Appendix A: Background Information for Carbon Monoxide

A.1 Toxicokinetics

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 oxyhaemoglobin and carboxyhaemoglobin 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 carboxyhaemoglobin 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.

Within the erythrocyte, carbon monoxide binds with hemoglobin to form carboxyhemoglobin (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 240–250-fold that of molecular oxygen (Roughton 1970). One part of carbon monoxide and 245 parts of oxygen would form equal parts of oxyhaemoglobin and carboxyhaemoglobin (50% of each), which would be achieved by breathing air containing 21% oxygen and 650 mg carbon monoxide/m^3 (570 ppm).

Distribution

Due to its high affinity for hemoglobin, most absorbed carbon monoxide will be found in the blood as carboxyhemoglobin, and therefore present in all tissues of the body. However, carbon monoxide can dissociate from carboxyhemoglobin and enter other tissues with heme-containing enzymes, including the heart and liver. About 15% of the body's CO is found outside of the blood (Coburn and Forman 1987; Longo 1977). A study by Hill et al. (1977) predicted similar levels of blood carboxyhaemoglobin (%) in a mother and fetus during prolonged exposures of the mother to carbon monoxide (34–340 mg/m^3 [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 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 (Stewart et al. 1970; Landaw 1973; Wagner et al. 1975). The half-life of disappearance of carbon monoxide from the blood in humans ranges from 2–6.5 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 carboxyhemoglobin (COHb), and subsequent hypoxia of oxygen-sensitive tissues. Background carboxyhemoglobin levels generally range from 1–2% in urban non-smokers, while smokers average 2–8% and may be as high as 18% COHb (Stewart et al. 1976).

The Coburn-Forster-Kane 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 CO exposure, with generally acceptable results (Peterson and Stewart 1970, 1975; Tikuisis et al. 1987; Benignus 1994). The equation is as follows:

$$\{A\,[HbCO]_t - (BV_{CO} + PI_{CO})\} \div \{A\,[Hb_{CO}]_0 - (BV_{CO} + PI_{CO})\} = e^{-tAVbB}$$

Where:
$A = P_{c,O2}/M[HbO_2]$
$B = 1/DL_{CO} + PL/VA$
DL_{CO} = diffusivity of the lung for CO
can also be written as $35(VO_2)e^{0.33}$ where VO_2 is the respiratory minute volume in liters/minute
$[HbCO]_t$ = ml of CO per ml blood at time t – this is generally the term to be solved for
$[HbCO]_0$ = ml of CO per ml blood at time 0 (approximately 0.8% COHb, or 0.0176 mg CO/ml blood for a non-smoker)
HbO_2 = ml of O_2 per ml blood, or $0.22-[HbCO]_t$
M = the ratio of the affinity of blood for CO relative to that of O_2, approximately 218
$P_{c,O2}$ = average partial pressure of O_2 in the capillaries, in mm Hg. Approximately 110.
PI_{CO} = partial pressure of CO in the inhaled air (in mm Hg)
PL = barometric pressure minus the vapor of water (i.e., 49) at body temperature, in mm Hg
t = exposure duration, in minutes
V_{CO} = rate of endogenous CO formation (approximately 0.007 ml/min)
Vb = blood volume, generally assumed to be 74 mg/kg body weight
VA = alveolar ventilation rate (ml/min), which can be estimated as $0.933\ V_E-132f$ where V_E is the minute volume (in ml/min) and *f* is the ventilation frequency

Cardiovascular Effects

Exposure to carbon monoxide, and the resulting increase in carboxyhemoglobin 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 2–7% (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 5–20% (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% (Aronow et al. 1984; Anderson et al. 1973; Adams et al. 1988; Kleinman et al. 1989; Allred et al. 1989); the chest pain in these cases is thought to be a response to mydocardial 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 CO exposures resulting in 3% or 5% COHb and the frequency of arrhythmias during rest or exercise. It therefore appears that at 5% COHb or below, humans are not at increased risk for ventricular arrhythmias, but that at higher COHb levels there may be a risk in sensitive populations.

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 CO exposure and angina pectoris, but not between CO exposure and electrocardiographic findings, in a group of Finnish foundry workers. In a follow-up study of the same population, no significant differences between CO 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 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 CO levels than the other two exposure populations.

A large number of reviews have evaluated the association between chronic CO exposure and the development of ischemic cardiovascular disease (Utell et al. 1994; Smith and Steichen 1993; Mennear 1993; Folinsbee 1992; Kristensen 1989; Weir and Fabiano 1982; U.S. EPA 1991, 1992). The reviews generally agree that acute 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) CO exposure and the development of cardiovascular disease.

Neurological Effects

Effects on the central nervous system 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 (Dolan 1985; Olson 1984; Benignus et al. 1987; Fawcett et al. 1992). Extremely high blood COHb levels (50–80%) result in severe neurological effects, including disorientation, seizures, coma, respiratory failure, and death (Dolan 1985; Olson 1984).

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 was present.

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 from 15–25% COHb in the mother produce reductions in birth weight, cardiomegaly, delays in behavioral development, and deficits in cognitive function (for review, see U.S. EPA 1991). Higher exposure levels, resulting in COHb levels of 48% or greater, resulted in maternal and fetal death.

A.3 Mechanisms of Action

The primary mechanism of action of carbon monoxide toxicity is the formation of carboxyhemoglobin. This reduces the amount of hemoglobin available to carry oxygen to the tissues, as well as interfering with oxygen release at the tissue level. These two factors combine to diminish cellular respiration, resulting in tissue hypoxia. Tissues sensitive to hypoxia, such as the lung, heart, and central nervous system, are particularly sensitive to the effects of carbon monoxide poisoning. 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.

A.4 Health Guidelines

ATSDR (2005) has not derived MRLs for carbon monoxide for any exposure duration or route.

EPA (2005) does not list an RfD, RfC, or cancer classification for carbon monoxide on IRIS.

National Air Quality Standards for CO 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 (US EPA 2000).

The World Health Organization (1999) recommends that CO exposures be kept to levels below which a COHb level of 2.5% would be generated. These correspond to 100 mg/m^3 (87 ppm) for 15 min, 60 mg/m^3 (52 ppm) for 30 min, 30 mg/m^3 (26 ppm) for 1 hour, or 10 mg/m^3 (9 ppm) for 8 hours.

IARC (2005) has not issued a carcinogenicity classification for carbon monoxide.

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

Hematological Effects

The primary effects of exposure of healthy individuals to carbon monoxide involve the formation of carboxyhemoglobin (COHb), the result of binding of carbon monoxide to the ferrous iron in hemoglobin. The formation of COHb, and resulting affinity of blood for oxygen, is responsible for the effects of carbon monoxide on other organs and tissues. As the earliest effects of carbon monoxide occur at 2% COHb (see cardiovascular effects, below), this effect was selected as the point of departure for deriving a TTD for hematological effects. According to Raub et al. (2000), and based on the CFK equation, the equilibrium CO concentration required to achieve 2% blood COHb is 10 ppm; this value was therefore used as the point of departure for the TTD. To the value of 10 ppm an uncertainty factor of 10, representing 3 for intrahuman variability and 3 for use of a minimal LOAEL, was applied to give the TTD of 1 ppm. A full factor of 10 was not used for intrahuman variability because variability in carboxyhemoglobin formation is expected to be small. **Normal values for carboxyhemoglobin are <2.3% of the total hemoglobin; 4-5% in heavy smokers.**

Cardiovascular Effects

Significant decreases in short-term maximal exercise duration have been noted in healthy men with blood COHb levels ranging from 2–7% (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 5–20% (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% (Aronow et al. 1984; Anderson et al. 1973; Adams et al. 1988; Kleinman et al. 1989; Allred 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. According to Raub et al. (2000), and based on the CFK equation, the equilibrium CO concentration required to achieve 2% blood COHb is 10 ppm; this value was therefore used as the point of departure for the TTD. To the value of 10 ppm an uncertainty

factor of 10, representing 3 for intrahuman variability and 3 for use of a minimal LOAEL, was applied to give the TTD of 1 ppm. A full factor of 10 was not used for intrahuman variability because variability in carboxyhemoglobin formation is expected to be small, and the 2% COHb level represents the bottom of the normal range of variability.

Neurological Effects

Effects on the central nervous system 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 being 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; 33ppm was therefore selected as the point of departure. To the LOAEL of 33 ppm, an uncertainty factor of 10 (3 for intrahuman variability, 3 for use of a minimal LOAEL) was applied to give the TTD of 3 ppm.

Summary (TTD for Carbon Monoxide)
TTD_{HEMATO} = 1 ppm
TTD_{CARDIO} = 1 ppm
TTD_{NEURO} = 3 ppm

A.6 References

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Appendix B: Background Information for Formaldehyde

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 μg/g of blood. Individual variability was markedly present. Immediately after a 40-minute exposure, mean blood concentration of formaldehyde was 2.77±0.28 μg/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.

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 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 μg/g of blood. Rats exposed to the 14.4 ppm air concentration of formaldehyde had blood concentrations of 2.25±0.07 μg/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 4 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 2-way exposures showed a 100% uptake of formaldehyde, while 1-way exposures of formaldehyde showed that retention was slightly lower than in the 2-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.

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 µg/g) were not significantly different from those of the exposed group. The 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 tenth 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. The metabolism of formaldehyde to formate (via formaldehyde dehydrogenase/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). Formaldehyde dehydrogenase (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 glutathione adduct of formaldehyde. If formaldehyde is not metabolized by FDH, then it can form cross linkages between proteins, 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 CO2. Formaldehyde dehydrogenase activity does not increase (i.e.,

not inducible) in response to formaldehyde exposure (Casanova-Schmitz et al. 1984); thus no increase in metabolism occurs.

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}CO2 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 that expired over the same period. 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.

Physiologically-Based Pharmacokinetic Models

Pharmacokinetic models describing the rate of formation of formaldehyde-induced DNA-protein cross links 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). Rates of formation of DNA-protein cross links 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 (EPA 1991). The models assume that rates of cross link formation are proportional to tissue concentration of formaldehyde and include saturable and nonsaturable elimination pathways, and that regional and species differences in cross link 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 cross links 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 cross link 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, b; 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 offgassing 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. 1986b), whereas monkeys exposed to 6 ppm formaldehyde show a wider distribution of similar epithelial lesions in the nose posterior to the vestibule and some extension of the lesions into the tracheal and bronchial regions (Monticello et al. 1989); (3) histochemical localization observations suggesting that regional differences in formaldehyde dehydrogenase, 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 cross link 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).

The Chemical Industry Institute of Toxicology and the U.S. EPA (CIIT 1998) are currently exploring options in using the CFD and pharmacokinetic models to extrapolate exposure-response relationships for formaldehyde-induced rat nasal tumors and related end points, such as rates of cellular proliferation in specific regions of the nasal epithelium, to derive estimates of cancer risk in humans exposed to inhaled formaldehyde. One approach being explored makes predictions for nasal and lung tumor risk in humans

exposed to inhaled formaldehyde using two-stage clonal-growth cancer models incorporating data on cell division rates, numbers of cell at risk, tumor incidence, and site-specific flux of formaldehyde (see also CIIT 1998; Conolly et al. 1992; Conolly and Andersen 1993; Morgan 1997). A second approach (a benchmark dose approach) makes predictions of nasal cancer risk in humans using curve fitting of relevant rat exposure-response data (e.g., nasal tumors or precursor lesions such as preneoplastic foci or squamous papillomas, rates of cellular proliferation, or rates of DNA-protein cross link formation) and CFD modeling and/or pharmacokinetic modeling for extrapolation purposes (CIIT 1998).

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. 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 or less) indicate that eye, nose, and throat irritation occurs at concentrations in the range of 0.4–3 ppm (Andersen and Molhave 1983; Bender et al. 1983; Day et al. 1984; Gorski et al. 1992; Krakowiak et al. 1998; Kulle et al. 1987; Pazdrak et al. 1993; Weber-Tschopp et al. 1977; Witek et al. 1986). 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. 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 restricted to lesions (squamous metaplasia and hyperplasia) in the epithelium of the upper respiratory tract (Chang et al. 1983; Monticello et al. 1989; Morgan et al. 1986a, 1986b; Rusch et al. 1983; Woutersen et al. 1987; Zwart et al. 1988).

Studies of animals exposed for life to formaldehyde in air or drinking water also show that formaldehyde primarily damages tissue at portals-of-entry (i.e., the upper respiratory tract and the gastrointestinal tract); evidence for toxic effects at distant sites is less consistent. 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 2 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 (Soffriti 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.

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).

More than 40 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 only equivocal evidence of a relationship between formaldehyde and nasopharyngeal cancer in humans, and even less convincing evidence for extrarespiratory cancer.

The apparent restriction of formaldehyde-induced noncancer and cancer effects to portals-of-entry 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 weight of evidence indicates that distant site effects from formaldehyde may occur only when the capacity for local disposition of formaldehyde is exceeded.

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. 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 cross links 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 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 formaldehyde dehydrogenase activity, overwhelming the natural protection against formaldehyde, and allowing the unmetabolized intact molecule to exert its effects locally. 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 capacity for local disposition of formaldehyde is exceeded.

It has been demonstrated that formaldehyde can form cross links 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 cross links in the respiratory tract measured in male Fischer 344 rats as well as in Rhesus monkeys; concentrations of DNA-protein cross links were greatest in the middle turbinate

tissues and lowest in the nasopharyngeal tissues, with some evidence of cross link 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. 1990; 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 (mucociliary clearance, 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 (Basler et al. 1985; Donovan et al. 1983; Grafstrom et al. 1985, 1993; Rithidech et al. 1987; Snyder and Van Houten 1986; Valencia et al. 1989; Woodruff et al. 1985; Yager et al. 1986). DNA-protein cross links 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) has 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 nine (three for the use of a minimal LOAEL and three for human variability).

ATSDR (1999) has derived an intermediate-duration inhalation MRL of 0.03 ppm for formaldehyde. 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 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).

ATSDR (1999) did not derive an acute-duration oral MRL for formaldehyde.

ATSDR (1999) derived an intermediate-duration oral MRL of 0.3 mg/kg/day for formaldehyde, based on a NOAEL of 25 mg/kg/day for gastrointestinal effects in rats exposed to formaldehyde in drinking water (Til et al. 1988). The LOAEL was 125 mg/kg/day. An uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) was applied to the NOAEL to derive the MRL.

ATSDR (1999) derived a chronic-duration oral MRL of 0.2 mg/kg/day for formaldehyde, based on a NOAEL of 15 mg/kg/day for gastrointestinal effects in male rats exposed to formaldehyde in drinking water (Til et al. 1989). The LOAEL was 82 mg/kg/day. An uncertainty factor of 100 (10 for

extrapolation from animals to humans and 10 for human variability) was applied to the NOAEL to derive the MRL.

IRIS (U.S. EPA 2005) does not report a chronic RfC for formaldehyde.

IRIS (U.S. EPA 2005) reports a chronic RfD of 0.2 mg/kg/day for formaldehyde, based on a NOAEL of 15 mg/kg/day for reduced body weight gain and altered gastrointestinal histopathology in male rats exposed to formaldehyde in drinking water (Til et al. 1989) and an uncertainty factor of 100.

IRIS (U.S. EPA 2005) presently classifies formaldehyde in carcinogenicity group B1 (probable human carcinogen). The inhalation unit risk for formaldehyde is 1.3E-5 $(ug/m^3)^{-1}$. IARC (2003) concluded that formaldehyde is carcinogenic to humans (Group 1), on the basis of sufficient evidence in humans and sufficient evidence in experimental animals. According to NTPs Eleventh Report on Carcinogens (2005), Formaldehyde (gas) is reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals.

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

Respiratory Effects

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).

Cancer

IRIS (U.S. EPA 2005) presently classifies formaldehyde in carcinogenicity group B1 (probable human carcinogen). The inhalation unit risk for formaldehyde is 1.3E-5 $(ug/m^3)^{-1}$.

Summary (TTD for Formaldehyde)
MRL_{RESP}=0.008 ppm

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Appendix C: Background Information for Methylene Chloride

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 (DiVincenzo et al. 1972; MacEwen 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. 1986a). 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 rats' fetuses. After acute 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 cytochrome P-450 enzymes (CYP2E1) and the other by a theta glutathione-S-transferase (GSSTl-1). The P-450 pathway (MFO) produces carbon monoxide (leading to carboxyhemoglobin formation) and carbon dioxide via formyl chloride (Gargas et al. 1986; Stewart et al. 1972) and the glutathione pathway (GST) produces carbon dioxide via a postulated glutathione conjugate (S-chloromethyl glutathione) and formaldehyde. The MFO pathway is a high affinity-low capacity pathway with a metabolic rate of 47 µmol/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.

Physiologically based pharmacokinetic (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 lung, the blood system, and the nervous system are the major target organs of toxicity associated with exposure to methylene chloride.

Respiratory Effects

Asphyxia was determined to be the cause of death in the case of a male worker who was subjected to acute inhalation exposure (concentration unknown) for 1 hour (Winek et al. 1981); the autopsy revealed bilateral pulmonary congestion with focal hemorrhage. Respiratory symptoms (cough, breathlessness, chest tightness) were reported in only 4 of 33 cases of acute 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 time-weighted average (TWA) to which these workers were exposed ranged from 18–1,200 mg/m^3.

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 to 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.

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 1986). 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 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 Clara cells correlated well with the activity of cytochrome P-450 monooxygenase in Clara cells, as assessed immunocytochemically in the whole lung, and biochemically in freshly isolated Clara cells. Nasal cavity squamous metaplasia was observed in rats exposed intermittently to 1,000 ppm methylene chloride in the NTP (1986) bioassay.

Hematological Effects

In humans, average blood COHb levels measure less than 1% in an atmosphere free of carbon monoxide, and less than 4% in a normal atmosphere. Blood COHb concentrations were about 30% higher than normal in two cases of lethal poisoning following acute 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 meters 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 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 authors to be suggestive of compensatory hematopoiesis (Ott et al. 1983b). 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 the 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 the 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 inhalation exposure to methylene chloride that were reported to occupational health authorities in the United Kingdom between 1961 and 1980 involved depression of the central nervous system (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 inhalation exposure to methylenechloride-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- to 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 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 central nervous system 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 neurologic effects, as measured by responses to questions relating to neurotoxicity (e.g., recurring severe headaches, numbness/tingling in hands of 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 more than 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 to 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 authors concluded that overall no effects on the central nervous system were attributable to chronic, low-level exposures to methylene chloride, a finding they reported as being consistent with that of Cherry et al. (1981).

Acute studies in animals are consistent with findings in humans that methylene chloride affects the central nervous system. 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 of 5,000 ppm or greater (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).

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; Lanes et al. 1993; Ott et al. 1983a). Some occupational studies (Cantor et al. 1995; Cocco et al. 1999; Gibbs et al. 1996; Heineman et al. 1994) 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.

In mice and rats, inhalation of very high levels of methylene chloride significantly increased the incidence of liver and lung cancer (Mennear et al. 1988; NTP 1986) and benign mammary gland tumors (fibroadenomas or adenomas) (Mennear et al. 1988; Nitschke et al. 1988a; NTP 1986). 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 the NTP (1986) study, groups of 50 animals of each sex were exposed to methylene chloride by inhalation 6 hours/day, 5 days/week for 102 weeks. F344/N rats were exposed to 0, 1,000, 2,000, or 4,000 ppm of methylene chloride and B6C3F1 mice were exposed to 0, 2,000, or 4,000 ppm. At or above 2,000 ppm, the incidence of liver tumors (mostly hepatocellular adenomas or carcinomas) in mice was significantly higher than in chamber and historical control groups (NTP 1986); at 4,000 ppm, the incidence of liver tumors was highly significant ($p \leq 0.001$). The incidence of combined benign and malignant liver tumors was high (67–83%) in the treated animals. There was also a statistically significant increase in the incidence of lung tumors in mice ($p < 0.001$) exposed at 2,000 ppm or above; these tumors were primarily alveolar/bronchiolar adenomas or carcinomas. The incidence of combined benign and malignant lung tumors was 54–85% in the treated animals. The NTP (1986) report concluded that there was "some evidence of carcinogenicity" in male rats and "clear evidence of carcinogenicity" in female rats, based on the increased incidence of benign mammary neoplasms following 2 years of inhalation exposure to methylene chloride. The report concluded that there was "clear evidence for carcinogenicity" for methylene chloride chronic inhalation exposure, based on the increased incidence of alveolar/bronchiolar neoplasms and of hepatocellular neoplasms in mice.

In two related studies, Kari et al. (1993) and Maronpot et al. (1995b) 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. (1995b) 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. (1995b) 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. (1995b) 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) have 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 Clara 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 Clara cell correlated well with the activity of cytochrome P-450 monooxygenase in the Clara cell, as assessed immunocytochemically in the whole lung and biochemically in the freshly isolated Clara cell (as determined by ethoxycoumarin O-dealkylation and aldrin epoxidation).

Over 13 weeks (5 days/week) of exposure, the acute Clara 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 authors suggest that the reason

for the decrease or disappearance of the lesion was due to an adaptation/tolerance in the Clara cell to methylene chloride that was linked to a marked decrease of methylene chloride metabolism by cytochrome P-450 pathways. Glutathione transferase (GST) activity in the Clara 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. CO 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 of 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 (De Jongh 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 cytochrome P-450 2E1 and glutathione S-transferase-mediated (GST) pathway. The MFO pathway is oxidative and appears to yield carbon monoxide as well as considerable amounts of carbon dioxide. The glutathione-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 glutathione in the conjugative pathway. Neither formyl chloride nor the glutathione conjugate of methylene chloride has been isolated or characterized, although Green (1997) reports that their formation is entirely consistent with available information on glutathione-mediated metabolism. Distribution of methylene chloride metabolism between these pathways is dose dependent. The MFO pathway is a high affinity, limited-capacity pathway which 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 less than 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 has demonstrated that: methylene chloride induction of bacterial mutagenicity is expressed more strongly in *Salmonella typhimurium* TA 1535 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 TA 100 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 TA 100 (Graves et al. 1994a); and bacterial testing with 3 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 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 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 glutathione conjugate, putatively chloromethylglutathione. Graves and Green (1996) also concluded that these results suggested that the mechanism for methylene chloride tumorogenicity in the mouse liver was likely to be genotoxic and mediated by the GSH pathway. Observed species differences in liver tumorogenicity 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. 1995b).

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 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 derived a chronic-duration MRL of 0.3 ppm for methylene chloride, based on a NOAEL of 50 ppm 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 hour/day, 5 day/week) resulting in a NOAEL[ADJ] of 8.92 ppm. Whereas the MRL was derived based on hepatic effects (extrarespiratory), a human equivalent concentration (HEC) was calculated. Since the ratio of the blood:air partition coefficient in the rat to the blood:air partition coefficient in the human was > 1, the value of 1.0 was used to calculate the NOAEL[HEC] (EPA 1994). This resulted in an MRL of 0.3 ppm by dividing the NOAEL of 8.92 ppm by an uncertainty factor of 30 (3 for extrapolation from animals to humans, and 10 for human variability).

ATSDR (2000) derived an acute-duration oral MRL of 0.2 mg/kg/day for methylene chloride, based on by route-to-route extrapolation of the inhalation data from Winneke (1974), using a PBPK model (Reitz et al. 1997). For acute neurological effects, the associated dose measure was defined as the peak concentration of methylene chloride in brain tissue (mg/L of brain tissue) of humans exposed to 300 ppm of methylene chloride for 4 hours by inhalation. The modified PBPK model calculated that the administered inhalation dose was equivalent to 3.95 mg of methylene chloride per L of brain tissue. The equivalent administered human concentration in drinking water that will produce the same neurological

effects was 565 mg of methylene chloride/L. Using a daily drinking water consumption value of 2L and an average human body weight of 70 kg, the LOAEL was calculated to be 16 mg/kg/day. An acute oral MRL was calculated by dividing the LOAEL (16 mg/kg/day) by an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability), to yield 0.2 mg/kg/day.

ATSDR (2000) did not derive an MRL for intermediate-duration oral exposure to methylene chloride.

ATSDR (2000) derived a chronic-duration MRL of 0.06 mg/kg/day for methylene chloride, based on a NOAEL of 6 mg/kg/day in rats exposed to methylene chloride in the drinking water for 104 weeks (Serota et al. 1986). The chronic oral MRL was calculated by dividing the NOAEL (6 mg/kg/day) by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

EPA (IRIS 2004) does not currently list an RfC for methylene chloride.

EPA (IRIS 2004) derived a chronic RfD of 0.06 mg/kg/day for methylene chloride (dichloromethane), based on a NOAEL of 5.85 mg/kg/day for histological alterations of the liver from an unpublished study. An uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) was applied to derive the RfD.

EPA (IRIS 2004) presently classifies dichloromethane as group B2 (probable human carcinogen), 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. The oral slope factor for methylene chloride is 7.5E-3 per mg/kg/day, and the inhalation unit risk is 4.7E-7 per $\mu g/m^3$.

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

Neurological Effects

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 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). 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. The value of 0.6 ppm is similar to the intermediate- and chronic-duration MRLs, both of which are 0.3 ppm.

Hepatic Effects

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 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).

Hematological Effects

ATSDR derived a chronic-duration MRL of 0.3 ppm for methylene chloride, based on a NOAEL of 50 ppm 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 hour/day, 5 day/week) resulting in a NOAEL[ADJ] of 8.92 ppm. Whereas the MRL was derived based on hepatic effects (extrarespiratory), a human equivalent concentration (HEC) was calculated. Since the ratio of the blood:air partition coefficient in the rat to the blood:air partition coefficient in the human was > 1, the value of 1.0 was used to calculate the LOAEL[HEC] (EPA 1994). This resulted in an MRL of 0.3 ppm by dividing the LOAEL of 8.92 ppm by an uncertainty factor of 30 (3 for extrapolation from animals to humans, and 10 for human variability).

Respiratory Effects

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 rats chronically exposed to 1000 ppm methylene chloride; more severe effects were seen in rats and mice at higher exposure levels (up to 10,000 ppm). As rodents are obligate nose breathers, it is possible that they will be particularly sensitive to nasal effects, relative to humans. Therefore, the 500 ppm NOAEL value from humans was used to derive the TTD. The NOAEL was divided by an uncertainty factor of 10, representing intrahuman variability, to derive the TTD_{RESP} of 50 ppm.

Cancer

EPA (IRIS 2004) presently classifies dichloromethane as group B2 (probable human carcinogen), 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. The oral slope factor for methylene chloride is 7.5E-3 per mg/kg/day, and the inhalation unit risk is 4.7E-7 per $\mu g/m^3$.

Summary (TTD for Methylene Chloride)
MRL_{NEURO}=0.6 ppm
$MRL_{HEPATIC}$=0.3 ppm
MRL_{HEMATO}=0.3 ppm
TTD_{RESP} = 50 ppm

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Appendix D: Background Information for Nitrogen Dioxide

D.1 Toxicokinetics

The uptake of NO_2 has been assessed following inhalation exposure in humans. Absorption was between 81–90% of the total NO_2 exposure in healthy human volunteers briefly exposed to an NO/ NO_2 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 an average uptake of 72% at rest and 87% during exercise (Bauer et al. 1986). The uptake of NO_2 in animal studies is similarly extensive, with near-complete absorption in acute studies. For example, Kleinman and Mautz (1987) determined that total respiratory absorption in dogs exposed to up to 5 ppm of NO_2 was 85% at rest, and nearly 100% with high ventilation rates. In animal studies, the uptake of NO_2 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, NO_2 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 NO_2 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 NO_2 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

Respiratory Effects

Exposures of healthy subjects to 4 ppm or below for up to 2 hours have generally been without noticeable effects on lung function (Hackney et al. 1978; Goings et al. 1989), although some studies have noted changes in airway responsiveness in healthy volunteers exposed to 2 ppm NO_2 or greater, particularly when challenged with methacholine (Abe 1967; Beil and Umer 1976; Mohsenin 1988; Von Nieding et al. 1977, 1979). Exposure to similar levels of NO_2 (~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; Boushey et al. 1988; Devlin et al. 1992; Frampton et al. 1989; Sandstroem et al. 1989, 1990).

Asthmatics or patients with COPD are much more sensitive to effects of NO_2, with changes in airway function generally reported at 0.3 ppm or greater (Bauer et al. 1986; Morrow & 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 NO_2 (>7520 $\mu g/m^3$, 4.0 ppm) can decrease arterial oxygen partial pressure (PaO_2) in exposed humans (Von Nieding & Wagner 1977; Von Nieding et al. 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 NO_2 required.

NO_2 has been shown to elicit a variety of respiratory effects in animal studies. Exposures to 5 ppm of NO_2 or greater (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 NO_2 continuously for 1 week, with a peak exposure of 2 ppm for one 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 NO_2 (<1 ppm) may result in increased macrophage-mediated clearance (Schlessinger and Gearhart 1987; Schlesinger 1987a,b). Long-term exposure (6 months) of baboons to 2 ppm NO_2 resulted in decreased macrophage migration, suggestive of impaired clearance (Greene and Schneider 1978). Changes in lung morphology generally do not occur below 5 ppm NO_2 in animal studies.

Immunological Effects

Animal studies have identified changes in immunological endpoints as a sensitive endpoint for exposure to NO_2; however, such effects have in most cases been localized to the lung. Richters & Damji (1988, 1990) noted decreases in the total proportion of T-cells in mice exposed to 0.25 ppm NO_2 for 7 or 36 weeks. Subchronic or chronic exposure to 1–2 ppm has resulted in other immunologic changes, including decreases in circulating IgG, IgM, and IgA levels, hemolytic activity of complement, and splenic natural killer cell activity (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; Gardner et al. 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 NO_2 concentrations for shorter durations (Coffin et al. 1977; Erlich and Henry 1968; Gardner et al. 1977a,b; Ito 1971; McGrath and Oyervides 1985).

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, NO_2 in the body quickly reacts to nitrous and nitric acids or reactive nitrogen species (including peroxynitrite). These reactions may result in a variety of changes, including cellular damage, lipid peroxidation, interaction with cellular proteins and thiols, depending on the susceptibility of cellular molecules to nitrogen radical interaction. Persinger et al. (2002) has recently published a review of basic molecular mechanisms of nitrogen dioxide-induced lung injury.

D.4 Health Guidelines

ATSDR (2005) has not derived MRLs for nitrogen dioxide for any exposure duration or route.

EPA (2005) does not list an RfD, RfC, or cancer classification for nitrogen dioxide on IRIS.

IARC (2005) has not issued a carcinogenicity classification for nitrogen dioxide.

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

Respiratory Effects

Short-term exposures of healthy adults to NO_2 have noted small changes in pulmonary function at concentrations as low as 2 ppm (Abe 1967; Beil and Umer 1976; Mohsenin 1988; Von Nieding et al. 1977, 1979). Asthmatics and people with COPD, who represent a sensitive population for the respiratory effects of NO_2, have generally shown no effects at 0.25 ppm and below (Joerres 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 NO_2-induced pulmonary changes in asthmatics is therefore 0.25 ppm. Since it occurs in a sensitive population, no uncertainty factor was applied for intrahuman variability. However, since the exposure duration was short (<1 hour), an uncertainty factor of 3 was applied to adjust for longer-duration exposures. The TTD was therefore 0.25 ppm ÷ 3 = 0.08 ppm.

Summary (TTD for Nitrogen Dioxide)
TTD_{RESP} = 0.08 ppm

D.6 References

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Appendix E: Background Information for Tetrachloroethylene

Tetrachloroethylene (C_2Cl_4, CASRN 127-18-4) is a commercially important chlorinated hydrocarbon solvent and chemical intermediate used as a dry cleaning and textile-processing solvent and for vapor degreasing in metal-cleaning operations. Tetrachloroethylene has been found in at least 771 of the 1,430 current or former EPA National Priorities List (NPL) hazardous waste sites (HazDat 1996). An estimate of the current end-use pattern for tetrachloroethylene is as follows: 55% for chemical intermediates, 25% for metal cleaning and vapor degreasing, 15% for dry cleaning and textile processing, and 5% for other unspecified uses (Chemical Profile 1995). A survey conducted by the International Fabricare Institute in 1989 indicated that 88.4% of the dry cleaners in the United States use tetrachloroethylene (Andrasik and Cloutet 1990) as the primary solvent.

Although the use of tetrachloroethylene in the dry cleaning and degreasing industries makes this chemical a potential hazard for exposed workers, casual contact by the general population with dry-cleaned clothing may pose a risk as well. One study showed that the storage of newly dry-cleaned garments in a residential closet resulted in tetrachloroethylene levels of 0.5–2.9 mg/m^3 (74–428 ppb) in the closet after 1 day, followed by a rapid decline to 0.5 mg/m^3 (74 ppb) which persisted for several days (Tichenor et al. 1990). Initial "airing out" of the clothes for 4–8 hours had little effect on the resulting emissions, presumably because diffusion through the fabric, rather than surface evaporation, was rate-limiting. A study of nine homes into which 10 or fewer freshly dry-cleaned garments were introduced showed an increase in tetrachloroethylene levels in the air of seven homes (Thomas et al. 1991). The increases ranged from 2 to 30 times the levels before the introduction of the garments, and the magnitude of the increase was highly correlated with the number of garments divided by the house volume. Tetrachloroethylene levels in personal breathing space and expired air of residents were also monitored and found to be generally correlated with indoor air concentrations. An investigation of different methods for reducing tetrachloroethylene retention in dry-cleaned fabrics found that, while airing at 20°C for several hours had little effect, airing at 45°C greatly reduced retention time, and thus was recommended as a way to reduce consumer exposure from garments (Guo et al. 1990).

E.1 Toxicokinetics

Results from human and animal studies indicate that inhaled tetrachloroethylene is rapidly and efficiently absorbed by the lungs (ATSDR 1997). 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 tetrachloro-

ethylene 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 1997). 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 hours, 30–40 hours, 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- to 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 more than 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 1997). 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 CYP2B1/2and 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- to 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 hydrase, and another involving initial conjugation of tetrachloroethylene with glutathione via glutathione transferase (ATSDR 1997). The glutathione 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 tetrachloro-ethylene, but it has been proposed that the trichloroethanol derives from metabolism of trichloroethylene contamination of tetrachloroethylene rather than metabolism of tetrachloroethylene (ATSDR 1997). Evidence is available that mice have a greater hepatic capacity for total tetrachloroethylene metabolism than rats, which in turn have a higher capacity than do 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 1997). 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 controlled conditions indicate that neurological effects are the most predominant and sensitive effects of tetrachloroethylene (ATSDR 1997). 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 1997). Studies of animals exposed *in utero* (via oral exposure of mothers) indicate that tetrachloroethylene can adversely influence the developing nervous system, but studies to examine possible associations between occupational exposure of humans to tetrachloroethylene and increased risks for birth defects in offspring or reproductive effects such as menstrual disorders and spontaneous abortions provide only suggestive evidence that these types of effects may occur in humans (ATSDR 1997). Limitations of the human reproductive and developmental toxicity studies include confounding exposures to other chemicals, inability to adjust for confounding factors, and lack of exposure data for individuals in the studies.

Cardiovascular Effects

Based on analogy to other low molecular weight halogenated hydrocarbons, cardiac arrhythmias (associated with sensitization of the heart to epinephrine) from acute high level exposures to tetrachloroethylene may be expected to occur in humans. However, ATSDR (1997b) reviewed only one case of cardiac arrhythmia in a dry cleaning worker exposed to tetrachloroethylene, and a study of beagle dogs exposed to 5,000 or 10,000 ppm tetrachloroethylene found no evidence of heart sensitization to epinephrine.

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) or liver effects in cases of people acutely exposed to high levels (e.g., enlarged liver or elevated serum ALT activity) (ATSDR 1997). 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 1986). Nephropathy was observed in rats and mice exposed to gavage doses ≥471 and 386 mg/kg/day, respectively (NCI 1977).

Hepatic Effects

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 1997). For example, hepatocellular degeneration and necrosis was 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 1986). 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 1986). 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).

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 predominately by the parent material (ATSDR 1997). 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 tetrachloroethylene 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 1997).

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 glutathione conjugates via β-lyase catalysis in the kidney (ATSDR 1997). 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, glutathione conjugation, competes for tetrachloroethylene as a substrate. The relevance of the observed rat kidney effects to humans has been questioned because glutathione 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-2μ-globulin, a protein that is produced in male rats but not in female rats or humans of either sex (ATSDR 1997). 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 nonpretreated rats (Moslen et al. 1977). The relevance of tetra-

chloroethylene-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 1997; Lake 1995).

E.4 Health Guidelines

ATSDR (1997b) derived an acute inhalation MRL of 0.2 ppm for tetrachloroethylene based on a NOAEL of 10 ppm and a LOAEL of 50 ppm for neurological effects (e.g., performance deficits in tests of vigilance and eye-hand coordination) in volunteers exposed 4 hours/day for 4 days (Altmann et al. 1992), and an uncertainty factor of 10 for human variability.

ATSDR (1997b) derived a chronic-duration inhalation MRL of 0.04 ppm for tetrachloroethylene based on a LOAEL of 15 ppm for significantly prolonged reaction times in women who worked in dry cleaning shops for an average period of 10 years (Ferroni et al. 1992) and an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability). Signs of mild kidney damage (increased urinary levels of lysozyme and β-glucuronidase) were found in another study of workers exposed to an average concentration of 10 ppm for an average of 14 years (Franchini et al. 1983). ATSDR (1997b) considered nervous system effects to be a more appropriate basis for the MRL and noted that the significance and adversity of the mild kidney effects were not clear.

ATSDR (1997b) did not derive an intermediate-duration inhalation MRL for tetrachloroethylene due to the lack of studies of neurological endpoints in humans exposed for intermediate durations. It was noted that liver enlargement was observed in mice exposed to 9 ppm, 24 hours/day for 30 days, but data in humans were considered more appropriate for MRL derivation because mice metabolize more tetrachloroethylene to trichloroacetic acid than humans and the peroxisomal proliferation response is greater in mice than humans.

ATSDR (1997b) derived an acute oral MRL of 0.05 mg/kg/day for tetrachloroethylene based on a LOAEL of 5 mg/kg/day for hyperactivity at 60 days of age in mice exposed to gavage doses for 7 days beginning at 10 days of age (Fredriksson et al. 1993) and an uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for extrapolating from animals to humans, and 10 for human variability).

ATSDR (1997b) did not derive intermediate- or chronic-duration oral MRLs for tetrachloroethylene due to the lack of suitable data. It was noted that intermediate-duration oral studies have observed liver

effects in rats and mice and kidney effects in male rats, but these effects were not considered appropriate for MRL derivation due to apparent differences between humans and rodents in metabolism of tetrachloroethylene and in peroxisomal proliferation response, and indications that the kidney effects in male rats may be associated with accumulation of α-2μ-globulin, a male rat-specific protein (ATSDR 1997).

EPA's IRIS database (EPA 2005) lists an RfD of 0.01 mg/kg/day for tetrachloroethylene based on a NOAEL of 20 mg/kg/day for hepatotoxic effects in mice exposed by gavage for 6 weeks (Buben and O'Flaherty 1985) and an uncertainty factor of 1,000 (10 for extrapolating from animals to humans, 10 for human variability, and 10 for extrapolating from subchronic exposure duration to chronic duration).

EPA's IRIS database (EPA 2005) does not list an RfC or a carcinogenicity assessment for tetrachloroethylene. As reviewed by ATSDR (1997b), the EPA Science Advisory Board in 1987 offered the opinion that the weight of evidence for tetrachloroethylene carcinogenicity was on a Group B2/C continuum (i.e., on the border between Group B2 and Group C). In 1991, another statement of this opinion was issued by the Science Advisory Board Executive Committee noting that tetrachloroethylene "should be considered to be an animal carcinogen, based on three endpoints in two species: liver tumors in male and female mice, kidney tumors in male rats, and, possibly, mononuclear cell leukemia in male and female rats" and that they did "not consider the evidence strong enough to classify this chemical as a probable human carcinogen." EPA has yet to present a more recent position on the weight-of-evidence classification for trichloroethylene carcinogenicity, but is expected to present, in 2001, an updated carcinogenicity assessment for tetrachloroethylene based on its 1996 Proposed Guidelines for Carcinogen Risk Assessment. NTP (2001) lists tetrachloroethylene as reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. IARC (1995) concluded that tetrachloroethylene is probably carcinogenic to humans (Group 2A) based on limited evidence in humans and sufficient evidence in experimental animals. IARC (1995) made the following notes to accompany its conclusions:

"Although tetrachloroethylene is known to induce peroxisome proliferation in mouse liver, a poor quantitative correlation was seen between peroxisome proliferations and tumor formation in the liver after administration of tetrachloroethylene by inhalation. The spectrum of mutations in proto-oncogenes in liver tumors from mice treated with tetrachloroethylene is different from that in liver tumors from mice treated with trichloroethylene. Several epidemiological studies showed elevated risks for oesophageal cancer, non-Hodgkin's lymphoma, and cervical cancer."

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

Neurological Effects

ATSDR (1997b) derived a chronic-duration inhalation MRL of 0.04 ppm for tetrachloroethylene based on a LOAEL of 15 ppm for significantly prolonged reaction times in women who worked in dry cleaning shops for an average period of 10 years (Ferroni et al. 1992). The LOAEL was duration-adjusted for continuous exposure to 3.6 ppm and an uncertainty factor of 100 (10 for the use of a LOAEL and 10 for human variability) was applied. Signs of mild kidney damage (increased urinary levels of lysozyme and β-glucuronidase) were found in another study of workers exposed to an average concentration of 10 ppm for an average of 14 years (Franchini et al. 1983). ATSDR (1997b) considered nervous system effects to be a more appropriate basis for the MRL and noted that the significance and adversity of the mild kidney effects were not clear.

Cardiovascular Effects

Data on the possible cardiovascular effects of tetrachloroethylene are not sufficient for derivation of a TTD_{CARDIO}.

Renal Effects

Signs of mild kidney damage (increased urinary levels of lysozyme and β-glucuronidase) were found in a study of workers exposed to an average concentration of 10 ppm for an average of 14 years (Franchini et al. 1983). This change was designated a minimal LOAEL, because the signs were mild; the duration-adjusted value is 2.4 ppm. The TTDRENAL was derived by applying an uncertainty factor of 30 (3 for a minimal LOAEL and 10 for intrahuman variability) to the LOAEL to give a TTDRENAL of 0.08 ppm.

Hepatic Effects

Long-term studies in rats have shown no hepatic effects at tetrachloroethylene concentrations up to 400 ppm (NTP 1986). 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 1986); exposure levels below 100 ppm were not evaluated. The 100 ppm LOAEL in mice was used to derive the hepatic TTD by applying an uncertainty factor of 1000 (10 for animal to human extrapolations, 10 for intrahuman variability, and 10 for use of a LOAEL) to give a $TTD_{HEPATIC}$ of 0.1 ppm.

Summary (TTD for Tetrachloroethylene)
MRL_{NEURO}=0.04 ppm
TTD_{RENAL}=0.08ppm
$TTD_{HEPATIC}$=0.1ppm

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