaction of nitrogen dioxide with water in the tissues to form nitrous and nitric acids (Goldstein et al. 1977; Saul and Archer 1983). Following high-level exposure (5–40 ppm) of nitrogen dioxide for 1 hour in mice, a concentration-dependent increase was seen in both NO_2^- and NO_3^- , which declined rapidly after the termination of exposure.

D.2 Health Effects

Cardiovascular Effects. Meta-analyses of epidemiological study results have identified increased risks of heart failure (Jia et al. 2023; Zhang et al. 2023) and stroke hospital admission and mortality (Niu et al. 2021) with nitrogen dioxide exposure. Although Mustafic et al. (2012) reported an increased risk of myocardial infarction with nitrogen dioxide via meta-analysis, Stanley Young and Kindzierski (2019) have called the results into question based on possible manipulation of p-values in the base publications. Nitrogen dioxide exposures are also associated with increased cardiovascular mortality (Chen et al. 2024) and increased mortality rates caused by circulatory disease, ischemic heart diseases, and cerebrovascular disease (Kasdagli et al. 2024). Analysis of human genome-wide association study (GWAS) data

identified no associations between acute myocardial infarction, heart failure, or stroke with nitrogen dioxide exposure (Wang et al. 2024).

Neurological Effects. Meta-analyses of epidemiological results involving nitrogen dioxide exposures have identified increased risks of dementia (Abolhasani et al. 2023; Jones et al. 2025; Tang et al. 2023), seizures (Antaya et al. 2024), and attention-deficit/hyperactivity disorder (ADHD) (Thygesen et al. 2020). Mixed results for Parkinson's disease (Hu et al. 2019; Xie et al. 2025), autism spectrum disorders (Amnuaylojaroen et al. 2024; Duque-Cartagena et al. 2024), and depression (Borroni et al. 2022; Fan et al. 2020) were identified via meta-analyses. Parasin et al. (2023) did not identify an association between nitrogen dioxide exposure and gross motor development in children. Nitrogen dioxide exposure was not associated with Alzheimer's disease (Fu and Yung 2020; Xie et al. 2025) or Alzheimer's disease dementia (Jones et al. 2025) in meta-analyses. Analysis of GWAS datasets identified mixed results for associations between Parkinson's disease and nitrogen dioxide exposure (Wang et al. 2024; Yi et al. 2025).

Respiratory Effects. Exposures of healthy subjects to ≤ 4 ppm for up to 2 hours have generally been without noticeable effects on lung function (Goings et al. 1989; Hackney et al. 1978), although some studies have noted changes in airway responsiveness in healthy volunteers exposed to ≥ 2 ppm nitrogen dioxide, particularly when challenged with methacholine (Abe 1967; Beil and Ulmer 1976; Mohsenin 1988; von Nieding and Wagner 1977, 1979). Exposure to similar levels of nitrogen dioxide (~ 2 ppm or greater for ≥ 2 hours) has resulted in increases in the number and/or percentages of pulmonary immune cell populations (lymphocytes and polymorphonuclear cells) in healthy volunteers (Becker et al. 1993; Devlin et al. 1992; Frampton et al. 1989; Sandstroem et al. 1989, 1990).

Asthmatics or patients with chronic obstructive pulmonary disease (COPD) are much more sensitive to effects of nitrogen dioxide, with changes in airway function generally reported at ≥ 0.3 ppm (Bauer et al. 1986; Morrow and Utell 1989; Roger et al. 1990), but with isolated reports of effects at concentrations as low as 0.12–0.14 ppm (Bylin et al. 1988; Koenig et al. 1985).

There have been isolated reports that higher levels of nitrogen dioxide ($> 7,520 \mu\text{g}/\text{m}^3$, 4.0 ppm) can decrease arterial oxygen partial pressure (PaO_2) in exposed humans (von Nieding and Wagner 1977, 1979) and cause a small decrease in systemic blood pressure (Linn et al. 1985). However, the impact of such changes is not clear, especially considering the generally high concentrations of nitrogen dioxide required.

In meta-analyses of epidemiological data, nitrogen dioxide exposure has been associated with increased respiratory mortality (Chen et al. 2024; Huangfu and Atkinson 2020) and increased mortalities from respiratory disease (Kasdagli et al. 2024), COPD (Huangfu and Atkinson 2020; Kasdagli et al. 2024), and acute lower respiratory infections (Huangfu and Atkinson 2020; Kasdagli et al. 2024). Nitrogen dioxide exposures were associated with increased children's respiratory disease outpatient visits (Zheng et al. 2022). Associations between nitrogen dioxide exposures and COPD were mixed, with increased risks identified in two studies (Chen et al. 2022; Zhang et al. 2018) and no association identified in one meta-analysis (Park et al. 2021). Nitrogen dioxide exposures are associated with increased risks of childhood asthma (Chen et al. 2022; Han et al. 2021) and asthma-related emergency room visits or hospitalizations (Zheng et al. 2015). Nitrogen dioxide exposures are not associated with acute exacerbation of idiopathic pulmonary fibrosis (Lan et al. 2024). Analysis of human GWAS data identified no associations between COPD or pneumonia with nitrogen dioxide exposure (Wang et al. 2024).

Nitrogen dioxide has been shown to elicit a variety of respiratory effects in animal studies. Exposures to ≥ 5 ppm of nitrogen dioxide (Giordano and Morrow 1972; Kita and Omichi 1974), but not ≤ 1 ppm (Schlesinger et al. 1987), result in changes in bronchial ciliated cells and a decrease in mucociliary clearance. Mice exposed to 0.5 ppm nitrogen dioxide continuously for 1 week, with a peak exposure of 2 ppm for 1 hour once/day, showed changes in macrophage morphology (Aranyi et al. 1976); exposure to lower levels did not result in altered macrophage morphology. Exposure to low levels of nitrogen dioxide (< 1 ppm) may result in increased macrophage-mediated clearance (Schlesinger 1987a, 1987b; Schlesinger and Gearhart 1987). Long-term exposure (6 months) of baboons to 2 ppm nitrogen dioxide resulted in decreased macrophage migration, suggestive of impaired clearance (Greene and Schneider 1978). Changes in lung morphology generally do not occur below 5 ppm nitrogen dioxide in animal studies.

Developmental Effects. Meta-analyses of epidemiological data have identified increased risks of heart anomalies, specifically coarctation of aorta (Chen et al. 2014; Hu et al. 2020; Vrijheid et al. 2011). Mixed results were obtained for preterm birth, with one study identifying an association with nitrogen dioxide exposures (Chen et al. 2022), while no associations were identified between preterm birth and nitrogen dioxide exposure in additional studies (Simoncic et al. 2020; Stieb et al. 2012). Nitrogen dioxide exposure was not associated with risks of low birth weight (Simoncic et al. 2020) or orofacial clefts (Huang et al. 2023b). Maternal exposures during gestation were associated with increased risks of atopic dermatitis, asthma, and hay fever in children (Ai et al. 2024). Shang et al. (2020) identified decreased children's global psychomotor and fine psychomotor scores with prenatal nitrogen dioxide exposures.

Immunological Effects. Animal studies have identified changes in immunological endpoints as a sensitive endpoint for exposure to nitrogen dioxide; however, such effects have, in most cases, been localized to the lung. Richters and Damji (1988, 1990) noted decreases in the total proportion of T-cells in mice exposed to 0.25 ppm nitrogen dioxide for 7 or 36 weeks. Intermediate- or chronic-duration exposure to 1–2 ppm has resulted in other immunologic changes, including decreases in circulating IgG, IgM, and IgA levels, hemolytic activity of complement, or splenic natural killer cell activity in mice, squirrel monkeys, and/or guinea pigs (Ehrlich et al. 1975; Fenters et al. 1973; Kosmider et al. 1973; Lefkowitz et al. 1986). Mice exposed continuously to 0.5 ppm, with 1 hour peak exposures of 1 ppm twice daily, for 15 days showed an increased mortality to streptococcus infection (Gardner 1980, 1982; Graham et al. 1987); this was not seen in mice exposed continuously to 0.05 ppm with peak exposures of 0.1 ppm. Similar effects on susceptibility to infection have been reported at higher nitrogen dioxide concentrations for shorter durations (Coffin et al. 1977; Gardner et al. 1977a, 1977b; Ito 1971; McGrath and Oyervides 1985).

Meta-analyses of epidemiological study results indicate that nitrogen dioxide does not increase the risk of death from SARS-CoV-2 infection (Houweling et al. 2024) but does increase the risk of eczema and childhood atopic dermatitis (Huang et al. 2023a) and risk of influenza infection incidence (Sun et al. 2024).

Cancer. Meta-analyses of epidemiological studies have identified increased risks of lung cancer (Chen et al. 2022; Hamra et al. 2015), breast cancer (Praud et al. 2023; Wei et al. 2021), and childhood acute lymphoblastic leukemia (Filippini et al. 2015) with nitrogen dioxide exposure. Nitrogen dioxide exposure is also associated with increased lung cancer mortality (Kasdagli et al. 2024). To date, the HHS, EPA, and IARC have not evaluated nitrogen dioxide's carcinogenic potential.

D.3 Mechanisms of Action

Nitrogen dioxide is a free radical gas, with a single unpaired electron on the nitrogen atom. As such, it is a highly reactive compound and capable of easily oxidizing cellular molecules. As described in Section D.1, nitrogen dioxide in the body quickly reacts to nitrous and nitric acids or reactive nitrogen species (including peroxyxynitrite). These reactions may result in a variety of changes, including cellular damage, lipid peroxidation, and interaction with cellular proteins and thiols, depending on the susceptibility of cellular molecules to nitrogen radical interaction. Persinger et al. (2002) is a published

review of basic molecular mechanisms of nitrogen dioxide-induced lung injury. Li et al. (2024) suggested that nitrogen dioxide or its reaction products may induce neurodegenerative diseases via mitochondrial dysfunction, oxidative stress, inflammation, and/or protein buildup.

D.4 Health Guidelines

ATSDR has not derived MRLs for nitrogen dioxide for any exposure duration or route.

EPA does not list an RfC or cancer classification for nitrogen dioxide on EPA (2024g). The NAAQS values for nitrogen dioxide are 53 ppb (0.053 ppm) with a 1-year averaging time and 100 ppb (0.1 ppm) with a 1-hour averaging time (EPA 2018).

For indoor air quality, WHO (2010b) established a 1-hour guideline of 200 $\mu\text{g}/\text{m}^3$ (106 ppb) based on minor alterations to pulmonary function in asthmatics at $\geq 500 \mu\text{g}/\text{m}^3$ and an annual average guideline of 40 $\mu\text{g}/\text{m}^3$ (21 ppb) based on an increased risk of childhood lower respiratory illness at $\geq 43 \mu\text{g}/\text{m}^3$. WHO (2021) has established a recommended AQG of 10 $\mu\text{g}/\text{m}^3$ (5.3 ppb) for nitrogen dioxide based on increased risks for all-cause and respiratory mortality (Huangfu and Atkinson 2020).

HHS, EPA, and IARC have not categorized the carcinogenicity of nitrogen dioxide.

D.5 Derivation of Target-Organ Toxicity Dose(s)

The endpoints of concern for nitrogen dioxide in this mixture are respiratory and immunological. These endpoints have sufficient animal data to allow derivation of TTDs. Additional endpoints of concern may be relevant for human health (see Section D.2 above) but insufficient quantitative data are available for these endpoints. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2018).

Respiratory Effects, Intermediate and Chronic Inhalation. Short-term exposures of healthy adults to nitrogen dioxide have noted small changes in pulmonary function at concentrations as low as 2 ppm (Abe 1967; Beil and Ulmer 1976; Mohsenin 1988; von Nieding and Wagner 1977, 1979). Asthmatics and people with COPD, who represent a sensitive population for the respiratory effects of nitrogen dioxide, have generally shown no effects at ≤ 0.25 ppm (Jörres and Magnussen 1990, 1991). Changes in pulmonary function in asthmatics begin at 0.3 ppm (Bauer et al. 1986) and progress with increasing

concentration. The NOAEL for nitrogen dioxide-induced pulmonary changes in asthmatics is therefore 0.25 ppm. Since it occurs in a sensitive population, a toxicodynamics uncertainty factor was not applied and a toxicokinetics uncertainty factor of 3 was applied to account for human variability. However, pulmonary changes in asthmatics may occur independent of exposure duration and the exposure duration for the critical study was short (<1 hour), a modifying factor of 3 was applied to adjust for longer-duration exposures. The TTD was therefore $0.25 \text{ ppm} \div (3 \times 3) = 0.03 \text{ ppm}$ and is applied to intermediate and chronic exposure durations.

Summary (TTD for Nitrogen Dioxide)

Intermediate-Duration Inhalation TTDs:

$$\text{TTD}_{\text{RESP}} = 0.03 \text{ ppm}$$

Chronic-Duration Inhalation TTDs:

$$\text{TTD}_{\text{RESP}} = 0.03 \text{ ppm}$$

D.6 References

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Appendix E. Background Information for Tetrachloroethylene

This appendix was written based primarily on the *Toxicological Profile for Tetrachloroethylene* (ATSDR 2019); primary references are cited for the reader's convenience in identifying pertinent studies. Where relevant, additional information was obtained from reviews and meta-analysis published after the profile. For additional information beyond what is presented here, the reader is referred to the toxicological profile or secondary source.

E.1 Toxicokinetics

Results from human and animal studies indicate that inhaled tetrachloroethylene is rapidly and efficiently absorbed by the lungs (ATSDR 2019). For example, in rats given nose-only inhalation exposures to 50 or 500 ppm for 3 hours, near steady-state exhaled breath concentrations were attained within about 20 minutes and were proportional to concentration (Dallas et al. 1994b). Total uptake of tetrachloroethylene increased with exposure concentration, but was not linearly proportional to concentration, consistent with an influence of saturable metabolism on pulmonary uptake. Studies with rats, mice, and dogs indicate that ingested tetrachloroethylene is rapidly and completely absorbed (Dallas et al. 1994a, 1995; Frantz and Watanabe 1983; Pegg et al. 1979). When applied to the skin as a liquid, tetrachloroethylene also is rapidly absorbed. Tetrachloroethylene was detected in exhaled breath of humans shortly after immersion of one thumb in liquid tetrachloroethylene; a peak concentration was attained after about 40 minutes of exposure (Stewart and Dodd 1964). Other human studies indicate, however, that skin absorption of tetrachloroethylene vapor contributes only a small portion of absorbed body burden compared with pulmonary absorption.

Once absorbed, tetrachloroethylene is distributed widely throughout the body, with preferential distribution to fatty tissue including maternal breast milk. Tetrachloroethylene is capable of crossing the placenta and reaching the developing fetus (ATSDR 2019). Estimated partition coefficients for tetrachloroethylene in human tissues and liquids are 10–20 for blood/air, 1,450–1,650 for fat/air, and 125–159 for fat/blood; these values are consistent with ready partition into blood from air and preferential distribution to fatty tissue. In humans exposed to airborne concentrations up to 144 ppm for 4 hours, exhalation of unmetabolized tetrachloroethylene was the predominant route of elimination (Monster et al. 1979). Urinary excretion of metabolites represented a small percentage (1–2%) of absorbed doses. Half-lives of tetrachloroethylene in highly perfused tissue, muscle tissue, and fatty tissue of humans have been

estimated at 12–16, 30–40, and 55 hours, respectively. In rats exposed to 10 ppm radiolabeled tetrachloroethylene, 68 and 3.6% of the absorbed radioactivity was exhaled as the parent material and carbon dioxide, respectively, over a 72-hour period; 24% of absorbed radioactivity was accounted for as nonvolatile urinary and fecal metabolites and 3–4% remained in the carcasses (Pegg et al. 1979). Metabolic saturation ensued with exposure to higher concentrations (600 ppm), as 88, 9, and 2% of the absorbed dose was accounted for by exhalation of parent chemical, urinary and fecal metabolites, and radioactivity remaining in the rat carcasses. The limited extent to which tetrachloroethylene is metabolized in rats is not dramatically influenced by induction of CYP isozymes. For example, in rats pretreated with phenobarbital before intraperitoneal injection with 1,474 mg/kg trichloroethylene/kg or 1,632 mg/kg tetrachloroethylene, rates of appearance of trichloroethylene metabolites in urine during 2-hour periods for up to 10 hours after injection were approximately 200–1,000-fold higher than rates for tetrachloroethylene metabolites (Ikeda and Imamura 1973). In contrast to humans and rats, mice appear to metabolize tetrachloroethylene more rapidly and completely. Following inhalation exposure of mice to 10 ppm radiolabeled tetrachloroethylene, urinary metabolites accounted for >80% of the absorbed dose (Schumann et al. 1980).

Metabolism of tetrachloroethylene to trichloroacetic acid, the principal metabolite, involves initial saturable catalysis by CYP isozymes to produce a reactive epoxide intermediate (tetrachloroethylene oxide), that can potentially bind to cellular macromolecules or rearrange to trichloroacetyl chloride (ATSDR 2019). Trichloroacetyl chloride is further oxidized to trichloroacetic acid. The liver is the predominant site of metabolism and CYP2B1/2 is an important isozyme in tetrachloroethylene metabolism. Pretreatment of rats with phenobarbital (an inducer of CYP2B1/2) or Aroclor 1254 (an inducer of CYP2B1/2 and 1A1/2 isozymes) before oral administration of 1,244 mg tetrachloroethylene/kg body weight increased the rates of urinary excretion of tetrachloroethylene metabolites by about 5–7-fold (Moslen et al. 1977).

Other metabolic pathways for tetrachloroethylene include one that leads from tetrachloroethylene oxide to oxalic acid and formic acid formation via catalysis by epoxide hydrolase and another involving initial conjugation of tetrachloroethylene with GSH via GST (ATSDR 2019). The GSH conjugate can be transported to the kidney where it can be hydrolyzed by β -lyase, producing a reactive thiol compound that is thought to bind to cellular macromolecules and lead to renal cytotoxicity. Small amounts of trichloroethanol have also been detected in the urine of workers exposed to tetrachloroethylene, but it has been proposed that the trichloroethanol derives from metabolism of trichloroethylene contamination of tetrachloroethylene rather than metabolism of tetrachloroethylene (ATSDR 2019). Evidence is available that

mice have a greater hepatic capacity for total tetrachloroethylene metabolism than rats, which in turn have a higher capacity than humans.

PBPK models have been developed to describe the disposition of tetrachloroethylene in mice, rats, and humans and to predict doses of proposed carcinogenic metabolites in target organs for the purpose of assessing human cancer risks based on rodent exposure-response data (ATSDR 2019). Further development to link models for different chlorinated hydrocarbons that share metabolic pathways may be useful to predict dispositional and toxicological outcomes of possible interactions.

E.2 Health Effects

Neurological Effects. Studies of occupationally exposed humans as well as of humans under acute-duration controlled conditions indicate that neurological effects are the most predominant and sensitive effects of tetrachloroethylene (ATSDR 2019). Observed effects include neurological symptoms such as headache, dizziness, and drowsiness in subjects exposed to 100 ppm for 7 hours, increased latency of pattern reversal visual-evoked brain potentials and performance deficits in tests of vigilance and eye-hand coordination in subjects exposed to 50 ppm, 4 hours/day for 4 days, and increased incidence of subjectively reported symptoms, such as dizziness and forgetfulness, in workers repeatedly exposed to average concentrations of about 20 ppm (ATSDR 2019).

Hepatic Effects. In controlled human exposure studies, no changes in serum levels of hepatic enzymes were identified following acute-duration exposures to up to 150 ppm tetrachloroethylene or following exposures to up to 100 ppm for 5.5 hours/day, 5 days/week for 11 weeks (ATSDR 2019). While some hepatotoxicity in workers exposed to tetrachloroethylene has been reported, results of studies evaluating effects of occupational exposures to tetrachloroethylene are likely confounded due to co-exposure to other chemicals.

Liver effects also have been observed in rats and mice repeatedly exposed to inhaled or ingested tetrachloroethylene, but mice appear more sensitive than rats (ATSDR 2019). For example, hepatocellular degeneration and necrosis were found in male mice exposed for 2 years to air concentrations ≥ 100 ppm, and increased liver tumors developed in both sexes of mice under these conditions (NTP 1986b). In contrast, rats exposed for 2 years to concentrations up to 400 ppm showed no increased incidence of non-neoplastic or neoplastic hepatic lesions (NTP 1986b). In shorter-term experiments, mice exposed for 14–28 days to 200 or 400 ppm in air showed hepatocellular vacuolization and proliferation of peroxisomes,

whereas rats under these conditions showed no proliferation of hepatic peroxisomes and less severe hepatocellular changes (i.e., hypertrophy) (Odum et al. 1988).

Renal Effects. Associations have also been made between human exposure to tetrachloroethylene and subtle renal effects in tetrachloroethylene-exposed workers (e.g., increased levels of enzymes or other proteins in urine) (ATSDR 2019). For example, a retrospective cohort study of drycleaning workers with tetrachloroethylene exposures identified an increased risk of hypertensive end-stage renal disease (Calvert et al. 2011). Renal effects have been observed in rats and mice chronically exposed to inhaled or ingested tetrachloroethylene. Rats and mice of both sexes exposed for 2 years to tetrachloroethylene air concentrations ≥ 200 and 100 ppm, respectively, showed dose-related renal tubular cell karyomegaly (nuclear enlargement) (NTP 1986b). Nephropathy was observed in rats and mice exposed to gavage doses ≥ 471 and 386 mg/kg/day, respectively (NCI 1977).

Cancer. In animal studies, tetrachloroethylene produced kidney cancer and mononuclear cell leukemia in rats following inhalation exposures and liver cancer in mice following gavage and inhalation exposures (ATSDR 2019). There are positive associations between tetrachloroethylene and bladder cancer incidence in humans, suggestive evidence that it causes lymphomas, and limited evidence for lung, liver, kidney, and cervical cancers. HHS (NTP 2021c) categorized tetrachloroethylene as *reasonably anticipated to be a human carcinogen* based on sufficient evidence in experimental animals, and IARC (2014) classified tetrachloroethylene to Group 2A (*probably carcinogenic to humans*) based on limited evidence in humans and sufficient evidence in experimental animals. EPA (2012) classified tetrachloroethylene as *likely to be carcinogenic to humans* based on conclusive evidence in experimental animals and suggestive evidence for carcinogenicity in humans.

E.3 Mechanisms of Action

Nervous system depression appears to be the most sensitive effect in humans from exposure to tetrachloroethylene, regardless of exposure route, and is thought to be caused predominantly by the parent compound (ATSDR 2019). Likely mechanisms of action include tetrachloroethylene-induced changes in the fatty acid pattern of neuronal membranes or the direct effect of incorporation of tetrachloroethylene in the membranes leading to an alteration in membrane structure and function. Possible contributions from metabolites cannot be conclusively ruled out but appear unlikely given the slow rates at which tetra-

chloroethylene is expected to be metabolized in humans. Trichloroethanol, a metabolite of trichloroethylene that is a potent neurotoxic agent, does not appear to be a metabolite of tetrachloroethylene (ATSDR 2019).

Liver and kidney effects observed in animals exposed to tetrachloroethylene have been proposed to be caused by reactive metabolic intermediates: a proposed reactive epoxide product of CYP catalysis in the liver; reactive oxygen species from proliferation of peroxisomes by trichloroacetic acid, the principal metabolite of tetrachloroethylene; and a reactive thiol product produced by hydrolysis of GSH conjugates via β -lyase catalysis in the kidney (ATSDR 2019). The latter reaction has been proposed to gain importance at high exposure concentrations when rates of elimination of the parent chemical in exhaled breath are maximized and CYP catalysis is saturated. The initial liver reaction leading to the thiol product, GSH conjugation, competes for tetrachloroethylene as a substrate. The relevance of the observed rat kidney effects to humans has been questioned because GSH conjugation activity was not detected in human liver preparations, β -lyase activities were low in human kidney preparations, and some of the kidney effects appear to be due to accumulation of α -2 μ -globulin, a protein that is produced in male rats but not in female rats or humans of either sex (ATSDR 2019). Evidence that metabolites may be involved in tetrachloroethylene hepatotoxicity includes the observation that pretreatment of rats with Aroclor 1254 before oral administration of 7.5 mmol tetrachloroethylene/kg (1,244 mg/kg) increased rates of urinary excretion of tetrachloroethylene metabolites and increased levels of serum AST compared with levels in non-pretreated rats (Moslen et al. 1977). The relevance of tetrachloroethylene-induced rodent liver effects to humans has been questioned based on evidence that humans produce little trichloroacetic acid from tetrachloroethylene (i.e., rates of total tetrachloroethylene metabolism in humans are low compared to rates in mice), mice and rats respond to trichloroacetic acid by induction of hepatocellular peroxisomes (that produce tissue damaging substances), and humans are relatively insensitive to the induction of hepatocellular peroxisomes (ATSDR 2019; Lake 1995).

E.4 Health Guidelines

ATSDR (2019) derived acute-, intermediate, and chronic-duration inhalation MRLs of 0.006 ppm for tetrachloroethylene based on an exposure duration-adjusted LOAEL of 1.7 ppm for color vision decrements in an occupational exposure study (Cavalleri et al. 1994), an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability), and a modifying factor of 3 for database deficiencies (inadequate information on low-dose immune system effects).

EPA (2012) derived an RfC of 0.04 mg/m³ (0.006 ppm) for tetrachloroethylene based on development of independent candidate chronic RfCs for two endpoints: altered reaction time and cognitive effects after occupational exposure (Echeverria et al. 1995) and altered color vision in occupationally-exposed adults (Cavalleri et al. 1994). EPA identified a LOAEL of 56 mg/m³ for the Echeverria et al. (1995) study and applied a total uncertainty factor of 1,000 (10 interindividual variability, 10 for extrapolation from a LOAEL to a NOAEL, and 10 for database uncertainty) to produce a candidate RfC of 0.056 mg/m³. EPA identified a LOAEL of 15 mg/m³ for the Cavalleri et al. (1994) study and applied a total uncertainty factor of 1,000 (10 interindividual variability, 10 for extrapolation from a LOAEL to a NOAEL, and 10 for database uncertainty) to produce a candidate RfC of 0.015 mg/m³. The RfC of 0.04 mg/m³ was identified as the midpoint between the two candidate RfCs of 0.056 and 0.015 mg/m³.

HHS (NTP 2021c) categorized tetrachloroethylene as *reasonably anticipated to be a human carcinogen* based on sufficient evidence in experimental animals. EPA (2012) classified tetrachloroethylene as *likely to be carcinogenic to humans* based on suggestive evidence for carcinogenicity in humans and conclusive evidence in experimental animals. EPA established an inhalation unit risk of 2.6x10⁻⁷ per µg/m³ for tetrachloroethylene. IARC (2014) classified tetrachloroethylene as *probably carcinogenic to humans* (Group 2A) based on sufficient evidence in experimental animals and limited evidence in humans.

E.5 Derivation of Target-Organ Toxicity Dose(s)

The noncancer endpoints of concern for tetrachloroethylene in this mixture are neurological and hepatic. TTDs are derived below for endpoints that are not the basis of the MRL, using the methods described by ATSDR (2018). The derivations are based primarily on data provided in ATSDR (2019) and, in particular, the LSE tables.

The POD_{HEC} for an extra-respiratory effect is calculated by multiplying the duration-adjusted point of departure by the ratio of the blood:gas partition coefficients in animals and humans [(Hb/g)_A / Hb/g)_H]. Since the partition coefficient for tetrachloroethylene in rodents is greater than in humans (see ATSDR 2019), a default value of 1 is used for the ratio.

Neurological Effects, Intermediate. ATSDR (2019) derived an intermediate-duration inhalation MRL of 0.006 ppm for tetrachloroethylene based on a LOAEL of 7.3 ppm for decreased color vision in tetrachloroethylene-exposed workers with an average exposure of 106 months (Cavalleri et al. 1994). The LOAEL was duration-adjusted for continuous exposure (8 hours/day, 5 days/week) to 1.7 ppm and

an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability) and a modifying factor of 3 (for database deficiencies) was applied.

Hepatic Effects, Intermediate. Kjellstrand et al. (1984) identified a LOAEL of 9 ppm based on liver enlargement and vacuolization of hepatocytes in mice exposed continuously (i.e., 24 hours/day) for 30 days. The LOAEL was converted to a LOAEL_{HEC} of 9 ppm as described above. Application of an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, and 10 for human variability) results in a TTD_{HEPATIC} of 0.03 ppm.

Neurological Effects, Chronic. ATSDR (2019) derived a chronic-duration inhalation MRL of 0.006 ppm for tetrachloroethylene based on a LOAEL of 7.3 ppm for decreased color vision in tetrachloroethylene-exposed workers with an average exposure of 106 months (Cavalleri et al. 1994). The LOAEL was duration-adjusted for continuous exposure (8 hours/day, 5 days/week) to 1.7 ppm and an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability) and a modifying factor of 3 (database deficiencies) was applied.

Hepatic Effects, Chronic. Long-term studies in rats have shown no hepatic effects at tetrachloroethylene concentrations up to 400 ppm (NTP 1986b). However, mice appear to be more sensitive, with hepatic degeneration and necrosis found in male mice exposed to ≥ 100 ppm tetrachloroethylene for 2 years (NTP 1986b); exposure levels < 100 ppm were not evaluated. The 100 ppm LOAEL in mice was duration-adjusted (6 hours/day, 5 days/week) to a LOAEL_{ADJ} of 18 ppm and converted to a LOAEL_{HEC} of 18 ppm as described above. Application of an uncertainty factor of 300 (3 for animal to human extrapolations with dosimetric adjustment, 10 for human variability, and 10 for use of a LOAEL) to the LOAEL_{HEC} results in a TTD_{HEPATIC} of 0.06 ppm. This value is higher than the intermediate-duration TTD_{HEPATIC} of 0.03 ppm identified in mice exposed continuously to 9 ppm for 30 days (Kjellstrand et al. 1984). Since chronic-duration exposure levels below the LOAEL of 100 ppm were not evaluated, the chronic-duration database is considered inadequate to identify a sensitive TTD_{HEPATIC}. Therefore, the adoption of the intermediate-duration TTD_{HEPATIC} of 0.03 ppm is recommended.

Summary (TTD for Tetrachloroethylene)

Intermediate-Duration Inhalation TTDs:

MRL_{NEURO} = 0.006 ppm

TTD_{HEPATIC} = 0.03 ppm

Chronic-Duration Inhalation TTDs:

MRL_{NEURO} = 0.006 ppm

TTD_{HEPATIC} = 0.03 ppm

E.6 References

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Appendix F. Database Query Strings for Combinations of Carbon Monoxide, Formaldehyde, Methylene Chloride, Nitrogen Dioxide, and Tetrachloroethylene

Information to prepare this profile was obtained via searches of the literature. The search objective was to identify noncancer and cancer toxicity, toxicokinetic, and interaction data from studies of humans and laboratory animals, as well as mechanistic studies using tissue, cell, or *in vitro* systems.

Initial searches of PubMed and Embase was conducted in November 2024 to identify references with records mentioning two or more of the four compounds of interest (carbon monoxide, formaldehyde, methylene chloride, nitrogen dioxide, and tetrachloroethylene) using Chemical Abstracts Service Registry Numbers (CASRN) and synonyms. Agency review documents were also collected for each of the compounds when available from IARC, EPA Integrated Risk Information System (IRIS) summaries and reviews, and National Toxicology Program (NTP). Table F-1 presents the CASRN and names of the compounds, as well as synonyms used in the search. The ATSDR Toxicological Profiles for carbon monoxide, formaldehyde, methylene chloride, nitrogen dioxide, and tetrachloroethylene were consulted to identify CASRN. Synonyms were generated by searching EPA's CompTox Chemicals Dashboard, Substance Registry Services, and Chemical Abstracts Service (CAS) Common Chemistry database.

Table F-1. Substances Searched for Joint Toxic Action Studies in PubMed and Embase

Component	CAS Registry number	Synonyms searched
Carbon monoxide	630-08-0	Carbon monooxide; Carbon monoxide; Carbon oxide; Carbonic oxide; HBI 002; KOHLENMONOXID; Kohlenoxyd; Kohlenstoffmonoxid; Koolmonoxyde; monoxido de carbono; Monoxyde de carbone; Oxyde de carbone; Wegla tlenek
Formaldehyde	50-00-0	Aldehyd mravenci; Aldehyde formique; Aldeide formica; Chlodithan; Chlodithane; Fannoform; Floguard 1015; FM 282; Formalaz; formaldehido; Formaldehyd; formaldehyde; Formaldehyde-12C; Formalin; Formalin-loesungen; Formalina; Formaline; Formalith; Formic aldehyde; Formol; FS 850A; Fyde; Lysoform; Methaldehyde; Methanal; Methyl aldehyde; Methylene glycol; Methylene oxide; Morbicid; Oplossingen; Optilyse; Oxomethane; Oxymethylene; Paraform; Sigma-F 8775; Superlysoform

Table F-1. Substances Searched for Joint Toxic Action Studies in PubMed and Embase

Component	CAS Registry number	Synonyms searched
Methylene chloride	75-09-2	Aerotherne; Bichloride, methylene; Chloride, methylene; chlorocarbon F 30; chlorure de methylene; Cloruro de Metileno; Dichloride, methylene; Dichlormethan; Dichloro-Methane; Dichloromethane; Dichloromethane; diclorometano; Distillex DS3; Driverit; F 30 (chlorocarbon); Freon 30; Khladon 30; M-Clean D; Metaclen; Methaclean U; methane dichloride; Methoklone; Methylenchlorid; methylene bichloride; Methylene chloride; Methylene dichloride; Methylenum chloratum; Metylenu chlorek; Narkotil; Nevolin; R 30 (refrigerant); refrigerant R 30; Salesthin; Solaesthin; Soleana VDA; Solmethine
Nitrogen dioxide	10102-44-0	Bioxido de Nitrogeno; dióxido de nitrogeno; Dioxyde d'azote; Nitrogen dioxide; Nitrogen oxide; Nitrogen peroxide; Nitrogen(IV) dioxide; Nitrosooxidanyl; Nitrosooxy; Peroxyde d' azote; Stickstoffdioxid; Stikstofdioxyde
Tetrachloroethylene	127-18-4	1,1,2,2-Tetrachloroethene; 1,1,2,2-Tetrachloroethylene; Ankilostin; Antisal 1; Antisol 1; Asahi Perchlor; Carbon bichloride; Carbon dichloride; Czterochloroetylen; Didakene; Dilatin PT; Dow-per; Ethene, 1,1,2,2-tetrachloro-; Ethene, tetrachloro-; ethylene tetrachloride; Ethylene, tetrachloro-; F 1110 (halocarbon); Fedal-Un; Freon 1110; HCO 1110; LXGL 15; Nema (VAN); Nema, veterinary; PCE (chlorohydrocarbon); Perawin; Perchloorethylene, per; PERCHLORAETHYLEN; Perchloraethylen, per; Perchlorethylene; Perchloroethene; Perchloroethylene; Perclene; Perchloroethylene; Percosolv; Percosolve; Perklone; Persa P 3; Tetracap; Tetrachlooretheen; Tetrachloraethen; Tetrachlorathen; Tetrachloorethylen; Tetrachloorethylen; Tetrachloro-Ethene; Tetrachloro-Ethylene; Tetrachloroethene; Tetrachloroethylene; Tetrachloroetene; tetrachloroetilen; Tetraguer; Tetraleno; Tetralex; Tetravec; Tetroguer; Tetropil

The query strings used for the literature searches are presented in Table F-2.

Table F-2. Database Query Strings

Database	search date	Query string
PubMed		
	11/2024	((("Carbon Monoxide"[mh] OR "Carbon Monoxide Poisoning"[mh] OR 630-08-0[rn] OR "Carbon monooxide"[tw] OR "Carbon monoxide"[tw] OR "Carbon oxide"[tw] OR "Carbonic oxide"[tw] OR "HBI 002"[tw] OR "KOHLENMONOXID"[tw] OR "Kohlenoxyd"[tw] OR "Kohlenstoffmonoxid"[tw] OR "Koolmonoxyde"[tw] OR "monoxido de carbono"[tw] OR

Table F-2. Database Query Strings

Database	search date	Query string
		<p>"Monoxyde de carbone"[tw] OR "Oxyde de carbone"[tw] OR "Wegla tlenek"[tw]) AND ((("Formaldehyde"[mh] OR 50-00-0[rn] OR "Aldehyd mravenci"[tw] OR "Aldehyde formique"[tw] OR "Aldeide formica"[tw] OR "Chlodithan"[tw] OR "Chlodithane"[tw] OR "Fannoform"[tw] OR "Floguard 1015"[tw] OR "FM 282"[tw] OR "Formalaz"[tw] OR "formaldehido"[tw] OR "Formaldehyd"[tw] OR "formaldehyde"[tw] OR "Formaldehyde-12C"[tw] OR "Formalin"[tw] OR "Formalin-loesungen"[tw] OR "Formalina"[tw] OR "Formaline"[tw] OR "Formalith"[tw] OR "Formic aldehyde"[tw] OR "Formol"[tw] OR "FS 850A"[tw] OR "Fyde"[tw] OR "Lysoform"[tw] OR "Methaldehyde"[tw] OR "Methanal"[tw] OR "Methyl aldehyde"[tw] OR "Methylene glycol"[tw] OR "Methylene oxide"[tw] OR "Morbicid"[tw] OR "Oplossingen"[tw] OR "Optilyse"[tw] OR "Oxomethane"[tw] OR "Oxymethylene"[tw] OR "Paraform"[tw] OR "Sigma-F 8775"[tw] OR "Superlysoform"[tw]) OR ("Methylene Chloride"[mh] OR 75-09-2[rn] OR "Aerothene"[tw] OR "Bichloride, methylene"[tw] OR "Chloride, methylene"[tw] OR "chlorocarbon F 30"[tw] OR "chlorure de methylene"[tw] OR "Cloruro de Metileno"[tw] OR "Dichloride, methylene"[tw] OR "Dichlormethan"[tw] OR "Dichloro-Methane"[tw] OR "Dichloromethane"[tw] OR "Dichoromethane"[tw] OR "diclorometano"[tw] OR "Distillex DS3"[tw] OR "Driverit"[tw] OR "F 30 (chlorocarbon)"[tw] OR "Freon 30"[tw] OR "Khladon 30"[tw] OR "M-Clean D"[tw] OR "Metaclean U"[tw] OR "Methaclean U"[tw] OR "methane dichloride"[tw] OR "Methoklone"[tw] OR "Methylenchlorid"[tw] OR "methylene bichloride"[tw] OR "Methylene chloride"[tw] OR "Methylene dichloride"[tw] OR "Methylenum chloratum"[tw] OR "Metylenu chlorek"[tw] OR "Narkotil"[tw] OR "Nevolin"[tw] OR "R 30 (refrigerant)"[tw] OR "refrigerant R 30"[tw] OR "Salesthin"[tw] OR "Solaesthin"[tw] OR "Soleana VDA"[tw] OR "Solmethine"[tw]) OR ("Nitrogen Dioxide"[mh] OR 10102-44-0[rn] OR "Bioxido de Nitrogeno"[tw] OR "dioxido de nitrogeno"[tw] OR "Dioxyde d'azote"[tw] OR "Nitrogen dioxide"[tw] OR "Nitrogen oxide"[tw] OR "Nitrogen peroxide"[tw] OR "Nitrogen(IV) dioxide"[tw] OR "Nitrosooxidanyl"[tw] OR "Nitrosooxy"[tw] OR "Peroxyde d' azote"[tw] OR "Stickstoffdioxid"[tw] OR "Stikstofdioxyde"[tw]) OR ("Tetrachloroethylene"[mh] OR 127-18-4[rn] OR "1,1,2,2-Tetrachloroethene"[tw] OR "1,1,2,2-Tetrachloroethylene"[tw] OR "Ankilostin"[tw] OR "Antisal 1"[tw] OR "Antisol 1"[tw] OR "Asahi Perchlor"[tw] OR "Carbon bichloride"[tw] OR "Carbon dichloride"[tw] OR "Czterochloroetylen"[tw] OR "Didakene"[tw] OR "Dilatin PT"[tw] OR "Dow-per"[tw] OR "Ethene, 1,1,2,2-tetrachloro-"[tw] OR "Ethene, tetrachloro-"[tw] OR "ethylene tetrachloride"[tw] OR "Ethylene, tetrachloro-"[tw] OR "F 1110 (halocarbon)"[tw] OR "Fedal-Un"[tw] OR "Freon 1110"[tw] OR "HCO 1110"[tw] OR "LXGL 15"[tw] OR "Nema (VAN)"[tw] OR "Nema, veterinary"[tw] OR "PCE (chlorohydrocarbon)"[tw] OR "Perawin"[tw] OR "Perchloorethyleen, per"[tw] OR "PERCHLORAETHYLEN"[tw] OR "Perchloraethylen, per"[tw] OR "Perchlorethylene"[tw] OR "Perchloroethene"[tw] OR "Perchloroethylene"[tw] OR "Perclene"[tw] OR "Percloroetilene"[tw] OR "Percosolv"[tw] OR "Percosolve"[tw] OR "Perklone"[tw] OR "Persa P 3"[tw] OR "Tetracap"[tw] OR "Tetrachlooretheen"[tw] OR "Tetrachloraethen"[tw] OR "Tetrachlorathen"[tw] OR "Tetrachlorethylen"[tw] OR "Tetrachlorethylene"[tw] OR "Tetrachloro-Ethene"[tw] OR "Tetrachloro-Ethylene"[tw] OR "Tetrachloroethene"[tw] OR "Tetrachloroethylene"[tw] OR "Tetracloroetene"[tw] OR "tetracloroetileno"[tw] OR "Tetrager"[tw] OR "Tetraleno"[tw] OR "Tetralex"[tw] OR "Tetravec"[tw] OR "Tetroguer"[tw] OR "Tetropil"[tw])) OR ((("Formaldehyde"[mh] OR 50-00-0[rn] OR "Aldehyd mravenci"[tw] OR "Aldehyde formique"[tw] OR "Aldeide formica"[tw] OR "Chlodithan"[tw] OR "Chlodithane"[tw] OR "Fannoform"[tw] OR "Floguard 1015"[tw] OR "FM 282"[tw] OR "Formalaz"[tw] OR "formaldehido"[tw] OR "Formaldehyd"[tw] OR "formaldehyde"[tw] OR "Formaldehyde-12C"[tw] OR "Formalin"[tw] OR "Formalin-loesungen"[tw] OR "Formalina"[tw] OR "Formaline"[tw] OR "Formalith"[tw] OR "Formic aldehyde"[tw] OR "Formol"[tw] OR "FS 850A"[tw] OR "Fyde"[tw] OR "Lysoform"[tw] OR "Methaldehyde"[tw] OR "Methanal"[tw] OR "Methyl aldehyde"[tw] OR "Methylene</p>

Table F-2. Database Query Strings

Database	search date	Query string
		<p>glycol"[tw] OR "Methylene oxide"[tw] OR "Morbicid"[tw] OR "Oplossingen"[tw] OR "Optilyse"[tw] OR "Oxomethane"[tw] OR "Oxymethylene"[tw] OR "Paraform"[tw] OR "Sigma-F 8775"[tw] OR "Superlysoform"[tw]) AND (("Methylene Chloride"[mh] OR 75-09-2[rn] OR "Aerothene"[tw] OR "Bichloride, methylene"[tw] OR "Chloride, methylene"[tw] OR "chlorocarbon F 30"[tw] OR "chlorure de methylene"[tw] OR "Cloruro de Metileno"[tw] OR "Dichloride, methylene"[tw] OR "Dichlormethan"[tw] OR "Dichloro-Methane"[tw] OR "Dichloromethane"[tw] OR "Dichoromethane"[tw] OR "diclorometano"[tw] OR "Distillex DS3"[tw] OR "Driverit"[tw] OR "F 30 (chlorocarbon)"[tw] OR "Freon 30"[tw] OR "Khladon 30"[tw] OR "M-Clean D"[tw] OR "Metaclen"[tw] OR "Methaclean U"[tw] OR "methane dichloride"[tw] OR "Methoklone"[tw] OR "Methylenchlorid"[tw] OR "methylene bichloride"[tw] OR "Methylene chloride"[tw] OR "Methylene dichloride"[tw] OR "Methylenum chloratum"[tw] OR "Metylenu chlorek"[tw] OR "Narkotil"[tw] OR "Nevolin"[tw] OR "R 30 (refrigerant)"[tw] OR "refrigerant R 30"[tw] OR "Salesthin"[tw] OR "Solaesthin"[tw] OR "Soleana VDA"[tw] OR "Solmethine"[tw]) OR ("Nitrogen Dioxide"[mh] OR 10102-44-0[rn] OR "Bioxido de Nitrogeno"[tw] OR "dioxido de nitrogeno"[tw] OR "Dioxyde d'azote"[tw] OR "Nitrogen dioxide"[tw] OR "Nitrogen oxide"[tw] OR "Nitrogen peroxide"[tw] OR "Nitrogen(IV) dioxide"[tw] OR "Nitrosooxidanyl"[tw] OR "Nitrosooxy"[tw] OR "Peroxyde d' azote"[tw] OR "Stickstoffdioxid"[tw] OR "Stikstoffdioxyde"[tw]) OR ("Tetrachloroethylene"[mh] OR 127-18-4[rn] OR "1,1,2,2-Tetrachloroethene"[tw] OR "1,1,2,2-Tetrachloroethylene"[tw] OR "Ankilostin"[tw] OR "Antisal 1"[tw] OR "Antisol 1"[tw] OR "Asahi Perchlor"[tw] OR "Carbon bichloride"[tw] OR "Carbon dichloride"[tw] OR "Czterochloroetylen"[tw] OR "Didakene"[tw] OR "Dilatin PT"[tw] OR "Dow-per"[tw] OR "Ethene, 1,1,2,2-tetrachloro- "[tw] OR "Ethene, tetrachloro- "[tw] OR "ethylene tetrachloride"[tw] OR "Ethylene, tetrachloro- "[tw] OR "F 1110 (halocarbon)"[tw] OR "Fedal-Un"[tw] OR "Freon 1110"[tw] OR "HCO 1110"[tw] OR "LXGL 15"[tw] OR "Nema (VAN)"[tw] OR "Nema, veterinary"[tw] OR "PCE (chlorohydrocarbon)"[tw] OR "Perawin"[tw] OR "Perchloorethyleen, per"[tw] OR "PERCHLORAETHYLEN"[tw] OR "Perchloraethylen, per"[tw] OR "Perchlorethylene"[tw] OR "Perchloroethene"[tw] OR "Perchloroethylene"[tw] OR "Perclene"[tw] OR "Perchloroetilene"[tw] OR "Percosolv"[tw] OR "Percosolve"[tw] OR "Perklone"[tw] OR "Persa P 3"[tw] OR "Tetracap"[tw] OR "Tetrachlooretheen"[tw] OR "Tetrachloraethen"[tw] OR "Tetrachlorathen"[tw] OR "Tetrachlorethylen"[tw] OR "Tetrachlorethylene"[tw] OR "Tetrachloro-Ethene"[tw] OR "Tetrachloro-Ethylene"[tw] OR "Tetrachloroethene"[tw] OR "Tetrachloroethylene"[tw] OR "Tetracloroetene"[tw] OR "tetracloroetileno"[tw] OR "Tetraguer"[tw] OR "Tetraleno"[tw] OR "Tetralex"[tw] OR "Tetravec"[tw] OR "Tetroguer"[tw] OR "Tetropil"[tw])) OR (("Methylene Chloride"[mh] OR 75-09-2[rn] OR "Aerothene"[tw] OR "Bichloride, methylene"[tw] OR "Chloride, methylene"[tw] OR "chlorocarbon F 30"[tw] OR "chlorure de methylene"[tw] OR "Cloruro de Metileno"[tw] OR "Dichloride, methylene"[tw] OR "Dichlormethan"[tw] OR "Dichloro-Methane"[tw] OR "Dichloromethane"[tw] OR "Dichoromethane"[tw] OR "diclorometano"[tw] OR "Distillex DS3"[tw] OR "Driverit"[tw] OR "F 30 (chlorocarbon)"[tw] OR "Freon 30"[tw] OR "Khladon 30"[tw] OR "M-Clean D"[tw] OR "Metaclen"[tw] OR "Methaclean U"[tw] OR "methane dichloride"[tw] OR "Methoklone"[tw] OR "Methylenchlorid"[tw] OR "Methylen bichloride"[tw] OR "Methylene chloride"[tw] OR "Methylene dichloride"[tw] OR "Methylenum chloratum"[tw] OR "Metylenu chlorek"[tw] OR "Narkotil"[tw] OR "Nevolin"[tw] OR "R 30 (refrigerant)"[tw] OR "refrigerant R 30"[tw] OR "Salesthin"[tw] OR "Solaesthin"[tw] OR "Soleana VDA"[tw] OR "Solmethine"[tw]) AND (("Nitrogen Dioxide"[mh] OR 10102-44-0[rn] OR "Bioxido de Nitrogeno"[tw] OR "dioxido de nitrogeno"[tw] OR "Dioxyde d'azote"[tw] OR "Nitrogen dioxide"[tw] OR "Nitrogen oxide"[tw] OR "Nitrogen peroxide"[tw] OR "Nitrogen(IV) dioxide"[tw] OR "Nitrosooxidanyl"[tw] OR "Nitrosooxy"[tw] OR "Peroxyde d' azote"[tw] OR "Stickstoffdioxid"[tw] OR</p>

Table F-2. Database Query Strings

Database	search date	Query string
		<p>"Stikstofdioxyde"[tw]) OR ("Tetrachloroethylene"[mh] OR 127-18-4[rn] OR "1,1,2,2-Tetrachloroethene"[tw] OR "1,1,2,2-Tetrachloroethylene"[tw] OR "Ankilostin"[tw] OR "Antisal 1"[tw] OR "Antisol 1"[tw] OR "Asahi Perchlor"[tw] OR "Carbon bichloride"[tw] OR "Carbon dichloride"[tw] OR "Czterochloroetylen"[tw] OR "Didakene"[tw] OR "Dilatin PT"[tw] OR "Dow-per"[tw] OR "Ethene, 1,1,2,2-tetrachloro-"[tw] OR "Ethene, tetrachloro-"[tw] OR "ethylene tetrachloride"[tw] OR "Ethylene, tetrachloro-"[tw] OR "F 1110 (halocarbon)"[tw] OR "Fedal-Un"[tw] OR "Freon 1110"[tw] OR "HCO 1110"[tw] OR "LXGL 15"[tw] OR "Nema (VAN)"[tw] OR "Nema, veterinary"[tw] OR "PCE (chlorohydrocarbon)"[tw] OR "Perawin"[tw] OR "Perchloorethyleen, per"[tw] OR "PERCHLORAETHYLEN"[tw] OR "Perchloraethylen, per"[tw] OR "Perchlorethylene"[tw] OR "Perchloroethene"[tw] OR "Perchloroethylene"[tw] OR "Perclene"[tw] OR "Percloroetilene"[tw] OR "Percosolv"[tw] OR "Percosolve"[tw] OR "Perklone"[tw] OR "Persa P 3"[tw] OR "Tetracap"[tw] OR "Tetrachlooretheen"[tw] OR "Tetrachloraethen"[tw] OR "Tetrachlorathen"[tw] OR "Tetrachlorethylen"[tw] OR "Tetrachlorethylene"[tw] OR "Tetrachloro-Ethene"[tw] OR "Tetrachloro-Ethylene"[tw] OR "Tetrachloroethene"[tw] OR "Tetrachloroethylene"[tw] OR "Tetracloroetene"[tw] OR "tetracloroetileno"[tw] OR "Tetraguer"[tw] OR "Tetraleno"[tw] OR "Tetralex"[tw] OR "Tetravec"[tw] OR "Tetroguer"[tw] OR "Tetropil"[tw])) OR (("Nitrogen Dioxide"[mh] OR 10102-44-0[rn] OR "Bioxido de Nitrogeno"[tw] OR "dioxido de nitrogeno"[tw] OR "Dioxyde d'azote"[tw] OR "Nitrogen dioxide"[tw] OR "Nitrogen oxide"[tw] OR "Nitrogen peroxide"[tw] OR "Nitrogen(IV) dioxide"[tw] OR "Nitrosooxidanyl"[tw] OR "Nitrosooxy"[tw] OR "Peroxyde d' azote"[tw] OR "Stickstoffdioxid"[tw] OR "Stikstofdioxyde"[tw]) AND ("Tetrachloroethylene"[mh] OR 127-18-4[rn] OR "1,1,2,2-Tetrachloroethene"[tw] OR "1,1,2,2-Tetrachloroethylene"[tw] OR "Ankilostin"[tw] OR "Antisal 1"[tw] OR "Antisol 1"[tw] OR "Asahi Perchlor"[tw] OR "Carbon bichloride"[tw] OR "Carbon dichloride"[tw] OR "Czterochloroetylen"[tw] OR "Didakene"[tw] OR "Dilatin PT"[tw] OR "Dow-per"[tw] OR "Ethene, 1,1,2,2-tetrachloro-"[tw] OR "Ethene, tetrachloro-"[tw] OR "ethylene tetrachloride"[tw] OR "Ethylene, tetrachloro-"[tw] OR "F 1110 (halocarbon)"[tw] OR "Fedal-Un"[tw] OR "Freon 1110"[tw] OR "HCO 1110"[tw] OR "LXGL 15"[tw] OR "Nema (VAN)"[tw] OR "Nema, veterinary"[tw] OR "PCE (chlorohydrocarbon)"[tw] OR "Perawin"[tw] OR "Perchloorethyleen, per"[tw] OR "PERCHLORAETHYLEN"[tw] OR "Perchloraethylen, per"[tw] OR "Perchlorethylene"[tw] OR "Perchloroethene"[tw] OR "Perchloroethylene"[tw] OR "Perclene"[tw] OR "Percloroetilene"[tw] OR "Percosolv"[tw] OR "Percosolve"[tw] OR "Perklone"[tw] OR "Persa P 3"[tw] OR "Tetracap"[tw] OR "Tetrachlooretheen"[tw] OR "Tetrachloraethen"[tw] OR "Tetrachlorathen"[tw] OR "Tetrachlorethylen"[tw] OR "Tetrachlorethylene"[tw] OR "Tetrachloro-Ethene"[tw] OR "Tetrachloro-Ethylene"[tw] OR "Tetrachloroethene"[tw] OR "Tetrachloroethylene"[tw] OR "Tetracloroetene"[tw] OR "tetracloroetileno"[tw] OR "Tetraguer"[tw] OR "Tetraleno"[tw] OR "Tetralex"[tw] OR "Tetravec"[tw] OR "Tetroguer"[tw] OR "Tetropil"[tw])) AND (2004:3000[edat] OR 2004:3000[crdt] OR 2004:3000[mhda] OR 2004:3000[dp]))</p>
Embase	11/2024	<p>Limit to (embase and yr="2004 -Current") ((Carbon monoxide/ or 630-08-0.rn. or (Carbon monooxide or Carbon monoxide or Carbon oxide or Carbonic oxide or HBI 002 or KOHLENMONOXID or Kohlenoxyd or Kohlenstoffmonoxid or Koolmonoxyde or monoxido de carbono or Monoxyde de carbone or Oxyde de carbone or Wegla tlenek).ti,ab,kf.) and ((Formaldehyde/ or 50-00-0.rn. or (Aldehyd mravenci or Aldehyde formique or Aldeide formica or Chlodithan or Chlodithane or Fannoform or Floguard 1015 or FM 282 or Formalaz or formaldehido or Formaldehyd or formaldehyde or Formaldehyde-12C or Formalin or Formalin-loesungen or Formalina or Formaline or Formalith or Formic aldehyde or Formol or FS 850A or Fyde or Lysoform or Methaldehyde or Methanal or Methyl aldehyde or Methylene glycol or Methylene oxide or</p>

Table F-2. Database Query Strings

Database	search date	Query string
		<p>Morbicid or Oplossingen or Optilyse or Oxomethane or Oxymethylene or Paraform or Sigma-F 8775 or Superlysoform).ti,ab,kf.) or (Dichloromethane/ or 75-09-2.rn. or (Aerothene or Bichloride, methylene or Chloride, methylene or chlorocarbon F 30 or chlorure de methylene or Cloruro de Metileno or Dichloride, methylene or Dichlormethan or Dichloro-Methane or Dichloromethane or Dichoromethane or diclorometano or Distillex DS3 or Driverit or F 30 chlorocarbon or Freon 30 or Khladon 30 or M-Clean D or Metaclen or Methaclean U or methane dichloride or Methoklone or Methylenchlorid or methylene bichloride or Methylene chloride or Methylene dichloride or Methylenum chloratum or Metylenu chlorek or Narkotil or Nevolin or R 30 refrigerant or refrigerant R 30 or Salesthin or Solaesthin or Soleana VDA or Solmethine).ti,ab,kf.) or (Nitrogen Dioxide/ or 10102-44-0.rn. or (Bioxido de Nitrogeno or dioxido de nitrogeno or "Dioxyde d'azote" or Nitrogen dioxide or Nitrogen oxide or Nitrogen peroxide or "Nitrogen(IV) dioxide" or Nitrosooxidanyl or Nitrosooxy or Peroxyde d' azote or Stickstoffdioxid or Stikstofdioxyde).ti,ab,kf.) or (Tetrachloroethylene/ or 127-18-4.rn. or (1,1,2,2-Tetrachloroethene or 1,1,2,2-Tetrachloroethylene or Ankilostin or Antisal 1 or Antisol 1 or Asahi Perchlor or Carbon bichloride or Carbon dichloride or Czterochloroetylen or Didakene or Dilatin PT or Dow-per or Ethene, 1,1,2,2-tetrachloro- or Ethene, tetrachloro- or ethylene tetrachloride or Ethylene, tetrachloro- or F 1110 halocarbon or Fedal-Un or Freon 1110 or HCO 1110 or LXGL 15 or Nema VAN or Nema, veterinary or PCE chlorohydrocarbon or Perawin or Perchloorethyleen, per or PERCHLORAETHYLEN or Perchloraethylen, per or Perchloroethylene or Perchloroethene or Perchloroethylene or Perclene or Perchloroetilene or Percosolv or Percosolve or Perklone or Persa P 3 or Tetracap or Tetrachloorethen or Tetrachloraethen or Tetrachlorathen or Tetrachloorethylen or Tetrachloorethylen or Tetrachloro-Ethene or Tetrachloro-Ethylene or Tetrachloroethene or Tetrachloroethylene or Tetracloroetene or tetracloroetileno or Tetraguer or Tetraleno or Tetralex or Tetravec or Tetroguer or Tetropil).ti,ab,kf.))) or ((Formaldehyde/ or 50-00-0.rn. or (Aldehyd mravenci or Aldehyde formique or Aldeide formica or Chlodithan or Chlodithane or Fannoform or Floguard 1015 or FM 282 or Formalaz or formaldehido or Formaldehyd or formaldehyde or Formaldehyde-12C or Formalin or Formalin-loesungen or Formalina or Formaline or Formalith or Formic aldehyde or Formol or FS 850A or Fyde or Lysoform or Methaldehyde or Methanal or Methyl aldehyde or Methylene glycol or Methylene oxide or Morbicid or Oplossingen or Optilyse or Oxomethane or Oxymethylene or Paraform or Sigma-F 8775 or Superlysoform).ti,ab,kf.) and ((Dichloromethane/ or 75-09-2.rn. or (Aerothene or Bichloride, methylene or Chloride, methylene or chlorocarbon F 30 or chlorure de methylene or Cloruro de Metileno or Dichloride, methylene or Dichlormethan or Dichloro-Methane or Dichloromethane or Dichoromethane or diclorometano or Distillex DS3 or Driverit or F 30 chlorocarbon or Freon 30 or Khladon 30 or M-Clean D or Metaclen or Methaclean U or methane dichloride or Methoklone or Methylenchlorid or methylene bichloride or Methylene chloride or Methylene dichloride or Methylenum chloratum or Metylenu chlorek or Narkotil or Nevolin or R 30 refrigerant or refrigerant R 30 or Salesthin or Solaesthin or Soleana VDA or Solmethine).ti,ab,kf.) or (Nitrogen Dioxide/ or 10102-44-0.rn. or (Bioxido de Nitrogeno or dioxido de nitrogeno or "Dioxyde d'azote" or Nitrogen oxide or Nitrogen peroxide or "Nitrogen(IV) dioxide" or Nitrosooxidanyl or Nitrosooxy or Peroxyde d' azote or Stickstoffdioxid or Stikstofdioxyde).ti,ab,kf.) or (Tetrachloroethylene/ or 127-18-4.rn. or (1,1,2,2-Tetrachloroethene or 1,1,2,2-Tetrachloroethylene or Ankilostin or Antisal 1 or Antisol 1 or Asahi Perchlor or Carbon bichloride or Carbon dichloride or Czterochloroetylen or Didakene or Dilatin PT or Dow-per or Ethene, 1,1,2,2-tetrachloro- or Ethene, tetrachloro- or ethylene tetrachloride or Ethylene, tetrachloro- or F 1110 halocarbon or Fedal-Un or Freon 1110 or HCO 1110 or LXGL 15 or Nema VAN or Nema, veterinary or PCE chlorohydrocarbon or Perawin or Perchloorethyleen, per or</p>

Table F-2. Database Query Strings

Database	search date	Query string
		<p>PERCHLORAETHYLEN or Perchloraethylen, per or Perchlorethylene or Perchloroethene or Perchloroethylene or Perclene or Percloroetilene or Percosolv or Percosolve or Perklone or Persa P 3 or Tetracap or Tetrachlooretheen or Tetrachloraethen or Tetrachlorathen or Tetrachlorethylen or Tetrachlorethylene or Tetrachloro-Ethene or Tetrachloro-Ethylene or Tetrachloroethene or Tetrachloroethylene or Tetrachloroetene or tetracloroetileno or Tetraguer or Tetraleno or Tetralex or Tetravec or Tetroguer or Tetropil).ti,ab,kf.)) or ((Dichloromethane/ or 75-09-2.rn. or (Aerothene or Bichloride, methylene or Chloride, methylene or chlorocarbon F 30 or chlorure de methylene or Cloruro de Metileno or Dichloride, methylene or Dichlormethan or Dichloro-Methane or Dichloromethane or Dichoromethane or diclorometano or Distillex DS3 or Driverit or F 30 chlorocarbon or Freon 30 or Khladon 30 or M-Clean D or Metaclen or Methaclean U or methane dichloride or Methoklone or Methylenchlorid or methylene bichloride or Methylene chloride or Methylene dichloride or Methylenum chloratum or Metylenu chlorek or Narkotil or Nevolin or R 30 refrigerant or refrigerant R 30 or Salesthin or Solaesthin or Soleana VDA or Solmethine).ti,ab,kf.) and ((Nitrogen Dioxide/ or 10102-44-0.rn. or (Bioxido de Nitrogeno or dioxido de nitrogeno or "Dioxyde d'azote" or Nitrogen dioxide or Nitrogen oxide or Nitrogen peroxide or "Nitrogen(IV) dioxide" or Nitrosooxidanyl or Nitrosooxy or Peroxyde d' azote or Stickstoffdioxid or Stikstofdioxyde).ti,ab,kf.) or (Tetrachloroethylene/ or 127-18-4.rn. or (1,1,2,2-Tetrachloroethene or 1,1,2,2-Tetrachloroethylene or Ankilostin or Antisal 1 or Antisol 1 or Asahi Perchlor or Carbon bichloride or Carbon dichloride or Czterochloroetylen or Didakene or Dilatin PT or Dow-per or Ethene, 1,1,2,2-tetrachloro- or Ethene, tetrachloro- or ethylene tetrachloride or Ethylene, tetrachloro- or F 1110 halocarbon or Fedal-Un or Freon 1110 or HCO 1110 or LXGL 15 or Nema VAN or Nema, veterinary or PCE chlorohydrocarbon or Perawin or Perchloorethyleen, per or PERCHLORAETHYLEN or Perchloraethylen, per or Perchlorethylene or Perchloroethene or Perchloroethylene or Perclene or Percloroetilene or Percosolv or Percosolve or Perklone or Persa P 3 or Tetracap or Tetrachlooretheen or Tetrachloraethen or Tetrachlorathen or Tetrachlorethylen or Tetrachlorethylene or Tetrachloro-Ethene or Tetrachloro-Ethylene or Tetrachloroethene or Tetrachloroethylene or Tetrachloroetene or tetracloroetileno or Tetraguer or Tetraleno or Tetralex or Tetravec or Tetroguer or Tetropil).ti,ab,kf.)) or ((Nitrogen Dioxide/ or 10102-44-0.rn. or (Bioxido de Nitrogeno or dioxido de nitrogeno or "Dioxyde d'azote" or Nitrogen dioxide or Nitrogen oxide or Nitrogen peroxide or "Nitrogen(IV) dioxide" or Nitrosooxidanyl or Nitrosooxy or Peroxyde d' azote or Stickstoffdioxid or Stikstofdioxyde).ti,ab,kf.) and (Tetrachloroethylene/ or 127-18-4.rn. or (1,1,2,2-Tetrachloroethene or 1,1,2,2-Tetrachloroethylene or Ankilostin or Antisal 1 or Antisol 1 or Asahi Perchlor or Carbon bichloride or Carbon dichloride or Czterochloroetylen or Didakene or Dilatin PT or Dow-per or Ethene, 1,1,2,2-tetrachloro- or Ethene, tetrachloro- or ethylene tetrachloride or Ethylene, tetrachloro- or F 1110 halocarbon or Fedal-Un or Freon 1110 or HCO 1110 or LXGL 15 or Nema VAN or Nema, veterinary or PCE chlorohydrocarbon or Perawin or Perchloorethyleen, per or PERCHLORAETHYLEN or Perchloraethylen, per or Perchlorethylene or Perchloroethene or Perchloroethylene or Perclene or Percloroetilene or Percosolv or Percosolve or Perklone or Persa P 3 or Tetracap or Tetrachlooretheen or Tetrachloraethen or Tetrachlorathen or Tetrachlorethylen or Tetrachlorethylene or Tetrachloro-Ethene or Tetrachloro-Ethylene or Tetrachloroethene or Tetrachloroethylene or Tetrachloroetene or tetracloroetileno or Tetraguer or Tetraleno or Tetralex or Tetravec or Tetroguer or Tetropil).ti,ab,kf.))</p>