

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:

$$\text{TTD}_{\text{HEMATO}} = 1 \text{ ppm}$$

$$\text{TTD}_{\text{NEURO}} = 4 \text{ ppm}$$

$$\text{TTD}_{\text{DEV}} = 1 \text{ ppm}$$

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:

$MRL_{RESP} = 0.03 \text{ ppm}$

$TTD_{DEV} = 0.2 \text{ ppm}$

Chronic-Duration Inhalation TTDs:

$MRL_{RESP} = 0.008 \text{ ppm}$

$TTD_{DEV} = 0.2 \text{ ppm}$

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; Tetraguer; 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 Percloroetilene 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 Tetracloroetene 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 Tetracloroetene 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 Tetracloroetene or tetracloroetileno or Tetraguer or Tetraleno or Tetralex or Tetravec or Tetroguer or Tetropil).ti,ab,kf.))</p>