EPA

TOXICOLOGICAL REVIEW

of

METHYL CHLORIDE

(CAS No. 74-87-3)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

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(DRAFT)

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard identification and dose-response assessment in IRIS pertaining to chronic exposure to methyl chloride. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of methyl chloride.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 513-569-7254.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

Chemical Manager

Mark Greenberg
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

Authors

James Riddle, Ph.D
Sciences International, Inc.
Alexandria, VA

Mark Greenberg
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

Internal EPA Reviewers

Hugh Tilson, Ph.D
National Health and Environmental Effects Research Laboratory
U.S. Environmental Protection Agency

Karl Jensen, Ph.D
National Health and Environmental Effects Research Laboratory
U.S. Environmental Protection Agency

John Lipscomb, Ph.D
National Center for Environmental Assessment
U.S. Environmental Protection Agency

1. INTRODUCTION

This document presents background information and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for dose-response assessments of potential noncancer health effects. The RfD is based on the assumption that thresholds exist for many toxic effects such as cellular necrosis, although they may not exist for other toxic effects such as some carcinogenic responses. It is expressed in exposure units of mg/kg-day, and generally represents an estimate (with an uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime following long-term or chronic exposure (e.g., seven years to a lifetime). The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate that permits the same degree of anticipated protection. This inhalation RfC considers toxic effects that may occur either in the respiratory system itself (portal-of-entry effects) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. This information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which any carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* results from the application of a low-dose extrapolation procedure and is presented as the cancer risk per mg/kg-day of exposure. The *unit risk* is the quantitative estimate of cancer risk expressed either per µg/L drinking water consumed or per $µg/m^3$ air breathed. Another form in which risk is presented is as a series of drinking water or air concentrations resulting in cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for methyl chloride has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidance documents that may have been considered in the development of this assessment include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a); *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b); *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c); *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991); *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a); *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996b); *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *The use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995a); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b); and *Policy for Risk Characterization at the U.S. Environmental Protection Agency (Memorandum of Administrator, Carol M. Browner* (U.S. EPA, 1995b).

Literature search strategies employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLIT, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Methyl chloride's CAS Registry Number is 74-87-3. It is also referred to as chloromethane or monochloromethane, and is or has been supplied in several purity grades: pure (≥ 99.5%), technical, and two "refrigerator" grades (HSDB, 1999; Lewis, 1993). Selected chemical and physical properties of methyl chloride are presented in Table 2-1.

Table 2-1. Selected physical and chemical properties of methyl chloride

Parameter	Data	Reference
Empirical formula	CH_3Cl	Lewis (1993)
Molecular weight	50.49	Budavari et al. (1989)
Melting point	-97.6 °C	Lewis (1993)
Boiling point	-23.7 °C	Lewis (1993)
Vapor pressure	3670 mm Hg at 20 °C	ATSDR (1998)
	4310 mm Hg at 25 °C	
Solubility in water	6263-7400 mg/L at 20 °C	U.S. EPA (1986d)
	4800-5325 mg/L at 25 °C	ATSDR (1998)
Log K_{OW}	0.91 (experimental)	ATSDR (1998)
Odor threshold	100 ppm	Sittig (1991)
Blood-air partition coefficient	2.12-2.49, 10 ppm exposure	Nolan et al. (1985)
(modeled human data)	1.71-1.83, 50 ppm exposure	
Conversion factors	1 ppm (v/v) = 2.064 mg/m^3	ATSDR (1998)
(in air at 25° C)	1 mg/m^3 = 0.4845 ppm (v/v)	

Methyl chloride is a colorless gas that compresses to a colorless liquid, both of which have been described as having a difficult-to-detect, nonirritating ethereal odor that is faintly sweet to the taste (Budavari et al., 1989; Lewis, 1993). It is moderately soluble in water, by which it is decomposed to methanol and hydrogen chloride; it is generally synthesized from these same two chemicals, and to a lesser extent from the chlorination of methane (ATSDR, 1998; Lewis, 1993; U.S. EPA, 1986d).

Methyl chloride is abundant in nature; the vast majority comes from natural sources such as the oceans, microbial fermentation, and biomass fires. Natural sources are thought to exceed anthropogenic sources by at least an order of magnitude, with much of the latter being produced and consumed industrially and not released into the environment (ATSDR, 1998; Farber and Torkelson, 1989; U.S. EPA, 1986d). Methyl chloride is principally used in the manufacture of silicones (72%), agrichemicals (8%), methyl cellulose (6%), quaternary amines (5%), butyl rubber (3%), and miscellaneous products (2%) such as tetramethyl lead and fluids used in thermometric and thermostatic equipment; it is no longer used significantly as a refrigerant or an anesthetic (ATSDR, 1998; Farber and Torkelson, 1989; Lewis, 1993; U.S. EPA, 1986d). Methyl chloride has a time-weighted average threshold limit value (TLV-TWA) of 50 ppm and a short-term exposure limit (STEL) of 100 ppm (ACGIH, 2000; ATSDR, 1998; OSHA, 1999); the OSHA permissable exposure limit (PEL) values are a 100 ppm TWA, and a 200 ppm ceiling with a 5-min maximum peak of 300 ppm in any 3-h period (OSHA, 1999; NIOSH, 1999).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

The principal route of human exposure to methyl chloride is inhalation. Methyl chloride is readily absorbed by the lungs and rapidly reaches a steady-state concentration in the blood, from which it is presumably distributed to most organs and tissues. Precise determinations of tissue distribution and equilibrium concentrations are complicated by methyl chloride's high volatility and rapid metabolism. Although cytochrome P-450-mediated oxidation seems to be involved to some degree in the metabolism of methyl chloride, substantial evidence indicates that conjugation with glutathione (GSH) is likely to be the principal first step in vivo. Direct dehalogenation of methyl chloride to formaldehyde by P-450 may occur, but P-450 oxidation of GSH-methyl chloride conjugation intermediates (e.g., S-methylcysteine or methanethiol) also could be involved in the formation of formaldehyde. The resulting formaldehyde then enters the "one-carbon pool," either directly or through conversion to formic acid, where it becomes available for macromolecular synthesis or is expired as CO_2. Very little methyl chloride is excreted unchanged, and most of that which is not used in various anabolic pathways or expired as CO_2 appears to be excreted in the urine. The various reported or hypothesized urinary

metabolites comprise several sulfur-containing compounds, all thought to be derived from the initial GSH conjugate (S-methylglutathione).

3.1 ABSORPTION

As noted, human absorption of methyl chloride is likely to occur through inhalation, although dermal absorption could constitute a minor contributing route of exposure. Ingestion is highly improbable. Data on absorption of methyl chloride are available only for inhalation exposure. Nolan et al. (1985) determined an in vivo blood:air partition coefficient for humans in the range of 2.12 to 2.49 at 10 ppm. Gargas et al. (1989) found a similar value (2.47) for the rat using an in vitro technique.

In a human behavioral study designed to explore the effects of methyl chloride and diazepam, administered singly or in combination, volunteers (sex unspecified) were exposed for 3 h to methyl chloride concentrations of 200 ppm (n = 24) or 100 ppm (n = 8) (Putz-Anderson et al., 1981). Levels of methyl chloride expired in alveolar breath reached equilibrium within the first hour of exposure, and were highly correlated with venous blood levels ($r = 0.85$, $n = 29$, $p < 0.01$). Blood samples were taken prior to and approximately half-way through (90 min) the exposure period. For the 200 ppm exposure group, the mean alveolar breath concentration (± standard deviation) of methyl chloride was 63 ± 23.6 ppm, while that in the blood was 11.5 ± 12.3 ppm. For the 100 ppm exposure group, breath concentrations were 36 ± 12 ppm and blood concentrations were 7.7 ± 6.3 ppm. These data reflect large inter-individual differences in apparent body burden, and the study authors noted that 3 of the 24 participants in the 200 ppm exposure group had methyl chloride breath concentrations of over 100 ppm, substantially higher than the remainder. Under these conditions, mean alveolar breath levels were proportionate to exposure concentrations, while blood levels were somewhat less so.

In another human study that utilized a complex exposure scheme, groups of 2-4 males were exposed for 7.5 h (Group I), 3 h (Group II) or 1 h (Group III) to concentrations of 0, 20, 100, “100f” (fluctuating among 50, 100, and 150 ppm for equivalent periods of time during the exposure period) or 150 ppm methyl chloride, 2-5 days/wk over the course of 6 weeks (Stewart

et al., 1980). Only a single concentration (counting 100f as a single concentration) was utilized in any given week, except the final week when two days of exposure to 150 ppm were followed by a single day of exposure to 0 ppm. Similarly, three groups of women were exposed for 7.5, 3 or 1 h (Groups I, II, and III, respectively) to 0 ppm methyl chloride for one day during Weeks 1 and 3, and to 100 ppm for five days during Week 2. For various reasons, including study drop-out, data are not available for every time point for each subject initially in the study, thus compromising the study's quality.

Breath samples obtained prior to, and 1 min, 15 min, 30 min, 1 h, 2 h, and 3 h after each exposure were analyzed for methyl chloride. Blood samples, taken pre-exposure, immediately "pre-exit" (from the exposure chamber), and 15 and 30 min post-exposure on Days 1 and 4 of each exposure week were also analyzed. Collectively, these data indicated that mean methyl chloride levels in expired alveolar breath were directly correlated with exposure concentrations immediately after exposure, although the proportionality tended to degrade at subsequent time points. One-minute post-exposure alveolar breath levels were approximately 40-55% of exposure concentrations. Of the 11 male and 9 female subjects, 3 males and 1 female were identified as "high responders" (or more appropriately, "slow metabolizers") whose alveolar breath levels of methyl chloride were 60-110% higher than their identically exposed peers immediately after exposure, and 3-6 fold higher at 1 h post-exposure. No inference was made to associate high blood levels with increased toxicity. Blood concentrations of methyl chloride were low even just prior to exiting the exposure chamber, declined rapidly, and were difficult to measure, especially for the 20 ppm exposure and for most time points other than pre-exit. Because of inter-individual variation and lack of complete data, generalizations are difficult to make. However, blood concentrations appeared to generally increase with exposure concentration (20 ppm < 100 ppm < 100f ppm < 150 ppm); for 7.5 h exposures to 100f or 150 ppm, pre-exit blood concentrations ranged from 3.2 to 8.2 ppm.

Methyl chloride concentrations were also measured in blood, expired breath, and alveolar breath samples taken from 6 male volunteers who were each exposed for 6 h to 50 and 10 ppm methyl chloride, on different days separated by 2 weeks (Nolan et al., 1985). Methyl chloride rapidly reached equilibrium levels in both expired air and blood during the first hour of exposure,

and these levels were proportional to exposure concentration. Of particular note, the authors reported no consistent relationship between methyl chloride concentrations in expired air and those in alveolar air. As in the previous study, a subpopulation of apparent high responders (or more appropriately, "slow metabolizers or rapid eliminators") was noted; two of the subjects were observed to have methyl chloride concentrations in expired air that were 1.5-2 times higher and venous blood levels 3 times higher than those of the remaining four subjects. Combining the respiratory minute volume (RMV) from a two-compartment pharmacokinetic model with the differences between inspired- and expired-air methyl chloride concentrations, the study authors calculated that the rapid and slow metabolizers, equivalent to the low and high responders identified previously by Stewart et al. (1980), absorbed 3.7 and 1.4 µg CH_3Cl/min/kg, respectively.

The pharmacokinetics of methyl chloride absorption and metabolism have also been studied in rats and dogs (Andersen et al., 1980; Landry et al., 1983a,b). Groups of 6-9 male Fischer 344 rats were exposed for approximately 2-3 h in a closed, atmosphere-recirculating chamber to various concentrations of different gases having low water solubility, including methyl chloride (Andersen et al., 1980). Disappearance of methyl chloride from the chamber was monitored, and the data were analyzed using a four-compartment (gas phase, blood and richly perfused tissues [RPT], poorly perfused tissues [PPT], and production of metabolites), steady-state pharmacokinetic model. Methyl chloride was characterized as having mixed uptake kinetics, comprised of both a slow first-order process and a rapid, but saturable, process. The former, thought to largely represent uptake into PPT, was fairly negligible for methyl chloride (rate constant = 0.027 kg^{-1} hr^{-1}), while the latter drove the overall uptake rate and was accounted for by the K_m describing generation of metabolites in the RPT (K_m = 630 ppm; V_{max} = 120 ppm/kg/h = 7.7 mg/kg/h). Thus, at low concentrations, absorption of methyl chloride would be essentially organ perfusion-limited and appear nearly first-order.

Using a dynamic, non-recirculating exposure chamber, Landry et al. (1983a) exposed male Fischer 344 rats for 6 h and male beagle dogs for 3 h to methyl chloride concentrations of 50 or 1,000 ppm (3 animals/species/concentration). Blood levels of methyl chloride were monitored during the exposure period and for several hours thereafter, and the data were found to be

adequately described by a linear, two-compartment open pharmacokinetic model (zero-order uptake, first-order compartmental input, blood included in the first compartment). In both rats and dogs, apparent steady-state blood levels were achieved within the first hour of exposure and were proportionate to exposure concentration. End-exposure methyl chloride blood levels were very similar for both species at exposure concentrations of 50 ppm (rat mean = 194 ng/g; individual dogs = 135, 171, and 177 ng/g) and 1,000 ppm (rat mean = 3,930 ng/g; individual dogs = 3,220, 3,760, and 4,080 ng/g). Methyl chloride blood levels in both species at the two exposure concentrations closely approximated the 20-fold ratio (1,000 ppm/50 ppm) expected for dose-independent uptake kinetics over the tested concentration range (rat ratio = 20.3; ratios for the two dogs exposed to both concentrations = 19 and 21). In a final expression of species similarity and relative concentration independence, mean steady-state blood:gas concentration ratios at 50 and 1,000 ppm were 1.8 and 1.9 for rats, respectively, and 1.5 and 1.8 for dogs.

In a follow-up study, Landry et al. (1983b) utilized another dynamic, non-recirculating exposure apparatus in conjunction with a whole-body plethysmography chamber to expose male Fischer 344 rats to methyl chloride concentrations of 50 or 1,000 ppm. The collected, digitized airflow data were used to calculate respiratory parameters (inhalation frequency, tidal volume, RMV). Methyl chloride uptake was calculated at 1.5 and 2 h of exposure using measurements in chamber supply and exhaust air, as well as chamber airflow rate. Incorporating RMV values permitted calculation of the ratios of methyl chloride concentrations in expired to inspired air. Virtually identical uptake rates at both time points indicated that apparent steady-state conditions had been achieved by 1.5 h of exposure for both test concentrations. Methyl chloride uptake rates of 0.20 and 3.27 nmol/min/g body weight (bw) for exposure concentrations of 50 and 1,000 ppm, respectively, displayed an approximate 16.5-fold difference, that was marginally different ($0.05 < p < 0.1$) from the 20-fold difference expected from a linear, dose-independent model of uptake. However, the ratios of methyl chloride concentrations in expired to inspired air were equivalent (0.86) for both exposure concentrations, apparently accounted for by a significant ($p < 0.05$) 20% reduction in RMV for the 1,000 ppm rats (143 mL/min) compared to the 50 ppm rats (179 mL/min).

The authors utilized these data, the methyl chloride blood concentration data from their earlier study (Landry et al., 1983a), and the addition of a metabolism component to their earlier two-compartment pharmacokinetic model, to suggest that under steady-state conditions, uptake is not strictly ventilation-limited. According to their model, in addition to a reduction in RMV at the higher exposure concentration, reductions in the first-order rate constants for metabolism (k_m, from 0.19 min^{-1} to 0.14 min^{-1}) and expiration (k_{1e}, from 1.38 min^{-1} to 0.96 min^{-1}) would also be expected. Thus the model estimated that for a 6-h exposure to 50 or 1,000 ppm of methyl chloride, the corresponding effective doses (the amounts remaining in the body at the end of exposure, plus the amounts metabolized) would be 3.8 and 67 mg/kg, respectively, or a fold-increase of 17.6. This increase is less than the 20-fold increase expected for linear, dose-independent uptake kinetics.

In summary, it appears that methyl chloride is readily absorbed during inhalation exposure and rapidly reaches steady-state levels in both expired air and in the blood of humans and laboratory animals. Of particular interest is the observation of two identifiable groups of humans who differ in their rate of metabolism: apparent slow metabolizers in contrast to apparent rapid metabolizers. Furthermore, in humans, rats, and dogs, absorption from the lungs at relatively low exposure concentrations (possibly up to 500-1,000 ppm) seems closely proportional to the exposure concentration. In addition to the studies with dogs described above (Landry et al., 1983a,b), older studies reviewed in ATSDR (1998) suggest that methyl chloride uptake in dogs may be proportional to exposure up to concentrations as high as 15,000 and 40,000 ppm.

3.2 DISTRIBUTION

A number of studies have directly or indirectly investigated methyl chlorides's distribution to tissue in Fischer 344 rats and dogs (Bus et al., 1980; Kornbrust et al., 1982; Landry et al., 1983a; Xu et al., 1990). As in humans, rapid and biphasic blood clearance was found in both Fischer 344 rats and beagle dogs after exposures to 50 or 1,000 ppm for 6 h (dogs) or 3 h (rats) (Landry et al., 1983a). Rapid and slower phase half-times were 4 and 15 min, respectively, for rats, and 8 and 40 min, respectively, for dogs.

Citing their earlier unpublished observations, Kornbrust et al. (1982) noted that after exposing rats to an unspecified inhalation dose of radio-labelled methyl chloride, over 45% was exhaled as $^{14}CO_2$ and only 6.6% as parent compound, while >14% of the radioactivity was still associated with the carcass after two days and nearly as much after five days. Persistent radioactivity was also observed in several major organs. Experiments were then conducted to determine whether these findings resulted from direct binding of methyl chloride to long-lived macromolecules, or from metabolic incorporation through normal anabolic pathways. Following exposure of male Fischer 344 rats for 6 h to 500 ppm of ^{14}C-labeled methyl chloride, uptakes of radioactivity into various tissues immediately post-exposure (expressed as µmole of CH_3Cl equivalents per gram wet weight of tissue; mean ± SD) were reported as: 4.13 ± 0.65 (liver), 3.43 ± 0.53 (kidney), 2.42 ± 0.24 (intestine), 2.29 ± 0.19 (testes), 1.21 ± 0.25 (lung), 0.71 ± 0.05 (muscle), and 0.57 ± 0.08 (brain).

In each case, 80% or more of the recovered radioactivity was acid-soluble, with the remainder variously distributed among deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein, and lipid macromolecules (Kornbrust et al., 1982). Increasing the exposure concentration to 1,500 ppm did little to alter the tissue distribution pattern, and on average increased the proportion of radioactivity found in the nucleotide fractions by less than 1.5-fold. While the total amounts of radioactivity associated with protein and lipid were generally an order of magnitude greater than those associated with nucleic acid, the specific activity (per nucleotide or amino acid) was much higher in nucleic acid in each tissue examined. In the liver, compared to immediately after exposure, the 24-h percentage of tissue radioactivity found associated with protein had increased from 9 to 48%, whereas acid-soluble radioactivity had decreased from 79 to 26%; smaller absolute increases were observed for lipid (9.5 to 13.1%), RNA (1.4 to 4.2%) and DNA (0.4 to 9.0%). Similar observations were reported for the other tissues examined, with the exception of the testes, in which acid-soluble radioactivity was retained to a much greater extent.

The study authors noted that this apparent shift of label to the macromolecular fractions was not due to their continued accumulation of radioactivity, but rather to a relatively more rapid loss of radioactivity in the acid soluble fraction. For liver, lung, kidney, and testes there were no

great changes from 0 to 24 h post-exposure in the amount of radioactivity associated with RNA or protein, but significant decreases with lipid and increases with DNA were noted, especially in liver. Methyl chloride's simple structure and the ubiquitous distribution of its ^{14}C atom suggested that metabolic incorporation, rather than direct alkylation, could be responsible for the label uptake into macromolecules. Therefore, several additional experiments involving macromolecular synthesis inhibitors and chromatography were conducted (Kornbrust et al., 1982). The results, discussed more fully in Sections 3.3 and 4.4.3, indicated that label incorporation into DNA, lipid, and protein macromolecules was largely dependent on their uninhibited biosynthesis. Furthermore, label in DNA and lipid appeared to be anabolically incorporated into purine bases and phospholipids.

Thus, the observed distribution of methyl chloride's carbon atom into macromolecules of the various organs and tissues examined appears mainly to result from its entry into the one-carbon pool used for biosynthesis, rather than from methyl chloride's direct interaction with cellular macromolecules. As the fraction of acid-soluble radioactivity attributable to unmetabolized methyl chloride was not determined in the examined tissues, the degree to which distribution of radioactivity reflects actual distribution of methyl chloride versus one-carbon pool metabolites is uncertain from these data.

Xu et al. (1990) compared the alkylation of hemoglobin (Hgb) in Fischer 344 rats by ^{14}C-labelled methyl chloride, methyl bromide, and methyl iodide. Following 24 h of inhalation exposure in a closed system injected with 45 µmol of test agent, the investigators found a "covalent binding index" (pmol bound/mg Hgb per µmol administered/kg bw) for methyl chloride of 145 ± 16 (SD, n = 5), only about 11 and 15% of the comparable indices for methyl bromide and methyl iodide, respectively. Although these results were presented in terms of alkylation of Hgb, it appears likely, given the findings of Kornbrust et al. (1982) discussed above, that much of the relatively small amount of methyl chloride-derived "covalently bound" radioactivity may be the result of anabolic incorporation of label from the one-carbon pool.

In another study, male Fischer 344 rats (6/dose) were exposed for 6 h to 0, 50, 225, 600, or 1,000 ppm of ^{14}C-labeled methyl chloride (Landry et al., 1983a). Total (nonvolatile) and

nonextractable ^{14}C activities were quantified in liver, kidneys, and testes (low activity or inadequate tissue supply precluded measurement in other tissues), and nonprotein sulfhydryl (NPSH) levels were measured in these tissues and in epididymis, blood, brain, and lung. On an activity per mg tissue basis, the tissue order of ^{14}C association was liver > kidney > testes. Accumulation of nonvolatile activity was closely proportionate to exposure concentration. In contrast, accumulation of nonextractable activity clearly demonstrated evidence of saturation in liver and kidney at exposures of 600 and 1,000 ppm, and in the testes at 1,000 ppm. This nonlinearity likely reflects the saturation of a metabolic process, perhaps for the incorporation of methyl chloride-derived one-carbon pool elements into macromolecules. Levels of NPSH were reduced in a dose-dependent manner in liver, kidney, testis, and epididymis tissues at exposure concentrations of 225, 600, and 1,000 ppm, suggesting that methyl chloride was distributed to these tissues and subsequently metabolized via a postulated glutathione conjugation pathway (see Section 3.3).

In a study described only in abstract (Bus et al., 1980), maternal and fetal tissue from pregnant Fischer 344 rats that had been exposed for 6 h to 1,500 ppm of methyl chloride on Gestation Day 19 were examined for NPSH depletion at 0, 2, 4, and 8 h after termination of exposure. Relative to control values, maternal liver and kidney NPSH levels were maximally depressed immediately after exposure (to 14.9 and 27.4% of control values, respectively), returning to normal by 8 h; fetal placental NSPH, also maximally depressed at 0 h (to 87.5% of the control value), returned to normal within 4 h. In contrast, fetal liver and carcass NPSH levels were not maximally depressed until 2 h post-exposure (to 66.8 and 71.0% of control values, respectively), and had not returned to normal levels by 8 h (to 86.5 and 92.6% of control values, respectively). These data suggest that methyl chloride can be distributed transplacentally to various fetal tissues. However, the details of this study were not located in a peer-reviewed report.

3.3 METABOLISM

Several early studies concerning the metabolism of methyl chloride demonstrated increased levels of formaldehyde in the blood of rats exposed by inhalation, as well as the in vitro reaction

of methyl chloride with human plasma albumin to form S-methylcysteine upon hydrolysis, and with NPSH (principally GSH) in human erythrocytes and in rat tissue homogenates of liver, kidney, and brain, to form S-methylglutathione and S-methylcysteine (reviewed in Farber and Torkelson, 1989; U.S. EPA, 1986d). The rat tissue conversions were shown to be enzyme catalyzed.

In a study by van Doorn et al. (1980), S-methylcysteine was detected in the urine of six chemical workers who were exposed to methyl chloride for 8 h a day during a 7-day shift, although the levels were very low in two who had the highest exposure. The researchers calculated that in four of the workers nearly all the "retained" methyl chloride (i.e., that not exhaled unchanged or as CO_2) was excreted as S-methylcysteine, whereas the other two excreted less than 10% of retained methyl chloride. No methylmercapturic acid was detected in the urine. Citing unpublished data from their laboratory, Kornbrust and colleagues noted, based upon recovery of activity from rats exposed by inhalation to radiolabelled methyl chloride for 6 h, the major (45-50%) final metabolite was CO_2 (Kornbrust et al., 1982; Kornbrust and Bus, 1983). Collectively, such observations suggested the potential involvement of both oxidation (possibly cytochrome P-450 mediated) and GSH conjugation pathways in the metabolism of methyl chloride.

As previously described (Section 3.2), inhalation exposure to methyl chloride has been found to cause in vivo depletion of NPSH in various tissues of male, pregnant female, and fetal Fischer 344 rats (Bus et al., 1980; Dodd et al., 1982; Landry et al., 1983a). Levels of NPSH were maximally depressed in maternal liver, maternal kidney, and fetal placental tissue immediately after an exposure of the dams to 1,500 ppm for 6 h, but not until 2 h later in fetal liver and carcass (Bus et al., 1980). Depressed values returned to control levels within 4-8 h after exposure, and alteration of maternal blood NPSH was not observed. Landry et al. (1983a) exposed male Fischer 344 rats for 6 h to methyl chloride concentrations of 0, 50, 225, 600, or 1,000 ppm, and observed dose-related depletions of NPSH in liver, kidney, testis, and epididymis of 225-1,000 ppm. Relative to control levels, residual NPSH was lowest in the liver and highest in the testis (13 and 70%, respectively, after 1,000 ppm). Examined only at the lowest and highest exposure concentrations, NPSH depletion was not observed in the blood, brain, or lung.

Kornbrust and Bus (1984) found that brain NPSH was reduced in male F344 rats when exposed for 6 h to methyl chloride levels of 1,500 ppm and greater.

In a similar study, Dodd et al. (1982) exposed male Fischer 344 rats for 6 h to methyl chloride concentrations of 0, 100, 500, or 1,500 ppm. Tissue levels of NPSH were not significantly reduced at 100 ppm, nor was blood NPSH at any concentration. However, concentration-dependent depleted levels relative to control values were observed in liver (41 and 17%), kidney (59 and 27%), and lung (55 and 30%) at 500 and 1,000 ppm, respectively. Depletion of NPSH was dependent on duration of exposure, with levels being successively lower after 1, 2, 4 and 6 h of exposure; even at 1,500 ppm, levels had recovered to approximately 90-95% of control values within 8 h after exposure in the liver and kidney, and to 80-85% in the lungs. Pretreatment of the rats with Aroclor-1254 did not significantly alter the methyl chloride-induced depletions of NPSH, despite significantly increasing activity levels in the liver of glutathione-S-alkyltransferase (GSH-SAT) and ethoxycoumarin O-deethylase (EOD). Similarly, pretreatment with SKF-525A, an inhibitor of microsomal enzymes, did not significantly change methyl chloride depletion of NPSH in liver or lung, although it appeared to ameliorate the effect in kidney tissue ($p < 0.01$). In general, these findings suggest that enzymatic formation of reactive metabolites of methyl chloride is not responsible for the observed NPSH depletion, and that GSH-SAT, if involved, is not the rate-limiting enzyme in the process.

As previously discussed (Section 3.2), when male Fischer F344 rats were exposed for 6 h to 500 or 1,500 ppm of ^{14}C-labeled methyl chloride, 8-20% of the radioactivity recovered from whole tissue homogenates of lung, liver, kidney, testes, brain, muscle, and intestine was associated with acid-insoluble material (Kornbrust et al., 1982). While the majority of label was found in protein and lipid, on a per residue basis the concentration of ^{14}C was generally an order of magnitude higher in RNA and DNA. Chromatographic analysis of extracted DNA and lipid indicated that label was detectably associated only with unmodified purine bases and phospholipids, and most association of label with protein was prevented by pretreatment of the rats with the protein synthesis inhibitor, cycloheximide. Similarly, the folic acid antagonist methotrexate (MTX) inhibited ^{14}C uptake into DNA, RNA, non-lipid acid-insoluble, and lipid

fractions relative to control values (by 94, 65, 64, and 47%, respectively), while increasing that associated with acid-soluble material by 55%. Pretreatment of the rats with methanol was reported to inhibit association of label with acid-insoluble material and expired CO_2 by approximately 66%, although pretreatment with ethanol (a competitive inhibitor of methanol metabolism), 3-amino-1,2,4-triazole (a specific inhibitor of catalase-dependent methanol oxidation), or 4-methylpyrazole (an alcohol dehydrogenase inhibitor) were without significant effect. Collectively, these results suggest that the association of methyl chloride carbon atoms with tissue macromolecules results primarily not from alkylation or other direct processes, but rather through metabolism to the one-carbon pool. Methyl chloride did not appear to be converted to methanol, although metabolism to formaldehyde or formate remained a plausible fate; methanol's metabolism to these same two compounds could explain it's inhibitory effect on the incorporation of methyl chloride-derived carbon into macromolecules and CO_2 (i.e., through dilution of methyl chloride's contribution to the one-carbon pool).

When male Fischer rats were exposed for 3 h to 4,000, or 10,000 ppm of methyl chloride, formate accumulation was not observed in the blood, and only a nonsignificant ($p > 0.05$) increase was observed in urine at the high dose (Kornbrust and Bus, 1982). In contrast, as little as 50 mg/kg bw of methanol injected intraperitoneally (ip) produced significant ($p < 0.05$) formate increases in both blood and urine. Pretreatment of the rats with nitrous oxide (which inhibits methionine synthetase and blocks the regeneration of tetrahydrofolate [THF] necessary for formate oxidation) or with MTX (which blocks the synthesis of THF from folate by binding to the enzyme dihydrofolate reductase) resulted in significantly elevated blood and urine levels of formate. These pretreatments were also observed to inhibit the incorporation of radioactivity into cellular macromolecules from either ^{14}C-labeled methyl chloride or formate. Furthermore, when rats were pretreated with nitrous oxide and injected ip with sodium formate (to dilute formate derived from methyl chloride), then exposed to ^{14}C-labeled methyl chloride, the amount of label exhaled as CO_2 was approximately halved, while that excreted in the urine was approximately doubled. The study authors thus concluded that methyl chloride is metabolized to formate, with subsequent incorporation into macromolecules or conversion to CO_2 via folate-dependent single-carbon pathways, and that this is likely to be a quantitatively significant in vivo process. Since exposure to methyl chloride alone does not result in elevated formate levels, but can

deplete tissue NPSH (Bus et al., 1980; Dodd et al., 1982; Landry et al., 1983a) and thus presumably inhibit the GSH-requiring enzyme formaldehyde dehydrogenase that oxidizes formaldehyde to formate, Kornbrust and Bus (1982) speculated that methyl chloride's toxicity might in part result from an accumulation of formaldehyde.

In further studies, Kornbrust and Bus (1983) showed that metabolism of methyl chloride to formaldehyde by rat liver microsomes occurred at a relatively low rate, was dependent on the presence of NADPH (or to a lesser degree, NADH), was approximately doubled by pretreating the rats with the microsomal enzyme inducers phenobarbital or 3-methylcholanthrene (3-MC), and was inhibited by addition to the microsomal mix of the P-450 inhibitors SKF-525A, carbon monoxide, metyrapone, or hexobarbital, suggesting an in vitro role for cytochrome P-450. Pretreatment of rats with SKF-525A, Aroclor 1254, or 3-MC did not significantly alter the in vivo uptake of label from $^{14}CH_3Cl$ into liver macromolecules, but phenobarbital increased uptake into liver lipid and acid-insoluble material by 35 and 28%, respectively. In vivo metabolism of $^{14}CH_3Cl$ to $^{14}CO_2$ was increased 19% by phenobarbital pretreatment and decreased 30% by SKF-525A pretreatment, but neither agent had any effect on urinary metabolite radioactivity. Pretreatment with diethylmaleate, which depletes GSH, inhibited the incorporation of label into liver macromolecules by 70-85% and reduced the occurrence of labeled CO_2 and urinary metabolites by 50-60%. In conjunction with data from studies discussed previously, these results indicate that in vivo metabolism of methyl chloride to formate is GSH-dependent, and not principally via direct P-450 mediated oxidation, although P-450 may be involved in a later, nonrate-limiting step. When S-methylcysteine was administered prior to $^{14}CH_3Cl$ exposure, the amount of label expired as CO_2 was decreased by 65%, but the amount excreted in urine was nearly doubled. This suggests that S-methylcysteine is an early intermediate in the metabolism of methyl chloride (exogenously added S-methylcysteine would dilute the ^{14}C-labeled S-methylcysteine derived from $^{14}CH_3Cl$, thus reducing the amount of label available for metabolism to formate and CO_2, and increasing the amount available for urinary excretion). The scheme for methyl chloride's metabolism presented in Figure 1 is based upon an original diagram by Kornbrust and Bus (1983) and later slightly modified by Hallier et al. (1990).

Liver and kidney cytosolic enzymes from several strains of mice (C3H, C57B16, $B6C3F_1$, $C3B6F_1$) and from Fischer 344 rats were incubated in head-space vials with 1,000 ppm of methyl chloride, and the decline in methyl chloride concentration monitored by gas chromatography as a measure of in vitro metabolism of the compound, presumably mediated via glutathione-S-transferase (GST) (Hallier et al., 1990). The metabolic turnover in kidney enzyme preparations from either rats or mice was found to be approximately 40-50% of that in the liver enzyme preparations, while the liver or kidney rates found for female mouse preparations were generally 20-50% higher than those for males (Hallier et al., 1990). Turnover in male rat preparations was significantly lower, about 10-30% of the rates seen in those from male mice. Hepatic and renal glutathione (GSH + GSSG) levels were monitored in $B6C3F_1$ mice during the course of a 6-h exposure to 1,000 ppm of methyl chloride (Hallier et al., 1990). Glutathione depletion occurred rapidly in both tissues, but was more rapid in liver than in kidney (e.g., down to approximately 5-10% versus 45-60% of control values, respectively, after 1 h of exposure; levels had fallen to approximately 1-5% after 6 h of exposure in both tissues). In contrast to earlier work reporting the conjugation of methyl chloride to GSH in human erythrocytes, these researchers also demonstrated that mouse and rat erythrocyte cytoplasm did not metabolize methyl chloride under conditions of in vitro exposure to 1,000 ppm. Finally, hepatic and renal levels of cytochromes P-450, P-420 and b5 were determined in the unexposed four strains of mice (Hallier et al., 1990). Levels of all three cytochromes in liver, as well as P-452 in kidney, were similar in males and females. However, kidney levels of P-450 and b5 were substantially lower in females than in males.

In contrast to the in vitro findings that methyl chloride metabolism was greater in female than male $B6C3F_1$ mice, Jäger et al. (1988) found that GST activity in liver from male $B6C3F_1$ mice (controls and those exposed to 100 or 1,000 ppm) was over 2-fold higher than that in liver from female mice or Fischer 344 rats of either sex, while kidney GST activities displayed species, but not sex, differences (activities not reported). Activities of FDH, potentially involved in methyl chloride's metabolism (i.e., in the conversion of formaldehyde to formate, see Figure 1), were reported to be twice as high in mouse liver (12 nmol/min/mg protein) as in rat liver, with no differences by sex observed (Jäger et al., 1988). Kidney FDH activities were similar in both sexes of both species (approximately 3.5 nmol/min/mg protein).

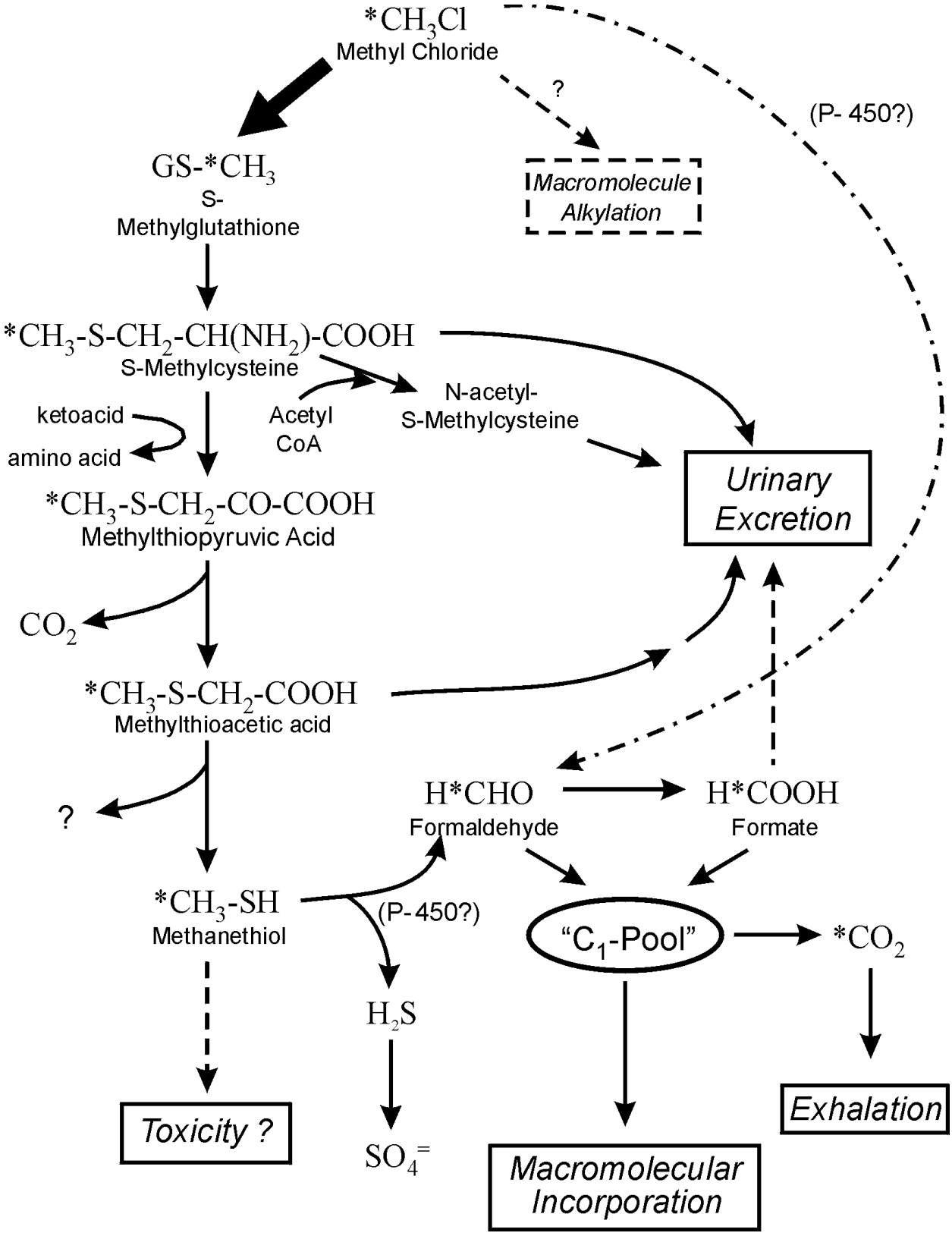

Figure 1. Scheme for metabolism of methyl chloride (after Kornbrust and Bus, 1983; Hallier ea al., 1990; Landry et al., 1983a)

A variety of isoenzymes constitute the GSTs, which catalyze the conjugation of GSH with a wide range of electrophilic, mainly hydrophobic compounds, and four multigene classes of soluble GSTs have been recognized in humans (Kempkes et al., 1996; Warholm et al., 1994). Human erythrocytes have been demonstrated to have GST activity toward methyl chloride, likely that of one of the two theta (θ) class isoenzymes, GSTT1-1 (Kempkes et al., 1996; Peter et al., 1989a,b; Warholm et al., 1994). In contrast, such erythrocyte GST activity (or any erythrocyte metabolic activity towards methyl chloride) has not been demonstrable in rats (Fischer 344, Sprague Dawley, or Wistar), mice ($B6C3F_1$), bovines, pigs, sheep, or rhesus monkeys (Peter et al., 1989b; Hallier et al., 1990). The GST activity towards methyl chloride in erythrocyte preparations from 45 German volunteers (20 male, 25 female) was examined by incubating them in head-space vials and monitoring the disappearance of methyl chloride and the formation of S-methlyglutathione ($GS\text{-}CH_3$) (Peter et al., 1989a,b). Two populations were distinguished by the results: 60% (12/20 males, 15/25 females), referred to as "conjugators," displayed GST(θ) activities of 0.45-5.26 nmol/min/1.5×10^{10} erythrocytes; the remaining 40% (8/20 males, 10/25 females), or "non-conjugators," displayed no detectable activity. Furthermore, no activity was observed with eight other substrates, including vinyl chloride, chloroethane, and methylene chloride (Peter et al., 1989a), nor was any $GS\text{-}CH_3$ generated when GST(π) from human placenta was incubated with methyl chloride (Peter et al., 1989a,b).

In the study by Kempkes et al. (1996), 15% (6/40) of the subjects tested were classified genotypically (using polymerase chain reaction [PCR] methodology) as non-conjugators (i.e., were homozygous for a deletion in the erythrocyte GSTT1 gene), whereas 17.5% (7/40) were classified phenotypically on the basis of erythrocyte GSTT1-1 enzyme activity towards methyl chloride. Distribution of erythrocyte GST(θ) activity in 208 Swedes (177 males, 31 females) towards methyl chloride indicated that 88.9% (185/208) were conjugators, while 11.1% (23/208) were non-conjugators (Warholm et al., 1994). Further analysis indicated that 42.8% (89/208) were "high activity" conjugators and 46.2% (96/208) were "low activity" conjugators. These groups were interpreted to represent individuals as either homozygous (+/+) or heterozygous (+/-), respectively, for the functional GST(θ) allele (i.e., of the GSTT1 gene). Allelic frequencies of the functional and defective alleles in this population were calculated to be 0.659 and 0.341, respectively. Results were not gender-dependent, and the distribution of GSTM1, the gene for

another polymorphic glutathione-S-transferase, GST(μ), was unrelated to GSH conjugation with methyl chloride. These authors cite unpublished results from their laboratory that indicate GST(θ) is probably also expressed in human liver. Collectively, these studies reinforce those discussed elsewhere (Sections 3.1, 3.2, and 3.4) that describe two human subpopulations in terms of methyl chloride absorption/distribution and excretion, although the implications of these findings for human toxicological risk are not clear. In a study designed to determine if erythrocytes from individuals with a homozygous deletion of the GSTT1 gene (null genotype) were more susceptible to oxidant challenge than controls, Onaran et al. (2000) separated 64 Turkish men and women into two groups on the basis of presence or absence of the GSTT1 genes. When collected erythrocytes were exposed to cumene hydroperoxide, levels of malondialdehyde and carbonyl levels were used for evidence of lipid peroxidation. Results indicated that those with the null genotype were no more susceptible to oxidant challenge than controls.

Peter et al. (1989c) demonstrated a possible role for intestinal microflora in the metabolism and toxicity of methyl chloride, at least in rodents. They found that feces taken from various sections of male Fischer 344 rat intestine, when incubated with S-methylcysteine or to a lesser degree with GS-CH_3 (early intermediates in methyl chloride's proposed metabolic pathway; see Figure 1), were able to later generate the toxic intermediate compound, methyl mercaptane (methanethiol). Feces from male B6C3F_1 cecum or colon were able to carry out this conversion only to a limited extent using S-methylcysteine as substrate, and not at all using GS-CH_3. The authors speculated on the possibility that methyl chloride's acute toxicity might in part result from biliary excreted GS-CH_3, perhaps mediated via methanethiol.

Of the more than 30 enzyme forms of cytochrome P-450 present in most species, in vitro studies have indicated that oxidation of methyl chloride to formaldehyde is mediated by cytochrome P-450 IIE1 (CYP2E1) in humans (Guengerich et al., 1991) and rodents (Dekant et al., 1995). CYP2E1 is constitutively expressed in human and rodent liver, is inducible by ethanol and other small molecule substrates for its activity, has a catalytic specificity that appears generally conserved among species, and, in humans, appears to be found extrahepatically in significant amounts only under conditions of induction or diabetes (Dekant et al., 1995; Gonzalez

and Gelboin, 1994; Guengerich and Shimada, 1991; Guengerich et al., 1991). Using human liver microsomal enzyme preparations in conjunction with selective indicator substrates and inhibitors of CYP2E1, as well as specific antibodies raised against human CYP2E1, Guengerich et al. (1991) concluded that this cytochrome P-450 isozyme was a major, if not the principal, catalyst involved in the oxidation of methyl chloride in humans. Citing Kornbrust and Bus (1983), however, these authors acknowledged that in the case of methyl chloride, CYP2E1-mediated direct oxidation to formaldehyde might constitute a detoxication reaction. As noted by Gonzalez and Gelboin (1994), while CYP2E1 levels have demonstrated some differences among individual human liver specimens, the variability is not as great as with other P-450s, making it unlikely that a polymorphism with respect to CYP2E1 expression exists in humans. Therefore, CYP2E1 levels would not appear to significantly contribute to differentiating the two human subgroups that have been identified with respect to methyl chloride metabolism (Nolan et al., 1985; Stewart et al., 1980; van Doorn et al., 1980).

Dekant et al. (1995) demonstrated a time, NADPH, and protein concentration-dependent oxidation of methyl chloride to formaldehyde in renal microsomes from the male Swiss-Webster CD-1 mouse. Citing earlier work by Hu et al. (1990) that identified an androgen-dependent CYP2E1 in male mouse kidney, Dekant and co-workers investigated microsome preparations from female or castrated male mice and found they had only 20-25% the activity of those from naïve males, while those from testosterone-treated females had 84%. These results, in conjunction with parallel findings for oxidation of the CYP2E1-specific substrate chlorzoxazone and for amounts of microsomal CYP2E1 protein (determined by Western blot analysis using rabbit anti-rat CYP2E1 IgG antibody), implicated a mouse renal CYP2E1 in the observed microsomal oxidation of methyl chloride. By contrast, methyl chloride- and chlorzoxazone-oxidizing activities in liver microsomes from male, female, and testosterone-treated females were similar, and over twice that observed in naïve male mouse renal preparations. Hepatic activity was inducible by ethanol pretreatment of the mice, whereas renal activity apparently was not. Additional experimentation demonstrated a nearly three-fold range in renal methyl chloride oxidation activities over the four male mouse strains (CD-1, C3H, C57BL/6J, and NMRI) examined. The C57BL/6J had the lowest activity and the CD-1 the highest. Sprague-Dawley rats displayed no detectable renal microsome activity, but did have an ethanol-inducible, sex-

independent activity in liver microsomes less than half that observed in CD-1 mice. The researchers discuss these findings in terms of a possible explanation for methyl chloride's renal tumorigenicity in male mice, but not in female mice or rats, and in terms of this tumorigenicity's relevance to human risk assessment given the lack of demonstrable CYP2E1 in humans to date.

While it appears established that methyl chloride is principally metabolized via a GSH-conjugation mediated pathway in rodents, the precise contribution of CYP2E1 to methyl chloride's in vivo metabolism and toxicity is less well understood (e.g., Kornbrust and Bus, 1983; Hallier et al., 1990; Dekant et al., 1995). Monitoring the declines of test agent compound concentrations in the exposure chamber, Ottenwälder et al. (1989) showed that uptake and metabolism of methylene chloride in mice was greatly reduced by pretreating the animals with specific inhibitors of cytochrome P-450; in contrast, they cite a personal communication from one of the authors indicating that neither inhibitor significantly affected the pharmacokinetics of methyl chloride. They also demonstrated that the uptake kinetics of 1,000 ppm methyl chloride was not significantly changed in the presence of 1,000 ppm methylene chloride, suggesting again that these two substances are principally metabolized by different pathways. However, after approximately 3 h of exposure (when the chamber concentration of methyl chloride had declined to 300-400 ppm), the data show a slight but consistent lag of methyl chloride uptake in the presence of methylene chloride. This small competitive effect could be another indication of a relatively minor (at least in terms of being rate limiting) in vivo role for CYP2E1 in the metabolism of methyl chloride.

The correlation of methyl chloride-induced GSH depletion and consequent lipid peroxidation was investigated in brain, liver, and kidney tissues of male B6C3F_1 mice and F-344 rats (Kornbrust and Bus, 1984). Over a range of 100-2,500 ppm, single 6-h exposures to methyl chloride induced substantial concentration-dependent NPSH decreases in all three tissues of both species, although the reductions were significantly more pronounced in mice than in rats. Depletion was greatest in liver and least in brain; relative to controls, NPSH levels at 2,500 ppm were approximately 2 and 15% in liver and 30 and 61% in brain for mice and rats, respectively. At 100 ppm, mouse liver NPSH was approximately 55% that of controls. Most of the depletion occurred during the first 30 min of exposure, with 2,500 ppm mouse liver levels at 7.8 and 2.1%

after 0.5 and 6.0 h, respectively. After a 6-h exposure to 1,500 ppm, NPSH had recovered to levels equal to or greater than controls in mouse liver and kidney, as well as rat liver (rat kidney data not presented), by 4 h post-exposure. However, recovery took much longer in brain, reaching only approximately 70% (mice) or 90% (rats) of control levels 18 h after the end of exposure. Repeated exposure to 2,000 ppm (6 h/day, 5 consecutive days) produced a steady decline in mouse brain NPSH when measured immediately after each exposure period, but not in kidney or liver, where complete recovery occurred during the 18-h intervals between each exposure episode.

When 9,000g supernatant fractions from the tissues were examined for their capacities to undergo lipid peroxidation (measured as thiobarbituric acid-reactive material, TRM), none was detected in the liver fractions from control mice or rats; however, substantial TRM levels were observed in the brain and kidney fractions of mice, and to a lesser extent, from rats. Exposure of rats to 2,000 ppm of methyl chloride for 6 h did not induce additional TRM in brain or kidney fractions, although a slight increase was seen in the liver fraction. In contrast, when mice were similarly exposed to 2,000 ppm, TRM was significantly increased in kidney (by 77%) and brain (by 11%) fractions, and was elevated in liver fraction to near that found in the brain fraction (~1.6 µmole/g wet weight). In mouse liver, when levels of NPSH were reduced by methyl chloride exposure to 20-30% or less of the control value, they were closely and inversely correlated with the in vitro levels of lipid peroxidation measured in liver 9,000g fractions. For comparison, it was observed that diethyl maleate (DEM) also decreased liver NPSH, but a near-lethal injection (2 mL/kg) was required to lower the level to 10%, and it was less effective at inducing in vitro lipid peroxidation (liver fraction TRM). Following a 6-h exposure to 1,500 ppm of methyl chloride, formation of liver fraction TRM was inhibited in a concentration-dependent manner to nearly non-detectable levels by the addition of 0.2-2.0 mM exogenous GSH. It was also inhibited by the addition of 2.0 mM EDTA, perhaps due to a sequestration of free iron (a good initiator of lipid peroxidation), and was not observed in animals that were allowed to recover for 4 h. Finally, using exhalation of ethane as an indicator, in vivo lipid peroxidation during a 6 h exposure to 2,500 ppm appeared to increase linearly during the last 3 h of exposure to a level over 25-fold greater than control levels, then to return to control levels by 1-h post-exposure. This study provides further evidence that depletion of GSH levels in target

tissues could be a significant aspect of methyl chloride's mechanism of toxicity, perhaps involving increased levels of lipid peroxidation, especially in the liver. The degree of GSH depletion and associated lipid peroxidation found in rat liver was less severe than that observed in mouse liver, which parallels the susceptibility to liver toxicity seen in these two species.

3.4 EXCRETION

Most of the limited number of studies providing information on the excretion of methyl chloride have been previously discussed to some extent in Sections 3.1-3.3. Relevant data from human studies is sparse. Stewart et al. (1980) exposed adults of both sexes to methyl chloride concentrations of 20-150 ppm according to various repetitive exposure regimens, and found that methyl chloride concentrations in alveolar breath had declined to less than several ppm within the first hour after cessation of exposure. Contrary to some reviews (e.g., ATSDR, 1998), this study did not analyze the subjects' urine for the presence of methyl chloride; rather, methanol (considered at the time a potentially significant metabolite of methyl chloride) was analyzed for, but not detected. This result is not surprising given the metabolic scheme for methyl chloride that is presented in Figure 1.

In a study of six workers exposed to average methyl chloride concentrations of 30-90 ppm during the course of a seven day work week, van Doorn et al. (1980) were able to demonstrate significantly elevated, yet variable, levels of urinary S-methylcysteine in four of the six subjects. Much lower levels were observed in the other two workers, a finding consistent with the conjugator and non-conjugator subpopulations discussed in Sections 3.1–3.3. Urinary excretion of methylmercapturic acid was not detected. As discussed earlier, upon exposure of six volunteers to 10 or 50 ppm of methyl chloride for 6 h, Nolan et al. (1985) were also able to distinguish two groups based upon steady-state levels of methyl chloride measured in blood and expired air, and rates of biphasic elimination from blood and expired air after cessation of exposure. The half-life of the slower, β-phase in blood was 90 min for the slow metabolizers (high steady-state levels), compared to 50 min for the rapid metabolizers (low steady-state levels). However, the investigators were not able to demonstrate a relationship between methyl chloride exposure and the level of S-methylcysteine measured in the urine.

Citing unpublished results from their laboratory, Kornbrust and colleagues reported that in rats after an inhaled dose (amount not specified) of $^{14}CH_3Cl$, only 6.6% was expired unchanged, while 45-50% was exhaled as CO_2 (Kornbrust et al., 1982; Kornbrust and Bus, 1983). Although these authors demonstrated that formate was generated during the metabolism of methyl chloride, increased urinary formate levels were not found in rats after a 3 h exposure to 4,000 ppm, and only a nonsignificant (at $p < 0.05$) doubling of urinary formate was seen after a 3-h, 10,000 ppm exposure. Increased urinary formate after methyl chloride exposure was only observed under conditions that inhibited folate-dependent formate metabolism or depleted GSH levels. Bus (1978) demonstrated that in rats exposed for 6 h to 1,500 ppm of $^{14}CH_3Cl$, of the total excreted radioactivity, 63.9, 32 and 3.9% were recovered in exhaled air, urine and feces, respectively (U.S. EPA, 1989, 1986d).

As discussed in Sections 3.1 and 3.2, Landry et al. (1983a) demonstrated that rats and dogs rapidly achieved steady-state blood concentrations of methyl chloride after exposures to 50 or 1,000 ppm, then underwent biphasic elimination of methyl chloride from the blood with α and β phase half-lives of 4 and 15 min, respectively, in the rat, and 8 and 40 min, respectively, in the dog. When rats were exposed for 6 h to 50-1,000 ppm of $^{14}CH_3Cl$, three urinary metabolites likely to be the product of GSH conjugation reactions were detected: N-acetyl-S-methylcysteine, methylthioacetic acid sulfoxide, and N-(methylthioacetyl)glycine. Excretion was most rapid during the first 5-h post-exposure, and was approximately proportional to exposure concentration. The amount of S-methylcysteine found in the urine of dogs was significantly ($p < 0.01$) greater after exposure to 1,000 ppm, but not 50 ppm, of methyl chloride.

In summary, much of the methyl chloride that is absorbed appears metabolized through a GSH-mediated pathway via formate to the one-carbon pool, from which it is either incorporated into tissue macromolecules or exhaled as CO_2. Under normal exposure conditions, little excess formate appears to be excreted in the urine, although several metabolites resulting from methyl chloride's conjugation with GSH are excreted in the urine. Little, if any, methyl chloride is excreted in the feces. Cytochrome P-450 oxidation of methyl chloride also occurs, but to a lesser extent than GSH-mediated reactions. The significance of polymorphism of GST-θ in human subpopulations to potential effects of methyl chloride is unclear.

4. HAZARD IDENTIFICATION

4.1 STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1 Mortality and Noncancer Effects

Methyl chloride acts principally as a depressant of the central nervous system (CNS), producing acute effects similar to, but less severe than, those of methyl bromide and methylene chloride. These effects result in a state somewhat like alcohol-induced drunkenness, although more persistent (ATSDR, 1998; Ellenhorn and Barceloux, 1988; Farber and Torkelson, 1989; Sittig, 1991). Typical signs and symptoms of intoxication have been described as appearing within 2-3 h of exposure and including headache, nausea, vomiting, painful neck, loss of appetite, diarrhea, dizziness, giddiness, blurred vision, ataxia, confusion, slurred speech, diplopia (double vision), tremors of the hands and lips, drooping eyelids and eye twitch, muscle spasms, convulsions and opisthotonus, cold and clammy skin, loss of memory, hallucinations, respiratory depression, unconsciousness, coma, and death (ATSDR, 1998; Ellenhorn and Barceloux, 1988; Farber and Torkelson, 1989; WHO, 1999; Sittig, 1991). Effects of longer-term, low-level exposure are thought to be generally, but not always, mild and reversible after a recovery period of days to months, and include fatigue or malaise, loss of appetite, headache, disequilibrium, blurred vision, confusion, anxiety, personality changes, short-term memory loss, vertigo, loss of coordination, weakness, pale skin, nausea, and vomiting (Ellenhorn and Barceloux, 1988; Sittig, 1991). These CNS effects are further discussed in Section 4.4.1.

As cited by Farber and Torkelson (1989), a review of the literature prior to 1955 by von Oettingen (1955) found 19 case reports describing fatalities as a result of a single or only a few severe exposures, and over 200 nonfatal cases. Typically these exposures resulted from leaking refrigerators or refrigeration systems, and probably involved very high concentrations of methyl chloride, perhaps in the vicinity of 30,000 ppm or greater (ATSDR, 1998; WHO, 1999). Citing Morgan Jones (1942), ATSDR (1998) indicates that short exposures to extremely high concentrations (600,000 ppm), while causing neurological effects, are not necessarily lethal.

Evidence suggests that in persons exposed to doses of methyl chloride sufficient to cause serious CNS effects, other organ systems including the heart, gastrointestinal tract, liver, kidneys, and lungs can also be adversely affected, although the cardiovascular and gastrointestinal effects may be secondary to CNS toxicity (ATSDR, 1998; WHO, 1999; Farber and Torkelson, 1989). Reported cardiovascular effects include tachycardia, increased pulse rate, low blood pressure, and electrocardiogram abnormalities; reported hepatic effects include clinical jaundice, cirrhosis of the liver, and impaired performance on the laevulose tolerance test; and reported renal effects include albuminuria, increased serum creatinine and blood urea nitrogen, proteinuria, anuria, and hematuria (ATSDR, 1998; WHO, 1999). In a review on the effects of environmental pollutants on taste and smell, olfactory loss (permanent anosmia or hyposmia) was identified as a result of chronic exposure to the chloromethanes, including methyl chloride (Schiffman and Nagle, 1992).

In a report submitted to the U.S. EPA, Dow Chemical Company (1992) presented a study of six cases of methyl chloride intoxication in workers who were chronically exposed to low levels (200-400 ppm) for at least 2-3 weeks prior to the onset of symptoms. Cases 1 and 2 were methyl chloride workers who worked together and were exposed to an 8-h time-weighted average (TWA) of up to 300 ppm. Case 1, a 54-year-old male, was admitted to the hospital with a diagnosis of symptomatic hypertension, and, according to his wife, had been displaying peculiar behavior for about 10 days (confusion, erratic driving, blurry vision, eating and swallowing difficulties, headaches, disturbed balance). Moderate hypertension (160/120) was the only abnormality observed upon hospital work-up. Three weeks after hospitalization, the patient still complained of headache and staggered upon first getting up from a chair. Some short-term memory deficit, balance instability, and hand tremor were noted two months after hospitalization. At three months the patient had improved, but continued showing hand tremor and displayed heightened nervousness. Case 2, a 58-year-old male who had been working 12-h shifts due to the absence of Case 1, was admitted to the hospital two weeks later with symptoms of delirium, confusion, disorientation, and combativeness. His hospital work-up was normal. Two months later, Case 2 continued complaining of short-term memory loss and increased nervousness, but after three months felt well enough to return to work.

Cases 3-6 were foam workers exposed for 2-3 weeks to an 8-h TWA of approximately 265 ppm methyl chloride (Dow Chemical Company, 1992). Case 3, a 33-year-old male, developed blurred vision, increased tiredness, nervousness, and stuttering, all of which resolved within six weeks after removal from work exposure to methyl chloride. Case 4, a 34-year-old male, experienced dizziness, weakness and numbness of his right arm, and a feeling of drunkenness. He felt improved after two days away from work, but after an additional week of work the symptoms returned, along with slurring of speech, difficulty in remembering what he wanted to say, loss of concentration, and staggering to the right. When work exposure to methyl chloride ceased, these symptoms resolved over the course of approximately one month's time. Case 5, a 24-year-old (presumably male), exhibited diplopia, dizziness, difficulty driving, staggering gait, and stuttering; his physical exam was normal except for mild hypertension (150/90). After his removal from work exposure to methyl chloride, the symptoms resolved within approximately two months. Case 6, a 29-year-old male, developed diplopia, nervousness, anorexia, vertigo, staggering gait, diarrhea, slurred speech, difficulty in remembering what he wanted to say, and feelings of a swollen tongue, and abnormal coordination. Blood pressure was slightly elevated when compared with his pre-employment status (150/100 versus 130/80), and his EEG revealed a predominance of slow beta waves that persisted for at least one month. Removal from work-place exposure to methyl chloride led to symptoms resolving within approximately two-and-one-half months.

As previously discussed (Section 3.1), Stewart et al. (1980) used a complex exposure regimen to expose groups of 2-4 male volunteers for 7.5 h (Group I), 3 h (Group II), or 1 h (Group III) to concentrations of 0, 20, 100, 100f (fluctuating among 50, 100 and 150 ppm for equivalent periods of time during the exposure period), or 150 ppm of methyl chloride, 2 to 5 days/week, over the course of 6 weeks. Only a single concentration (counting 100f as a single concentration) was utilized in any given week, except for the final week in which two days of exposure to 150 ppm were followed by a single day of exposure to 0 ppm. Similarly, three groups of women were exposed for 7.5, 3 or 1 h (Groups I, II, and III, respectively) to 0 ppm methyl chloride for one day during Weeks 1 and 3, and to 100 ppm for five days during Week 2. Each subject received a comprehensive medical exam prior to the beginning and after the study that inclued a complete history and physical exam, a complete blood count (CBC) and panel of

clinical chemistries, and a 12-lead electrocardiogram (EKG). The CBC and clinical chemistries were also performed at least weekly during the study, and brief medical exams (blood pressure, temperature, subjective signs/symptoms, urinalysis) were given each day prior to exposure. During exposure, EKGs were continuously monitored and recorded hourly. Blood, breath, and urine sampling analyses and results (for detection of methyl chloride and methanol) have been described in Sections 3.1 and 3.4. Blood carboxyhemoglobin was monitored at least weekly. Several neurological and cognitive tests were conducted, and are described later in Section 4.4.1. Cardiopulmonary function parameters that were monitored included RMV, vital capacity, functional residual capacity, metabolic rate, alveolar-capillary gas exchange, heart rate, and blood pressure, both at rest and, for some parameters, at two levels of dynamic muscular exercise. Electromyograms (EMGs) of the electrically-elicited monosynaptic reflex of the gastrocnemius muscle were obtained for Group I males and females and were subjected to visual analysis and measurement of stimulus-response latencies. Finally, individualized forms were provided for each volunteer to record any subjective responses that occurred during exposures or within 3 h thereafter.

All subjects were found to be in good health prior to and after the study, and daily medical surveillance revealed no abnormalities of temperature, blood pressure, clinical blood chemistries, CBCs, urinalyses, or EKGs that were attributable to any methyl chloride exposure. Blood carboxyhemoglobin levels gave no indication that methyl chloride was being converted to carbon monoxide in the body, nor was elevated methanol detected in any urine sample. Methyl chloride exposure had no major effect upon pulmonary mechanics as evidenced by generally normal pulmonary ventilation rates, and maximum and partial expiratory volumes and flow rates. As cardiopulmonary function is sensitive to metabolic rate, the data were also analyzed as a function of metabolic rate during resting or muscular exercising conditions. With the possible exceptions of arterial pH and P_{CO2}, cardiopulmonary functions remained within normal limits throughout the study. A slight respiratory acidosis was noted during some exposures, especially at 100f ppm, likely caused by an accompanying tendency toward alveolar hypoventilation. EMGs obtained during methyl chloride exposures were not significantly altered from those of controls, nor were subjective responses significantly different between control days and methyl chloride exposure days. The authors concluded that exposure to 20 to 150 ppm methyl chloride for up to 7.5 h/day

for 2-5 consecutive days produced no detrimental effects on the various physiological and clinical parameters measured in the study. As noted, the neurological and cognitive aspects of this study are discussed in Section 4.4.1.

In a cohort study by Rafnsson and Gudmundsson (1997), mortality and cancer patterns were investigated among a group of men exposed acutely to toxic levels of methyl chloride 32 years earlier in a boating incident. In 1963, methyl chloride leaked from a refrigerator aboard a fishing trawler, which was located under the quarters of 17 crewmen (deckhands); all but two exhibited symptoms of intoxication. Eleven other crewmen, including seven officers, had quarters farther astern and were exposed only while checking upon their sick crewmates in their quarters, or during repair of the refrigerator. Methyl chloride may have leaked from the refrigerator for 4 days prior to discovery and repair, and the first "incomprehensible" symptoms of intoxication appeared within the next 2 days. Earlier published studies documented the deckhands' illness, neurological signs, and clinical status after follow-up periods of 20 months and 13 years. One deckhand died within 24 h of the exposure, two others developed severe depression and committed suicide within the following 18 months, and one officer fell off the trawler 11 months later and was lost. This study's cohort consisted of 24 crew members, stratified into 18 deckhands and 6 officers, while the 120 referents, chosen with substantial care and controlled for age, occupation, social class and lifestyle factors, consisted of 90 deckhands and 30 officers. Various Icelandic statistics registers permitted a determination of the vital status of all the subjects, as well as causes of death for the deceased. Comparisons between crew and referent groups were based upon person-years at risk, and were expressed as either risk ratios (RR) or Mantel-Haenszel test point estimates (M-H), along with the corresponding 95% confidence intervals (CI).

Comparing the entire crew with all the referents, an excess in mortality from all causes was found: 2.2 (RR, CI = 1.1-4.2), or 2.0 (M-H, CI = 1.3-3.1). When stratified into deckhand and officer groups, RRs were 2.5 (CI = 1.0-5.7) and 2.2 (CI = 0.6-6.4), respectively. The crew also demonstrated an excess of mortality because of cardiovascular disease: 2.3 (RR, CI = 0.9-5.5), or 2.1 (M-H, CI = 1.2-3.8). Furthermore, this excess appeared more prominent in the more highly exposed deckhands (RR = 3.9, CI = 1.0-14.4) than in officers (RR = 1.7, CI = 0.3-6.4).

The overall crew-referent comparisons for mortality due to all cancers and to lung cancer were 1.6 (RR, CI = 0.3-6.0) or 1.5 (M-H, CI = 0.3-5.6), and 2.9 (RR, CI = 0.1-55.6) or 2.7 (M-H, CI = 0.1-52.6), respectively. Few cases of cancer were observed in the small crew cohort, with no cases of lung cancer occurring in the crew officer stratum. According to the International Cause of Diseases classification scheme, of the 14 deaths occurring in the exposed crew, 6 were due to myocardial infarction, 1 to hypertensive heart disease, and 1 to cerebral hemorrhage. The authors concluded that the long-term sequelae of severe, acute exposure to methyl chloride may include elevated mortality from cardiovascular disease. They offered some speculative discussion on alkylation by methyl chloride as a biological basis for this phenomenon, although other evidence suggests this explanation to be unlikely (Sections 3.2, 3.3, 4.4.3, and 4.4.4). The data may also be weakly suggestive of an elevated cancer mortality risk, although the RR and M-H confidence intervals were quite wide and included unity.

Holmes et al. (1986) conducted a retrospective cohort study of workers in a synthetic butyl rubber manufacturing plant to determine whether there was indication of increased mortality overall or from specific causes. The principal impetus for the study was worker exposure to methyl chloride, which was used in the manufacturing process. The cohort consisted of 852 male process workers (661 white, 191 non-white) who were employed at the facility for at least one month during the period from startup in 1943 to 1978, and was divided into groups identified (by job title, duties, location, etc.) as having the potential for high (H), medium (M) or low (L) exposure. Because of process changes that could have significantly affected (i.e., reduced) exposure potential, three time periods (1943-1950, 1951-1960, and 1961-1978) were also analyzed. The causes of death were coded by a trained nosologist according to the 8th Revision of the *International Classification of Diseases.* Standardized mortality ratios (SMRs) were calculated ([observed/expected deaths] × 100), along with 95% CIs, and a computer program was used to generate the expected numbers of death based upon application of United States age, race, sex, and cause-of-death rates to appropriately categorized cohort groups. Overall, in white male workers no excess mortality was found for all causes (120/146.1, SMR = 82, CI = 68-98), cancer (19/28.8, SMR = 66, CI = 40-103), circulatory system diseases (71/72.9, SMR = 97, CI = 76-123), or external causes of death, including accidents (15/16.2, SMR = 93, CI = 52-153). In general, similar results were found for non-white male workers, white male workers stratified

by employment period, and white males first employed during 1943-1950 further stratified by duration of exposure or potential degree (H, M, L) of exposure. In the 1943-1950 first employed white male group, while no individual SMRs were statistically significant, visual inspection of the limited data suggests some evidence of trending in SMRs by exposure potential for all causes (L = 56, M = 72, H = 89), cancer (L = 42, M = 45, H = 65), and circulatory system diseases (L = 48, M = 91, H = 108). Analysis for the statistical significance of these possible trends was not reported.

The researchers concluded that their data provide no evidence that chronic exposure to methyl chloride results in excess mortality from all causes of death, or from any specific cause. They noted that for the broad disease categories utilized, cohort RRs down to at least 2-3 would have been detectable, but that the study's power would be substantially reduced for cohort subgroups and rare (or more specific) causes of death. They also note that the total number of deaths observed in the cohort was significantly less than expected, a finding in agreement with most occupational cohort studies and attributable to the "healthy worker effect." Inspection of this study's data indicates that in the present instance, this healthy worker effect was largely accounted for by lower-than-expected cancer mortality, rather than by reduced cardiovascular mortality as is often the case. This fact was observed by Rafnsson and Gudmundsson (1997) when concluding that their findings of an association between methyl chloride exposure and excess cardiovascular mortality were not directly contradicted by this study. Given the possible trending of SMRs with degree of exposure potential discussed above, and the fact that cardiovascular SMRs for white male workers tended to approximate 100, this study appears not to preclude a possible methyl chloride effect on cardiovascular mortality that is superimposed on a healthy worker phenomenon (resulting from reduced cancer mortality). Finally, the exposure in Rafnsson and Gudmundsson (1997) was acute rather than chronic, as in Holmes et al. (1986), and appears to have involved substantially higher concentrations of methyl chloride.

The mortality of a cohort of 2,610 Louisiana chemical plant workers were analyzed by Olsen et al. (1989). With respect to non-cancer mortality, no excess mortality was observed when compared with United States, Louisiana, or local mortality rates for arteriosclerotic heart disease (SMRs = 39-46), cerebrovascular disease (SMRs = 92-124) or all external causes of

death, including accidents (SMRs = 38-48). These findings again demonstrate the healthy worker effect, along with the frequently seen contribution of reduced cardiovascular mortality. However, they are of minimal value in assessing the risks of methyl chloride exposure because the cohort was exposed to many other chemicals in addition to methyl chloride and no effort was made to stratify workers by exposure to specific chemicals or by level of potential exposure.

The literature contains other, mostly older, case reports and human studies that have been previously summarized (ATSDR, 1998; Farber and Torkelson, 1989; WHO, 1999). They provide additional descriptions of the CNS, cardiovascular, hepatic, and renal effects that can be caused in humans by exposure to methyl chloride. Many exposures appear to have been acute or of unknown duration; methyl chloride concentrations may have generally been known to be high or low, but rarely were defined with any specificity. Signs and symptoms appeared typically to have become noticeable within hours or a day or two of exposure, and generally the effects resolved within days or several months of the cessation of exposure. In some cases, however, the effects appeared to persist for years and rarely, perhaps, for a lifetime.

4.1.2 Cancer Effects

Few data were located pertaining to the carcinogenicity of methyl chloride in humans. As noted in Section 4.1.1, only very weakly suggestive evidence for an effect of acute, severe exposure to methyl chloride on mortality from all cancers or from lung cancer was seen in the trawler cohort study of Rafnsson and Gudmundsson (1997). As indicated by the large CIs that included unity, cohort and group sizes were just too small to generate reliable indications of carcinogenic potential. The butyl rubber worker cohort study (Holmes et al., 1986) that dealt with chronic exposure, demonstrated lower-than-expected incidences of cancer at all sites and at certain specified sites. For all categories but two, SMRs ranged from 25 to 75; the SMR of malignant neoplasms of the lung for non-white workers was 120, while that for all malignant neoplasms in white workers first employed during the period from 1951 to 1960 was 107. Both increases were statistically non-significant.

The deficiencies (exposure to multiple chemicals in addition to methyl chloride, lack of cohort stratification, etc.) of the Louisiana chemical worker study (Olsen et al., 1989), with respect to defining any effects specifically attributable to methyl chloride exposure, have also been noted in the previous section. When compared with United States, Louisiana, or local reference populations, the study did identify an increase in mortality due to cancers of the brain and CNS (SMRs = 333, 354, and 322, respectively), but CIs were large and the results were statistically non-significant. Similarly, mortality due to leukemia and aleukemia was non-significantly elevated versus United States and Louisiana populations (SMRs of 331 and 356, respectively), although the increase was statistically significant when compared with the local five-parish area (SMR = 492, CI = 101-1,437). However, there were only three observed cases and they did not share a common histology (chronic granulocytic leukemia, acute lymphoblastic leukemia, acute aleukemic myeloid leukemia); their respective durations of employment at the facility were 9.8, 1.2, and 2.8 years, and their job titles were different. Considering these facts, the researchers concluded that the leukemia deaths were not likely to be job-related. In any case, the study did not assess the degree of methyl chloride exposure or exposure to any of 21 other specifically identified chemicals used or made at the facility.

Dow Corning Corporation (1992) reported a follow-up case control study of respiratory cancers at one of its silicone production plants. Earlier retrospective mortality studies had established a non-significant excess of respiratory cancers for a cohort of 1,942 plant employees (30 observed/24.9 expected; SMR = 120.5, CI = 81.3-172.0), and a significant excess among long-term plant employees having $\geq$20 years of service (18 observed/8.5 expected; SMR = 211.9, CI = 131.9-351.6). The 41 index cases comprised all regularly employed persons at the plant from 1943 to 1980 who were identified as having primary respiratory cancer (i.e., cancers of the trachea, bronchus, lung, or pleura), while the referent group consisted of 4 matched controls for each case. Exposure to the following groups of substances were considered: (1) substances present in the plant known to cause pulmonary neoplasms (asbestos, chromium, cadmium, radiation from a cobalt-60 catalyst, nickel, arsenic compounds, and crystalline silica; radiation and arsenic compounds were subsequently excluded due to lack of index case exposure, and benzene was added because it was a known human carcinogen); (2) suspected respiratory carcinogens (dimethyl sulfate, formaldehyde, vinyl chloride, talc dust, and acetonitrile); and

(3) potential respiration hazards having significant plant exposure potential and/or limited evidence of safety in humans (chlorosilanes, methoxysilanes, amorphous silica, and methyl chloride). For each substance, each plant job was classified as having no contact, incidental contact, or routine contact. The plant was also divided into process areas and activity zones, and jobs were categorized by standardized titles for subsequent risk analysis.

When employment of any duration was considered, no plant zone had significantly elevated odds ratios (ORs) for respiratory cancer, although non-significant increases were seen in several zones. Despite the sparseness of available data, among employees with 5 or more years of service within any given zone, a significant excess was found in the Elastomers and Rubber Production zone (OR = 3.7, CI = 1.4-9.8), with lesser non-significant excesses found in several other areas. No significant excess, however, was found for any zone among employees having 10 or more years of service in any zone, although the data were very limited (<10% of the cases had $\geq$10 yr of service in any specific plant zone). Few cases held common job titles, and only several of the many job titles reached statistical significance ($p < .05$) or near significance; these associations were not considered etiologically relevant, however, because long-term employment in such positions was uncommon (often only 2-10 months). Statistically elevated ORs were found for plant exposures to several specific materials (e.g., acetonitrile, talc dust, dimethyl sulfate), but these were typically based upon incidental rather than routine contact, and upon more recent rather than the earlier time-frame exposures (i.e., within the past 5 years, rather than $\geq$20 years earlier) generally thought to be etiologically most relevant to respiratory cancer. Because methyl chloride exposure was fairly specific to silicones production plants, it was specifically focused on for any indication of association with increased respiratory cancer risk; no such association was found, and the authors concluded that the prevalence of methyl chloride exposure among the work force appeared sufficient to exclude any significant level of risk.

4.2 PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1 Subchronic and Chronic Oral Studies

Only a single subchronic study was located that employed an oral route of exposure, and no chronic studies were found. In a report submitted to EPA by Dow Chemical Company (1982), a study involving rabbits fed cold olive oil solutions of methyl chloride by gavage was briefly described. Data for two animals were reported, one receiving 60 doses at 40 mg/kg of methyl chloride over a period of 83 days, the second receiving 60 doses at 100 mg/kg over 85 days. It was noted that olive oil did not react with methyl chloride, and that larger doses were not practical because the volume of oil became too large, with rapid escape of methyl chloride gas from the oil causing considerable bloating. The low-dose animal showed no treatment related abnormalities, demonstrated normal weight gain, and upon microscopic examination, displayed an entirely normal liver, kidney, spleen, adrenal gland, and pancreas. The high-dose rabbit also appeared normal with steady weight gain during the exposure, and upon gross and microscopic autopsy, the liver, kidney, adrenal gland, and pancreas appeared normal, although fat depots were described as only fair. The spleen, however, was grossly observed to be somewhat enlarged and dark-colored, and microscopically displayed moderate congestion, phagocytosis, and slight hemosiderosis. This slight pathology of the spleen was considered of uncertain significance by the researchers.

4.2.2 Subchronic and Chronic Inhalation Studies

In a Dow Chemical Company 90-day inhalation study, McKenna et al. (1981a) exposed groups of CD-1 mice (10/sex/concentration), Sprague-Dawley rats (10/sex/concentration), and male Beagle dogs (4/concentration) for 6 h/day, 5 days/wk during a 93-95 day period (a total of 64-66 exposures) to methyl chloride (99.5% pure) concentrations of 0, 50, 150, or 400 ppm (0, 103, 310, or 826 mg/m^3). All animals were observed on each exposure day for signs of toxicity, and body weights were recorded at least weekly. Rodents (5/sex/species/concentration) periodically underwent a simple battery of tests to evaluate sensory and motor function. This neurological testing and the associated results are described more fully in Section 4.4.1.

Hematological parameters (RBC, PCV, Hgb, total and differential WBC) were evaluated in all rats and dogs immediately prior to the initiation of exposure and at study termination, as were urinalyses (specific gravity, pH, sugar, protein, ketones, bilirubin, urobilinogen, occult blood). Various clinical chemistry determinations were conducted at necropsy on all rats and dogs (blood urea nitrogen or BUN, alanine aminotransferase or ALT, aspartate aminotransferase or AST [dogs only], and alkaline phosphatase or AP). Two rats and 4 mice that died during exposure or were killed moribund were subjected to gross necropsy; 2 of the mice were also examined microscopically. At the study's end, gross necropsy was performed on 40 male and 38 female rats, 36 male and 38 female mice, and 16 male dogs; the rats and dogs were fasted overnight, mice were not. Eye examinations were conducted, and organ weights for liver, kidneys, heart, and testes were recorded. Representative specimens from most major organs and tissues (somewhat fewer from mice) were obtained for microscopic evaluation from 0 and 400 ppm groups for all dogs and most rodents. Cerebrum, cerebellum, spinal cord, peripheral nerves, and brain stem were examined. Liver specimens were also obtained from low- and intermediate-dosed animals. Statistical significance (using analysis of variance and Dunnett's test) was defined as $p < 0.05$.

The daily clinical observations failed to reveal any signs or symptoms in any animal exposure group attributable to methyl chloride. However, statistical analyses of the rodent sensory and motor function tests revealed various differences between control and methyl chloride-exposed rodents; these statistical differences were judged either unrelated to methyl chloride exposure or of suspect toxicological significance, and are further discussed in Section 4.4.1. Some statistical differences in mean body weights between control and exposed groups were observed, but these were not dose-dependent or consistent with exposure duration, and were thus considered incidental effects unrelated to methyl chloride exposure. The only mean or relative organ weight findings that appeared consistent with a methyl chloride exposure effect were small, statistically significant increases in mean relative liver weights for male rats and female mice at 400 ppm. There were no gross or histopathological observations supportive of hepatotoxicity in either group, but statistically non-significant trends toward increased mean relative liver weights were noted in 400 ppm male mice and in 150 ppm male and female mice. These observations, combined with the presence of equivocal histological evidence for liver

toxicity in male mice, suggested that the slight liver weight effects may have been attributable to methyl chloride exposure.

Upon hematological examination, 400 ppm male rats and male dogs each displayed small but statistically significant reductions in mean RBC. In rats, although this reduction was accompanied by a non-statistically significant decline in mean Hgb, similar findings were not seen in female rats; there were no indications of dose-response, and the mean RBC value was within the laboratory's historical range. In dogs, the reduced mean RBC relative to controls was also observed in the groups prior to the initiation of exposure, with no significant change found in pre- versus post-exposure RBC values for the 400 ppm dogs. Thus, the statistical RBC reductions seen in 400 ppm rats and dogs were both considered within the range of normal variability, apparently unrelated to methyl chloride exposure and of no toxicological significance. Similarly, the statistically significant reductions in mean AP activity noted in all methyl chloride-exposed dog groups relative to controls were also observed pre-exposure, and were therefore judged unrelated to methyl chloride exposure. No other clinical chemistry effects were noted. Urinary specific gravities were significantly reduced in 400 ppm male rats and in 6 of 10 of the 150 ppm female rats, but not in 400 ppm female rats. No other significant alterations in urinary parameters were observed, nor were any gross or microscopic pathological effects found in the kidneys. As a result, the authors considered the reduced urinary specific gravity an exposure-related effect, but were uncertain if it represented an adverse or simply a sub-clinical pharmacologic effect of methyl chloride.

Pathological examination of rodents dying or sacrificed prior to study termination revealed no indication that the deaths were related to methyl chloride exposure, nor were any of the gross or microscopic pathological changes observed in rats sacrificed at study termination—all were considered incidental and typical of spontaneously occurring natural lesions commonly observed in rats of that age and strain. Similarly, most lesions observed in the mice were considered incidental and unrelated to methyl chloride exposure. However, subtle changes were noted in the appearance of some hepatocytes from the livers of 5 of 10 male mice in the 400 ppm group. These changes involved altered tinctorial properties, perhaps due to altered vacuolation and basophilia, and were considered reversible. Similar changes were also observed in 2 of 9 control

mice, and in 1 of 7 of the 150 ppm male mice. All dogs had lesions in the lungs suggestive of parasitic nematode infection, as well as other nonspecific lesions not attributable to methyl chloride exposure. Slight hepatocytic swelling was found in 2/4 of the 400 ppm dogs, 1/4 of the 150 ppm dogs and 2/4 of the 50 ppm dogs, but not in control dogs. Because of the lack of an apparent dose response or any correlation with appreciably altered liver weights or clinical chemistry findings in individual dogs, this effect was also not considered exposure related. No clinically observable neuromuscular effects were observed nor were there any adverse effects on the CNS. In summary, this study failed to reveal any unequivocal evidence of toxicity related to methyl chloride exposure in mice, rats, or Beagle dogs, and suggests a NOAEL of 400 ppm for intermittent subchronic exposure.

A 90-day inhalation study was also conducted by Battelle for the Chemical Industry Institute of Toxicology (CIIT) in F-344 rats and B6C3F_1 mice (Mitchell et al., 1979a). This was conducted as a pilot study to select exposure levels for the 2-year chronic study described later. Animals (10/sex/species/concentration) were exposed for 13 weeks, 5 days/wk, 6 h/day to methyl chloride (99.5% pure) at concentrations of 0, 375, 750, or 1,500 ppm (0, 774, 1,549, or 3,098 mg/m^3). All animals were observed daily and body weights were recorded weekly. Pre- and post-exposure ophthalmoscopic exams were performed, and organ weights (heart, adrenals, brain, testes/ovaries, spleen, liver, kidneys, lungs, and pancreas) were taken at necropsy. Urine was collected during Week 13 for analysis (specific gravity, pH, glucose, ketones, occult blood, protein, urine sediment), and just prior to necropsy, blood samples were collected for hematology (RBC, MCV, Hgb, total and differential WBC, reticulocytes, mean corpuscular hemoglobin [MCH], MCH concentration [MCHC], bone marrow myeloid:erythroid [M:E] ratio) and clinical chemistry (glucose, BUN, ALT, AST, AP, creatinine phosphokinase [CPK]) determinations. Pathology examinations were conducted on tissues (including brain) from control and 1,500 ppm animals first; if a methyl chloride-related lesion was seen at the high concentration, lower concentrations were successively examined until the lesion was not found.

The study's summary reported that significantly lower body weights were observed in male and female 1,500 ppm rats during Weeks 3-13, and in male and female 750 ppm rats during Weeks 6-12. Increased relative liver weights were noted for male and female mice in the 750

and 1,500 ppm groups. Generally, any observed changes in hematological or clinical chemistry parameters were considered within normal expected ranges, or were not clearly dose response-related. However, increased AST levels were observed in male mice of the 1,500 ppm group. Vacuolar changes in the cytoplasm of hepatocytes were noted in the livers from 9 of 14 rats in the 1,500 ppm group (severe in 1, moderate in 5, mild in 2), from 7 of 18 rats in the 750 ppm group (severity distribution similar to control rats), and from 7 of 19 control rats (moderate in 1, mild in 6), and was approximately five times more prevalent in females than males. Additionally, hepatic infarctions were observed in 1 male mouse and 1 female rat at 1,500 ppm. Thus, effects on body weight (rats) and relative liver weight (mice) at 750 and 1,500 ppm, as well as on hepatic histology (mice and rats) at 1,500 ppm, were considered likely or potentially related to methyl chloride exposure. No histopathological effects in the brain in either the rat or mouse were observed.

Battelle subsequently conducted a 24-month, chronic inhalation study in F-344 rats and $B6C3F_1$ mice for CIIT (1981). Groups of animals (120/sex/species/concentration) were exposed 6 h/day, 5 days/wk, for up to 24 months to concentrations of 0, 50, 225, or 1,000 ppm (0, 103, 465, or 2,065 mg/m^3) of 99.97% pure methyl chloride. These concentrations were based in part on the results of the 90-day pilot study previously described (Mitchell et al., 1979a). Interim sacrifices and toxicological evaluations were scheduled for 6, 12, and 18 months after initiation of the study. Mortality records were kept, and clinical observations, ophthalmic examinations, body and organ weight determinations, hematology, clinical chemistry and urinalysis determinations, and necropsy and histopathological examinations were conducted on the same battery tissues described for the 90-day subchronic study. These tissues included spinal cord, brain, liver, lung, testes, epididymis, and spleen. Animals were first subjected to a complete gross pathology examination, then a pre-selected battery of tissues (including brain) were examined by light microscopy and pre-selected organs weighed. Porphyrin fluorescence evaluations and neurofunction examinations were also performed.

A six-month interim status report of this study was prepared by Mitchell et. al. (1979b). Observations were based upon animals (10/sex/species/concentration) sacrificed after 6 months of exposure. Biologically significant increases in ALT levels were observed in male and female

mice, but were not well correlated with individual animal histopathology. Similarly, decreased BUN values in some mice were not correlated with the presence of severe hepatic disease, and so were not considered of clinical significance. All other hematological variations were judged to be without clinical significance, and most other parameters were within normally expected ranges or did not exhibit convincing dose-response relationships. Significant decreases in absolute and relative kidney weights were found in 1,000 ppm male mice. Relative liver and kidney weights were significantly increased in male rats, as were relative lung weights in males at all concentrations and females at 1,000 ppm. In rats, chronic inhalation of methyl chloride appeared associated with testicular germ cell degeneration and sperm granulomas, interstitial pneumonia (1 male, 4 females), and subacute tracheitis in females. The levels at which these effects occurred were not stated. In mice, associations with focal acute scleritis, hepatocellular degeneration, splenic lymphoid depletion, and multifocal thymic lymphoid necrosis were found. The levels that these effects occurred in mice were not stated although it was indicated in the final report (CIIT, 1981) that they occurred at 1,000 ppm. Body weights were significantly depressed in 1,000 ppm male and female rats, in 1,000 ppm male mice, and in all concentration groups of female mice. No brain lesions were reported in the 6-month evaluation.

The overall results of this chronic study were presented in the final report by CIIT (1981). For mice, one exposure mistake was noted: on three consecutive days, the 50 ppm and 1,000 ppm groups were switched and received each other's exposure; although the effect of this error was considered negligible by the study authors, it may have affected the pathology results for spinal cord lesions (see below) in the 50 ppm group. Mouse mortality was increased in both sexes at the 1,000 ppm concentration, but was unaffected at 50 and 225 ppm; it was also elevated in males when compared with females as a result of male dominance fighting, which led to wounds around the prepuce and penile orifice that resulted in infection, urinary stasis, and death. Clinical signs suggestive of CNS toxicity (e.g., tremor, paralysis) were noted only in 1,000 ppm mice, consistent with the neurofunctional impairment and cerebellar lesions described below. No ophthalmic effects were observed in mice of either sex at any concentration, but neurofunctional impairment (clutch response) was found in nearly all 1,000 ppm mice of either sex after 18-22 months of exposure. This finding was supported by the histopathological observation of cerebellar lesions that first appeared in 1,000 ppm male and female mice at the

18-month sacrifice. The lesion was characterized by degeneration and atrophy of the cerebellar granular layer and was seen in 3 of 7 males and 6 of 8 females, and in 17 of 18 females sacrificed at 22 months. The lesion was also found in 1,000 ppm mice that died spontaneously between 0 and 17 months (15/24 males, 9/20 females), or between 18 and 22 months (45/47 males, 35/37 females). It did not occur in the 0, 50, or 225 ppm groups. In the 18-24 month spontaneous death category, 35/37 females and 45/47 males in the 1,000 ppm group had cerebellar granular cell atrophy that was more extensive at 24 months than at 18.

Thirteen of 18 females had minimal to moderate axonal swelling and degeneration of the lumbar spine nerves at the 22-month necropsy (only 1,000 ppm females examined). Similar lesions were found in 12/18 females in the thoracic spinal cord and 5/18 in the cervical spinal cord, lesions that were not present in 1,000 ppm animals at 18 months. At the 18-month interim necropsy, multifocal axonal swelling and degeneration (dorsal root of spinal cord) occurred in 7/10 female mice (1/5 in males) at 50 ppm and 7/10 in females (2/5 in males) at 225 ppm, but none at 1,000 ppm; this was not observed in controls. Similar lesions were seen in the cauda equina of the spinal cord at 18 months. Tabular incidence was 3/5 (males) and 3/10 (females) at both 50 and 225 ppm, respectively [incidence in text ranged from 4/5 males and 10/10 females at 50 and 225 ppm]; it also was observed in 3/7 males at 1,000 but not in any of the 8 females. This lesion occurred in 1/5 control males and 2/10 control females. It was indicated that axonal swelling and degeneration of minimal to mild severity was observed in all groups (not clear if this included controls) at 12 months.

The growth rate of male 1,000 ppm mice was significantly depressed through the first 18 weeks of exposure, with necropsy body weights significantly decreased in 1,000 ppm females at 6 and 12 months, and in 1,000 ppm males at 12 months. Among the statistically significant organ weight changes observed in male mice were: brain, absolute decrease (1,000 ppm, 6, and 12 mo); kidney, absolute decrease (1,000 ppm, 6 and, 12 mo); and liver and lungs, relative increase (1,000 ppm, 12 mo). Those in female mice included: heart, absolute increase (1,000 ppm, 12 mo) and relative increase (1,000 ppm, 12 and, 18 mo; 225 ppm, 24 mo); brain, absolute decrease (1,000 ppm, 6, 12, and 18 mo); liver, absolute increase (1,000 ppm, 18 mo) and relative increase (1,000 ppm, 6, 12, and 18 mo); and kidneys, relative increase (1,000 ppm,

12, and 18 mo). The relationship between brain weight decreases and the previously described cerebellar lesions was not known, nor were the reasons for the apparent sex-related difference in kidney weight response (although renal tumors were found only in males). Increased liver weight values, more prominent in female mice, did not correlate well with hepatic lesions, which were found more frequently in males.

Significantly elevated ALT levels were observed in male mice (1,000 ppm at 6, 12, and 18 mo; 50 and 225 ppm at 6 mo), which were consistent with histologically observed hepatocellular degeneration and necrosis at the 1,000 ppm concentration—lesions considered induced by methyl chloride exposure. Elevated ALT levels were also found in female mice at all methyl chloride concentrations at 6 and 12 months, but these were not correlated with any specific histopathological changes, and values were normal at 18 and 24 months. Significant reticulocytosis was observed in 1,000 ppm males at 6 months, and hypoglycemia in 50 and 1,000 ppm males at 12 months; all groups, including controls, were hypoglycemic at 24 months. These data were not correlated with any specific histopathological alterations. No other hematological, clinical chemistry, or urinalysis findings in mice could be related to methyl chloride. Porphyrin fluorescence evaluations of fresh liver impression smears made at necropsy were negative.

The hepatocellular lesions (vacuolization, karyomegaly, cytomegaly, multinucleation, degeneration) first noted at 6 months in 1,000 ppm male mice were found with increasing frequency at 12 and 18 months, and were seen in the majority of males suffering unscheduled deaths. In less severe form, they also became apparent in approximately one third of the 1,000 ppm females that died early during months 18-22. Renal tubuloepithelial hyperplasia and karyomegaly appeared in 1,000 ppm males at 12 months, subsequently increasing in incidence and severity until the last males in this group were sacrificed at 21 months. Renal tumors were significantly increased in 1,000 ppm males during months 12-21; 17 renal neoplasms were found in 13 animals (8 renal cortical adenomas, 4 adenocarcinomas, 2 papillary cystadenomas, 2 tubular cystadenomas, and 1 papillary cystadenocarcinoma). These were considered induced by methyl chloride exposure, as were 2 adenomas (statistically nonsignificant in and of themselves) occurring in 225 ppm males at 24 months. A statistically significant increase in

renal cortical cysts was seen at 18-22 months in 7 males and 1 female from the 1,000 ppm group, as well as in 1 male and 1 female from the 225 ppm group at 24 months. Also at 24 months, microcysts were observed in 6 males from the 50 ppm group, and 1 control male had a cyst. Although their precise relationship to each other and to the other renal lesions was not clear, renal cyst and microcyst formation was considered by the investigators to be methyl chloride-related. However, the low incidence in the 225 ppm group suggests that it may be a spontaneous lesion. Unpublished data (Johnson, 1988) from eight 2-year mouse studies indicate that the incidence values for renal microcysts from the CIIT (1981) study fall within Dow Chemical Company's historical control incidence for this strain. Seminiferous tubule atrophy and degeneration were also statistically significant and considered exposure-related in 1,000 ppm males. Finally, 1,000 ppm mice developed splenic atrophy and lymphoid depletion during months 6-22 that was considered related to methyl chloride exposure. Mice exposed to 225 ppm or less of methyl chloride developed little splenic lymphoid depletion or atrophy, but along with controls developed a high incidence of non-exposure-related splenic lymphoid hyperplasia, a relatively common condition in laboratory mice.

In rats, mortality was not significantly affected at any concentration of methyl chloride. Ophthalmologic examination revealed sporadic corneal cloudiness and opacity across all groups at 6 months that developed into a significant pattern in females, but not males, at 18 months. Twelve-month observations were complicated by a different lesion, "corneal haze," that appeared in control and 1,000 ppm rats, possibly a result of SDA virus outbreaks. By 24 months corneal cloudiness and opacity were similar in exposed and control rats of both sexes. Lenticular changes (prominent anterior lens sutures) were significant in both sexes at 18 months, although more males than females appeared affected at all concentrations. Both the corneal and lenticular lesions were considered possibly related to methyl chloride exposure. No neurofunctional impairments and no statistically or biologically significant alterations in hematological, clinical chemistry, or urinalysis parameters that could be attributed to methyl chloride exposure were found in rats. No clinical observations pertaining to neurotoxicity were recorded for rats, and as in mice, there was no indication that methyl chloride-exposed rats became porphyritic.

Growth rates for 1,000 ppm male and female rats, and for 250 ppm females, were significantly reduced during the first 24 weeks of exposure. Necropsy body weights were reduced for 1,000 ppm males and females at all sacrifice periods, and for 225 ppm males at 18 months; conversely, body weights were increased for 50 ppm males at the 24-month terminal sacrifice. Among the statistically significant organ weight changes observed in male rats were: heart, absolute increase (1,000 ppm at 12 mo) and relative increase (1,000 ppm at 12 and 18 mo, 225 ppm at 24 mo); brain, absolute decrease (1,000 ppm at 6, 18, and 24 mo) and relative increase (1,000 ppm at 12 and 18 mo, 225 ppm at 18 mo); testes, absolute decrease (1,000 ppm at 18 and 24 mo) and relative decrease (1,000 ppm at 24 mo); lungs, absolute increase (50 and 225 ppm at 6 mo) and relative increase (50, 225, and 1,000 ppm at 6 mo); adrenals, absolute decrease (1,000 ppm at 18 mo); liver, relative increase (1,000 ppm at 6 and 24 mo, 225 ppm at 12 mo); and kidneys, relative increase (1,000 ppm at 6, 12, 18 [right only], and 24 [left only] mo) and relative decrease (50 ppm at 24 mo). Those in female rats included: brain, absolute decrease (1,000 ppm at 18 and 24 mo), relative increase (1,000 ppm at 12 and 24 mo); liver, absolute decrease (1,000 ppm at 12 and 24 mo), relative decrease (50 ppm at 24 mo); kidneys, absolute decrease (1,000 ppm at 24 mo), relative decrease (50 ppm at 24 mo), and relative increase (1,000 ppm [left only] at 6 and 12 mo); lungs, absolute decrease (1,000 ppm at 24 mo) and relative increase (1,000 ppm at 6 mo); spleen, absolute decrease (1,000 ppm at 12 mo); and heart, relative increase (1,000 ppm at 12 and 24 mo). Decreased body weights appeared to be the major contributing factor in most instances where changes in absolute weights did not correlate with those in relative weights. No body or organ weight changes occurring at exposure concentrations of less than 1,000 ppm were considered to be biologically relevant by the authors. It was noted that weight alterations in the brain, kidney, and heart were not reflected in any clinical or pathological findings, although the decreased brain weights could be a reflection of a methyl chloride effect similar to that seen in mice. No explanation was apparent for the complex changes in liver weights, although they generally paralleled those of the kidneys in both males and females. Heart weight changes were considered probably not biologically significant, and appeared in a number of instances to have reflected low heart weights in the controls and/or low final body weights.

The testes were the only target organs examined in the rat that were considered to have significant gross or histopathological lesions related to methyl chloride exposure. Bilateral, diffuse degeneration and atrophy of the seminiferous tubules were only observed in 1/10 of 1,000 ppm males at 6 months, 4/10 of 1,000 ppm at 12 months, and 10/20 at 18 months (uni- and bilateral and signs of age-related interstitial cell hyperplasia and adenomas). In contrast, no testicular lesions were observed in the other exposure groups at 6 or 12 months. However, beginning with the 18-month interim sacrifice, interstitial hyperplasia and/or adenomas was apparent, particularly in the control and 225 ppm group, such that there was no exposure-response based on incidence.

At 24 months, all animals including controls had a background of interstitial cell hyperplasia and adenoma. Some histopathological observations suggested that methyl chloride exposure might increase interstitial cell hyperplasia while reducing the size of interstitial cell tumors. Epididymal sperm granulomas were seen in 2 male rats of the 1,000 ppm group at 6 months, and in 1 at 24 months. The small number of lesions, their time-course of appearance, and their observation in the control groups of other studies conducted in that laboratory precluded directly attributing sperm granuloma formation to methyl chloride exposure.

The testicular results in rats are consistent with a LOAEL of 1,000 ppm, based on early signs of seminiferous tubule degeneration and atrophy in the absence of age-related degeneration. A NOAEL of 225 ppm appears reasonable since tubule degeneration and atrophy at this exposure level occurred upon onset age-related hyperplasia and compressive adenomas. In mice, a LOAEL of 50 ppm, the lowest concentration tested, was identified based on an exposure-related increased incidence of axonal degeneration and atrophy in areas of the spinal cord.

4.3 REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

No human or animal studies on the reproductive or developmental effects of methyl chloride by the oral route of exposure were located.

No studies were located in which reproductive or developmental effects were examined in humans after inhalation exposure to methyl chloride that occurred in the absence of significant exposure to other chemicals. A report by Kucera (1968) has been cited as describing a single case of an infant born with sacral agenesis to a mother exposed during pregnancy to methyl chloride and ammonia (John et al., 1984), and as describing an association of sacral agenesis in five infants born to mothers having close contact during pregnancy to "trichloroethylene and methyl chloride, among other industrial chemicals…" (Schardein, 1993).

Huel et al. (1990) attempted to assess the potential adverse reproductive outcomes among former female workers in a New Mexico microelectronics assembly plant. After a matching process, 90 former worker-referent pairs were obtained that represented 302 and 324 pregnancies, respectively. The study appeared to identify an increased risk of spontaneous abortion after the beginning of employment among the female workers when compared to referents (OR = 5.6, $p < 0.01$; OR = 4.0, $p < 0.05$ after taking into account the increased risk for subsequent spontaneous abortions in women who suffered them during previous pregnancies prior to employment). However, methyl chloride was only one chemical among many to which the women were exposed (e.g., chlorofluorocarbons, chlorinated hydrocarbons, glycol ethers, isopropanol, acetone, toluene, xylene, "alcohol"). Women reportedly suffered symptoms of intoxication on a daily basis, but the layout and manufacturing procedures of the plant did not permit any firm conclusions regarding the association of this apparent elevated risk of spontaneous abortion with exposure to particular organic solvents, including methyl chloride, and therefore is of limited value.

In studies described elsewhere in greater detail (Sections 4.2.2 and 4.4.2), exposure of Sprague Dawley rats for 48-72 h to 500, 1,000, or 2,000 ppm (1,032, 2,065, or 4,130 mg/m^3) of methyl chloride resulted in epididymal toxicity (e.g., degeneration, inflammation, interstitial edema, decreased sperm counts, sperm granulomas, scarring, and obstructive changes) that was dose-related, persistent through 12 days of recovery, and in some animals accompanied by testicular atrophy and degeneration (Burek et al., 1981). Clearly dose-dependent testicular degeneration (reduced numbers of late-stage spermatids, separation and luminal sloughing of spermatocytes and early-stage spermatids, vacuolated germinal epithelium, multi-nucleated giant

cells) and accompanying epididymal lesions (e.g., reduced sperm counts, sloughed spermatocytes, giant cells, and cellular debris in tubules) were found in male F-344 rats exposed for 6 h/day on 9 of 11 days to 0, 2,000, 3,500, or 5,000 ppm (4,130, 7,228 mg/m^3) of methyl chloride (Morgan et al., 1982). During the course of the CIIT chronic inhalation study (Section 4.2.2) (Mitchell et al., 1979b; CIIT, 1981), degeneration and atrophy of the seminiferous tubules were observed in the testes of F-344 rats and B6C3F$_1$ mice at 1,000 ppm. The increased incidence of sperm granulomas in rats, especially after six months of exposure, was not considered by the investigators to be directly attributable to methyl chloride.

A number of additional studies have also explored the effects of subacute exposure to methyl chloride on the reproductive physiology and performance of adult male F-344 rats (Chapin et al., 1984; Working et al., 1985a,b; Working and Bus, 1986; Chellman et al., 1987). To characterize testicular and epididymal lesions and any associated effects on reproductive hormones, Chapin et al. (1984) exposed male F-344 rats to 3,500 ppm of methyl chloride, 6 h/day, for 5 days, and after an interim of 3 days, for an additional 4 days. By Day 9 after the initiation of exposure, delay of spermiation (suggesting late-stage spermatids or Sertoli cells may be initial targets) and the occurrence of periluminal bodies were observed in the testes to a minimal degree; these lesions increased in severity through Day 19, with degeneration (cellular vacuolation and exfoliation) of the seminiferous epithelium beginning on Day 13, and the occurrence of a few giant cells on Day 19. The occurrence of round, periodic acid-Schiff stain (PAS) positive bodies in the tubular lumina of regions 1 and 2 of the epididymis was noted on Day 9, and decreased luminal sperm density on Day 11; these lesions were also observed in epididymal region 3, but with a lag period of approximately 2 days, and their severity increased for about 4 days before beginning to moderate. Bilateral sperm granulomas were first observed in epididymal regions 5 and 6 on Day 7 (in 1 of 8 rats), increasing in frequency through Day 19 when they were found in all animals (6/6). The primary inflammatory response appeared to be directed against tubular epithelium, rather than extravasated sperm. After 5 days of exposure, circulating levels of testosterone were reduced from 120 ± 31 ng/mL in controls to <6 ng/mL. However, when challenged with human chorionic gonadotropin (hCG) to test Leydig cell function, or luteinizing hormone releasing hormone (LHRH) to test pituitary function, methyl chloride-exposed and control rats responded similarly (although somewhat less strongly in the

case of hCG) in terms of induced serum testosterone levels. Methyl chloride exposure for 6 h was also found to reduce NPSH levels in the testes, epididymis, and liver (relative to 0 h levels) by approximately 66, 79-87, and 92%, respectively, but not in blood, supporting a mechanism of enzyme-mediated conjugation of GSH with methyl chloride. After 8 weeks of recovery, about 80% of the tubules in 4/5 rats still showed no evidence of spermatogenesis.

Working et al. (1985a,b) conducted a two-part study on male Fischer 344 rats (80/group) that were exposed 6 h/day for 5 days to 0, 1,000, or 3,000 ppm (0, 2,065, or 6,195 mg/m^3) of methyl chloride, or were injected with 0.2 mg/kg bw of triethylenemelamine (TEM, positive control). Each of forty males per exposure group was bred weekly to a single, untreated female for 8 wk in order to assess mating performance and dominant lethality (Working et al., 1985a), while the remaining 40 males/group were used to evaluate sperm quality and testicular histopathology (Working et al., 1985b). In a smaller second experiment, F-344 males (40/group) were exposed 6 h/day for 5 consecutive days to either 0 or 3,000 ppm methyl chloride, then bred as described for just 4 wk prior to sacrifice. At the end of the 5-day exposure period, body weights were significantly reduced ($p < 0.05$) relative to controls in the 1,000 and 3,000 ppm groups (2 and 16%, respectively), but returned to control levels by post-exposure Weeks 3 and 4, respectively. The percentages of females mated by males in the 1,000 ppm and TEM groups were not significantly different from controls at any week post-exposure, but were reduced for experiment-one 3,000 ppm males during the first 2 weeks post-exposure (Week 2, $p < 0.05$). In the second experiment, the percentages of mated females for control and 3,000 ppm groups were not significantly different (4-week averages: controls, 85.6%; 3,000 ppm males, 91.0%). Fertility was not significantly reduced in 1,000 ppm males, but was significantly reduced in TEM (Week 2) and 3,000 ppm (Weeks 2 and 3, approximately 35-65% of control values) males; a small, statistically non-significant reduction persisted in the latter group through Week 16. At termination (Week 17), unilateral or bilateral sperm granulomas were observed in the epididymides of 30% (12/40) of the 3,000 ppm males, but not in any of the control, 1,000 ppm, or TEM males.

The dominant lethal test measures embryo loss in untreated female animals that have been mated with exposed males; this embryonic death results from genetic lesions (primarily structural

and numerical chromosome abnormalities) or, in some cases, cytotoxic effects that were induced in the male germ cells (Olshan and Faustman, 1993; Working and Chellman, 1989). Embryonic or fetal loss occurring after implantation (postimplantation loss) is considered a direct measure of genotoxic effects, whereas conceptus loss prior to implantation (preimplantation loss) may reflect either genotoxicity or cytotoxicity that adversely affects sperm function. In this study (Working et al., 1985a), exposure to 1,000 ppm methyl chloride did not cause any consistently significant change in dominant lethality parameters relative to controls. However, 3,000 ppm produced a small but significant ($p < 0.05$) increase in postimplantation loss of 9.5% for post-exposure Week 1 matings; thus, the relevant genetic lesions appeared to be induced only in sperm residing in the vas deferens and epididymis during methyl chloride exposure. In contrast, increases of preimplantation loss (approximately 14-94% over controls) were observed for all 8 weeks of mating; the maximum increase was for Week 2, and increases were statistically significant for Weeks 2-4, 6, and 8. Significantly elevated rates of preimplantation loss were observed for matings of TEM-treated males during post-exposure Weeks 2 and 3, and of postimplantation loss during Weeks 1-5. Dominant lethal genetic effects typically demonstrate a coupling of increased postimplantation loss with decreased preimplantation loss as matings progress through the post-exposure period; this pattern was observed with TEM, but not with methyl chloride.

In the companion study (Working et al., 1985b), no consistent differences in sperm quality or testicular histopathology parameters relative to controls were observed in males from the 1,000 ppm methyl chloride or TEM groups. In the 3,000 ppm group, testicular weight was significantly depressed ($p < 0.05$) at Weeks 3-8 post-exposure, recovering to near-control levels by Week 16. Beginning at Week 2, just over 50% (20/39) of the animals displayed gross or microscopic sperm granulomas in the caudal epididymis; these increased in size and severity over time, and were unilateral in some cases, bilateral in others. Histologically, the testes evidenced a delay in spermiation, chromatin margination in round spermatids, epithelial vacuolation, luminal exfoliation of spermatogenic cells, and multinucleated giant cells. Testicular spermatid head count was significantly depressed during Weeks 2-8. While sperm count in the vas deferens began declining by Week 2, it was significantly reduced during Weeks 3-8, and still appeared somewhat depressed at Week 16. The frequencies of intact and motile sperm observed during Weeks 1-6 were generally significantly depressed (3.5-69% of

controls), but had recovered by the end of 16 weeks. Furthermore, increases in morphologically abnormal sperm isolated from the vasa deferentia were seen during the first 5 weeks post-exposure; these increases were significant ($p < 0.05$) for Weeks 1-3, dramatically peaking at approximately 70% for Week 3. These companion studies indicate that exposure for 6 h/day on 5 consecutive days to 3,000 ppm of methyl chloride resulted in toxicity to sperm located in the epididymis and vas deferens, which resulted in a small increase in dominant lethality expressed as postimplantation loss, presumably due to genetic lesions. Exposure also caused toxic effects to spermatids, primary spermatocytes, and spermatogonial stem cells in the testes, as well as chronic epididymal inflammation. Because of this inflammation, the resulting sperm granulomas, and the atypical pattern of pre- and postimplantation fetal loss induced by methyl chloride, the authors speculated that the observed increases in infertile matings and preimplantation loss may be largely the result of failure of fertilization because of low sperm number and poor sperm quality (i.e., cytotoxic rather than genotoxic mechanisms). The reproductive NOAEL and LOAEL, based upon the parameters described in these companion studies (Working et al., 1985a,b), are 1,000 and 3,000 ppm, respectively.

To further explore the potential role of cytotoxicity in the preimplantation losses induced by methyl chloride, Working and Bus (1986) conducted a similar study in which male F-344 rats were exposed to 0 (n = 30), 1,000 (n = 10), or 3,000 ppm methyl chloride (n = 20), 6 h/day for 5 consecutive days. Positive control males (n = 10) received a single ip injection of 0.2 mg/kg TEM on exposure Day 5. Following the exposure period, each male was bred weekly for up to 8 weeks to 2 untreated females. Twelve hours post-mating, females were sacrificed and their ova recovered and scored as fertilized or unfertilized. The fertilization rate from control matings was approximately 88% (602/683), and rates were not significantly depressed from this level in the 1,000 ppm (80%) or TEM (77%) groups. However, fertilization rate was significantly depressed ($p < 0.05$) in the 3,000 ppm group through the 8 weeks of mating, ranging from 3.4% (14/415, Week 2) to 72.3% (159/221, Week 8). Furthermore, the 8-week patterns of unfertilized ova in this study and preimplantation loss in the dominant lethal assay coincided, with the percentage of unfertilized ova equaling or exceeding that of preimplantation loss for each post-exposure week of mating. By contrast, unfertilized ova could account for only a little more than a third of the preimplantation losses induced by the genotoxicant, TEM. When fertilized ova were cultured

in vitro for 24 h, only the TEM-exposed ova displayed a significant decrease from controls (85 versus 96%) in the percentage of ova that underwent cleavage. Collectively, these data support the authors' conclusion that methyl chloride-induced preimplantation loss in the dominant lethal assay results from cytotoxicity-mediated failure of fertilization, rather than from genotoxic effects.

An additional study conducted by the same laboratory (Chellman et al., 1987) explored whether the observed cytotoxic effects on sperm were the result of methyl chloride's effects on the testes or on the epididymides. Groups of male F-344 rats (18/group) were exposed 6 h/day for 5 consecutive days to 0 or 3,000 ppm methyl chloride, with or without concurrent exposure to the anti-inflammatory agent 3-amino-1-[m-(trifluoromethyl)phenyl]-2-pyrazoline (BW755C), 10 mg/kg by ip injection, 1 h pre- and post-exposure. Earlier studies indicated that co-treatment with BW755C prevented methyl chloride's induction of both epididymal inflammation and the increased postimplantation loss observed in the dominant lethal assay for matings at 1 and 2 weeks post-exposure (Chellman et al., 1986a). These findings suggested that methyl chloride's induction of epididymal inflammation may be responsible for producing the DNA damage that results in postimplantation loss, perhaps via the generation of reactive oxygen intermediates (Chellman et al., 1986a, 1987; Working and Chellman, 1989). In contrast, BW755C did not mitigate the methyl chloride-induced increases in preimplantation loss that were observed beginning at Weeks 2 and 3 post-exposure.

In this study (Chellman et al., 1987), exposure to 3,000 ppm methyl chloride significantly reduced the post-exposure relative organ weights of the seminal vesicles (Week 1), epididymides (Weeks 2 and 3) and testes (Week 3); similar reductions also were observed in rats co-exposed to methyl chloride and BW755C, but not in controls or animals treated only with BW755C (except for Week 2 seminal vesicles in the latter). Exposure to methyl chloride resulted in testicular histopathology characterized by delayed spermiation, disorganization, and destruction of the seminiferous epithelium, and marked, persistent decreases in the number of mid- to late-stage spermatids in the testes; again, co-exposure to BW755C did not prevent these degenerative changes. Daily sperm production in the testes was significantly reduced during Weeks 1-3 by methyl chloride exposure, with or without BW755C. Methyl chloride-exposed males displayed

visible uni- or bilateral epididymal sperm granulomas (4/18 animals), and inflammatory cells, debris, and nucleated cells (8/18 animals) in the epididymal tubular lumina; no such effects were observed in the methyl chloride + BW755C group. Sperm numbers were normal in the vas deferens of the methyl chloride-exposed group at Weeks 1 and 2, but were drastically reduced at Week 3; percentages of motile sperm were significantly reduced at Weeks 1 and 2, severely so at Week 3, when the percentage of morphologically abnormal sperm was also greatly increased (99 versus 2% in controls). Co-exposure to BW755C did not prevent these adverse effects on sperm. Given these findings, in conjunction with known epididymal transit times for sperm in F-344 rats, the authors concluded that the preimplantation losses observed at Weeks 2 and 3 post-exposure (and later) in dominant lethal experiments likely resulted from methyl chloride's cytotoxic effects on sperm located in the testes during exposure, rather than in the epididymides.

A two-generation reproduction study using F-344 rats was conducted by Hamm et al. (1985). Animal groups (40 males, 80 females/concentration) were exposed by inhalation to 0, 150, 475, or 1,500 ppm (0, 310, 981, or 3,098 mg/m^3) methyl chloride, 6 h/day, 5 days/wk for 10 weeks. Exposure was then increased to 7 days/wk, and each F_0 male was mated with two F_0 females during the course of 2 weeks (1 female/wk). After the two-week breeding period, 10 F_0 males/group were necropsied and the remaining 30/group were each bred for 2 weeks to two unexposed females; after an additional 10 weeks of recovery, 10 more F_0 males/group were necropsied and the remaining 20/group were each bred to two unexposed females; finally, after an additional 18 weeks of recovery, another 10 F_0 males/group were necropsied and the remaining 10/group were each bred to two unexposed females. Pregnant F_0 females, bred to F_0 males during Weeks 10-12, continued to be exposed to methyl chloride, except from Gestation Day 18 through Postnatal Day 3; exposure was resumed 6 h/day, 7 days/wk during Postnatal Days 4-28, when pups were weaned. Pups were never directly exposed to methyl chloride. After weaning, 15 F_1 pups/sex and 25 F_0 females were necropsied. The same exposure regimen, but without the recovery breeding protocol, was utilized for F_1 matings (40 males, 80 females for 0 and 150 ppm; 23 males, 46 females for 475 ppm).

Body weight gains were depressed (10-20%) at all weekly weighings after 2 weeks of exposure for both sexes in the 1,500 ppm group, and in the 475 ppm group (5-7%) after Day 57.

Exposure-related lesions were observed only in 1,500 ppm F_0 males, and included degeneration and atrophy of the seminiferous tubules (10/10 males examined), as well as epididymal sperm granulomas (3/10); the testes of these animals weighed only approximately half as much as those of control and lower-dose groups. The number of females that mated (as evidenced by the presence of copulation plugs) did not vary by exposure group, but no litters were produced in exposed or unexposed females that were sired by 1,500 ppm males. The number of males proven fertile was also statistically lower in the 475 ppm group. No consistently significant differences in litter size, sex ratio, pup viability, survival, or growth were observed in the 150 or 475 ppm groups relative to controls. Body weight for the 1,500 ppm males remained significantly lower than for controls during the first 9 weeks of recovery, when it then became comparable. After 10 weeks of recovery, the percentage of fertile 475 ppm males was not significantly different from controls, and that of 1,500 ppm males had partially recovered (25 versus 75% for controls); further improvement in the 1,500 ppm group was not seen after an additional 18 weeks of recovery. Also, testicular weights in the 1,500 ppm group remained less than in controls, but no epididymal sperm granulomas were observed. Methyl chloride exposure (150 or 475 ppm) did not cause statistically significant effects on F_1-generation fertility, although 475 ppm litters had a decreased percentage of males (41 ± 16% versus 51 ± 18% in controls, 53 ± 13% 150 ppm litters) and reduced pup weights for Days 14-21 (but not earlier or later). Based upon a marginal reduction in the number of males proven fertile, the two-generation reproductive LOAEL in this study was 475 ppm with a corresponding NOAEL of 150 ppm. As noted by the authors, the actual NOAEL may in fact have been closer to the 475 ppm concentration, where the reduction in number of fertile males was only statistically significant when matings with exposed (2) and unexposed (2) females were combined, and not when considered separately.

Wolkowski-Tyl et al. (1983a,b) conducted evaluations of the structural teratogenicity of methyl chloride in rats and mice after inhalation exposure. Groups of bred female F-344 rats (25/concentration) and female C57BL/6 mice bred to C3H males (33/concentration) were exposed 6 h/day to methyl chloride concentrations of 0, 100, 500, or 1,500 ppm (0, 206, 1,032 or 3,098 mg/m^3). Rats were exposed from Gestation Days 7 through 19, then were sacrificed on Gestation Day 20; mice were exposed from Gestation Days 6 through 17 with sacrifice on

Gestation Day 18, except for the 1,500 ppm group, in which the animals were sacrificed in extremis during Gestation Days 10-14. In rats, no behavioral toxicity in the dams was seen at any concentration of methyl chloride, but at 1,500 ppm there was evidence of both maternal toxicity (depressed weight gain during exposure and body weight at sacrifice) and fetal toxicity (reduced male and female fetal body weights, reduced female crown-rump length at Gestation Day 20). There were no exposure-related effects on implantations, resorptions, dead or live fetuses, or sex ratio; the authors speculated that methyl chloride's effects were expressed late in gestation and were probably secondary to maternal toxicity. No exposure-related fetal malformations were observed (external, visceral, or skeletal), although some evidence of delayed ossification was observed in the 1,500 ppm fetuses. The study authors concluded that methyl chloride was not teratogenic in pregnant female F-344 rats exposed to maternally and fetotoxic concentrations during the critical periods of fetal and embryo development.

Pregnant mice of the 1,500 ppm group displayed urogenital bleeding and CNS dysfunction (hunched posture, walking on "tip-toes", tremors, imbalance) commencing on Gestation Days9 (the fourth day of exposure), of severity that required premature sacrifice on Gestation Days 10-14 (exposure Days 5-9). Upon necropsy, these animals exhibited necrosis of neurons in the internal granular layer of the cerebellum. No reproductive parameters were significantly affected by methyl chloride exposure. Statistically non-significant trends for increasing body weights and crown-rump lengths with increasing concentration were seen in male and female fetuses; the only significant finding ($p < 0.05$) was increased crown-rump length in male fetuses of the 100 ppm group. No significant, exposure-related external or skeletal anomalies were observed in the fetuses of either the 100 or 500 ppm group. However, visceral examination of 50% of the mouse fetuses revealed a small but statistically significant increase in the incidence of a heart anomaly in the 500 ppm group. This lesion involved the tricuspid valve, chordae tendineae, and papillary muscles in the right atrioventricular septum (6 fetuses), or the bicuspid valve, chordae tendineae, and papillary muscles in the left atrioventricular septum (3 fetuses). The lesion was found in 6 of 17 litters and in both sexes (3 males, 6 females), with no single fetus having both sides involved; 1 litter had right and left side involvement, and 5 of 6 affected litters also had fetuses with normal appearing hearts.

To further explore this apparent teratogenic effect in $B6C3F_1$ fetuses, 74-77 female C57BL/6 mice impregnated by C3H males were exposed 6 h/day from Gestation Days 6 through 17 to 0, 250, 500, or 750 ppm (0, 516, 1,032, or 1,548 mg/m^3) of methyl chloride (Wolkowski-Tyl et al., 1983b). Beginning on the seventh day of exposure (Gestation Day 12), females in the 750 ppm group displayed ataxia, tremors, convulsions, and hypersensitivity to touch or sound; prior to the scheduled sacrifice, 6 of these animals died and 1 was killed in extremis. When compared with controls, survivors of this group were the only ones to exhibit significant reductions in body weight gain during gestation, body weight on Gestation Day 18, and absolute weight gain (weight gain minus gravid uterine weight). No exposure-related differences were observed in pregnancy rate, gravid uterine weight, maternal liver weight, numbers of implantations, resorptions, dead fetuses, nonlive (dead plus resorbed) fetuses, live fetuses, sex ratio, or mean fetal weight per litter. However, statistically significant ($p < 0.05$) and concentration-related trends were observed for the numbers and percents of malformed or affected (nonlive plus malformed) fetuses per litter. All but one malformation (an umbilical hernia, 250 ppm group) were found in the heart and included reductions in the number of papillary muscles and/or chordae tendineae on the right associated with the tricuspid valve, globular hearts, tricuspid valve defects, small right ventricle, and white deposits (calcium-containing) in the left ventricular wall. Defects were more common in females (23) than males (14), with 1 fetus in the 500 ppm group and 3 in the 750 ppm group exhibiting multiple heart malformations. Incidences of malformations were: 0 ppm (3/433 fetuses, 3/58 litters), 250 ppm (7/458 fetuses, 6/62 litters), 500 ppm (11/444 fetuses, 7/62 litters), and 750 ppm (17/400 fetuses, 14/56 litters). Relative to controls, there were statistically significant increases in the numbers of 750 ppm litters, male fetuses, and female fetuses with malformations; increases across all groups were seen for the numbers of fetuses with any malformations ($p < 0.025$) and with heart malformations ($p < 0.005$). The researchers concluded that in pregnant C57BL/6 female mice and their $B6C3F_1$ conceptuses, methyl chloride exposure during gestation (Gestations Days 6-17) was maternally toxic at 750 ppm and teratogenic at 750 and 500 ppm, causing heart malformations. No maternal, embryo or fetotoxicity, nor any teratogenicity, was observed at the 250 ppm concentration.

Findings of teratogenicity in the mouse have been questioned (John-Greene et al., 1985). These authors discuss the unusual nature and rarity of the heart lesion described by Wolkowski-Tyl et al. (1983a,b), its apparent dissimilarity to lesions reported in human and hamster hearts, its relatively low incidence in methyl chloride-exposed animals and its occurrence in controls, the relatively uncommon use of the $B6C3F_1$ hybrid in teratogenicity testing, the technical difficulties involved in detecting the lesion without introducing artifacts, and the interanimal variability in appearance of the papillary muscles. Furthermore, when conducting blindly-scored studies on animals exposed continuously to 300 ppm for 24 h (Gestation Days 11.5-12.5, the developmental stage they considered the most critical for cardiac structures), or 1,000 ppm for 12 h (Gestation Days 11.5-12), John-Greene et al. (1985) were unable to demonstrate the lesion's occurrence. In response, Tyl (1985) acknowledged and shared some of these concerns, but pointed out that the lesion was found in two studies using different personnel and examination procedures, and in one study was confirmed by another pathologist and staff, and that great care was used in cutting the hearts for examination. Tyl (1985) also pointed out the differences in exposure regimen, 6 h/day for Gestation Days 6-17 versus 12-24 continuous hours on Gestation Day 11.5 to Gestation Day 12-12.5, discussing in particular why exposure beyond Gestation Day 12-12.5 could be critical for generation of the lesion. Until further work is reported, methyl chloride should prudently be considered a mouse teratogen.

Finally, the report by Bus et al. (1980) previously discussed in Sections 3.2 and 3.3 indicates that methyl chloride can cross the placenta and cause potentially adverse effects in the fetus. Pregnant F-344 rats were exposed on Gestation Day 19 for 6 h to 1,500 ppm methyl chloride. Maternal liver and kidney NPSH levels were maximally depressed immediately after the end of exposure, but had returned to normal by 8 h. Fetal placental NPSH, also maximally depressed at 0 h post-exposure, returned to normal within 4 h, but fetal liver and carcass NPSH decreases were not maximal until 2 h post-exposure. These decreases were substantial (66.8 and 71.0%, respectively) and had not yet returned to normal by 8 h post-exposure.

4.4 OTHER STUDIES

4.4.1 Neurological Studies

The study on male and female volunteers by Stewart et al. (1980), previously discussed in Sections 3.1-3.4 and 4.1.1, also included several neurological and cognitive tests on its subjects, who were exposed sedentarily for up to five consecutive days, up to 7.5 h/day, to methyl chloride concentrations of 0, 20, 100, "100f" (fluctuating 50-150), or 150 ppm. During the first 5 and last 10 min of exposure, each subject performed modified Romberg and heel-to-toe equilibrium tests (standing on one leg with arms at sides for $\geq$3 sec, and walking heel-to-toe in a straight line for ~5 ft, first with eyes open and then with eyes shut) that were videotaped. Group I subjects also had spontaneous electroencephalograms (EEGs) and visual evoked responses (VERs) recorded 4 times/day, 3 days/wk during their 7.5 h/day, 5 day/wk exposure regimen. EEGs were visually inspected, and VER wave complexes were analyzed for amplitude, latency, and wave form; data from the three methyl chloride exposure concentrations were compared with those from the 0 ppm control sessions. Groups I and II also received a battery of five cognitive tests on Days 1, 3, and 5 of each exposure week, beginning 2-3 h after the commencement of exposure. In order of performance, the tests included: (1) a 10- and 30-sec time estimation test; (2) the Marquette Time Estimation Test, involving a series of light and tone stimuli; (3) a coordination test (Flanagan Aptitude Classification Tests, 7A, Coordination); (4) a two-part arithmetic test involving addition and subtraction, then multiplication and division; and (5) a visual inspection test involving spotting the number "3" from a page containing rows of random numbers.

No significant neurological abnormalities were observed in any of the male or female individuals exposed to methyl chloride, even at 150 ppm for 7.5 h/day, 2 days/1 wk, or 100 ppm (or 100f ppm) for 7.5 h/day, 5 days/1 wk. Equilibrium remained subjectively normal during exposures, as did EEG recordings and VER tracings. No statistically significant effects of methyl chloride exposure on the cognitive tests were apparent, with one exception. Group I males (exposed for 7.5 h/day) changed from overestimating the length of the light stimulus on the Marquette test, to underestimating it with increasing methyl chloride concentration. However, the researchers considered this finding to be spurious because the same shift was not

observed for the sound stimulus, nor did it occur in the Group II (3-h exposure) male subjects. They also drew attention to the lack of any observed decrement in arithmetic task performance, noting that while this result appeared to differ from the findings of Repko and colleagues (see below), the arithmetic tasks in this study were not part of a multi-tasking regimen as they were in the Repko study. The researchers concluded that several days exposure of male or female volunteers to as much as 150 ppm methyl chloride for 7.5 h/day did not result in any biologically significant decrements in their neurological or cognitive functioning.

The Dow Chemical Company (1992) report on six cases of methyl chloride intoxication in humans, previously discussed in Section 4.1.1, is one of numerous case history studies reviewed by Repko (1981), although the published version was reviewed (Scharnweber et al., 1974). Collectively, these case histories are summarized to demonstrate that symptoms of methyl chloride exposure indicative of CNS toxicity in humans include headache, nausea, double vision, mental confusion, vomiting, anuria, nervousness, emotional instability, ataxic gate, low blood pressure, insomnia, anorexia, vertigo, tremor, convulsions, increased pulse and respiration rates, coma, and eventual death. As previously noted, generally (but perhaps not always) these symptoms seem completely reversible, although some memory loss of events during exposure may remain. Repko (1981) also notes that several case histories and medical reports have described specific degeneration of portions of the CNS (cerebral cortex to the sacral part of the spinal cord), frontal and parietal atrophy, lipoid-filled histocytes in the leptomeninges of the cerebral hemispheres, generalized hyperemia of the cerebral cortex, nerve cell degeneration, etc.

Also reviewed by Repko (1981) is an earlier study (Repko et al., 1976) that attempted to quantitatively investigate the neurological and behavioral effects of low-level occupational exposure to methyl chloride. A group of 122 methyl chloride workers was compared with another group of 49 workers presumably not exposed to methyl chloride. The mean ambient air concentration of methyl chloride was determined to be 33.6 ppm. Both groups were reported to be asymptomatic with respect to either acute or chronic methyl chloride intoxication, and neurological examinations revealed no significant differences between them, including dysrhythmias determined from EEG tracings. Quantitative test results were reported to be correlated with ambient air levels of methyl chloride and with urine pH, but not with breath

levels of methyl chloride. Group comparison suggested that methyl chloride exposure adversely affected performance on cognitive time-sharing tasks and magnitude of finger tremor, but not on various psychological and personality tests. These results implied that methyl chloride exposure can cause "a decrease in speed and accuracy of simple tasks and a dimintion [*sic*] of the ability to time-share in more complex performance or work situations…at ambient levels of methyl chloride below 100 ppm" (Repko, 1981). However, the study has been severely criticized (Farber and Torkelson, 1989) on the grounds that the control group was much younger than the exposed group, the controls were measured later in the study and at a different location, and the methyl chloride workers had previously been exposed to higher concentrations of methyl chloride, thus confounding the relationship of any observed effects to the reported low-level exposure (33.6 ppm). Anger and Johnson (1985) also suggested that the subjective opinion of the neurologist that 58% of the control subjects (versus 57% of the exposed group) were abnormal in some way was problematic, and they expressed concern that the data interpretation was based upon t-tests that were uncorrected for the total number of dependent variables. Nonetheless, given the large number of significant differences, these reviewers considered it likely that chronic exposure to methyl chloride at concentrations approximating 35 ppm resulted in some worker behavioral changes.

Putz-Anderson et al. (1981) studied the behavioral effects on 56 humans (39 males, 17 females; 8 or 12/group) of inhaling 0, 100, or 200 ppm of methyl chloride for 3 h, with or without concurrent ingestion of 10 mg of diazepam (Valium). Participants were evaluated on three performance tests: a visual vigilance task (similar to an automated version of Mackworth's Clock Test), a dual task (tone detection and eye-hand compensatory tracking), and a time discrimination task. Three performance indices were obtained: threshold performance level (TPL), which approximated a 70% accuracy level; response time (RT); and number of response blocks (RB), defined as a longer-than-usual RT (i.e., greater than the mean RT plus two SDs of the baseline data). The net decline in mean performance across the three tasks was 4% for 200 ppm methyl chloride (marginally significant, $p < 0.053$), while for diazepam it was a significant 10.1% ($p < 0.01$); no potentiation was observed, as the performance decrement for exposure to 200 ppm methyl chloride plus10 mg diazepam was 13.5%, or essentially additive. Only the visual vigilance TPL and the time discrimination RB indices were sensitive indicators

of methyl chloride impairment, whereas all indices except the time discrimination RB were significantly affected by diazepam exposure. In line with other studies described in the toxicokinetic sections, these authors found that 3 of 24 subjects exposed to 200 ppm had significantly higher breath levels (> 100 ppm) of methyl chloride than did the remainder, and that blood levels were highly correlated with breath levels. This study indicates a marginal behavioral effect of a 3-h exposure to 200 ppm of methyl chloride (behavioral testing of the 100 ppm group was not performed), an effect that was not potentiated by concurrent exposure to the commonly prescribed CNS depressant diazepam (effects were merely additive).

Neurotoxic effects resulting from inhalation exposure to methyl chloride have also been described in a number of animal studies. As described in Section 4.4.2, Morgan et al. (1982) exposed groups of F-344 rats (10/sex/concentration) 6 h/day to 0, 2,000, 3,500, or 5,000 ppm (0, 7,228 or 10,325 mg/m^3) methyl chloride for 5 days, then for another 4 days after a 2-day break (i.e., on Days 1-5 and 8-11). Among other effects in the 5,000 ppm group, forelimb ataxia was observed in most females and in about half the males on Day 3, hindlimb paralysis developed in 2 males and 1 female on Day 5, and 1 female suffered convulsions during the Day 11 exposure. Following the Day 5 exposure, 13 rats were sacrificed in extremis (5,000 ppm; 6 males and 5 females; 3,500 ppm; 2 females), but the survivors seemed to tolerate the methyl chloride exposure much better during the second week (Days 8-11). At the high concentration, 3 of 10 males and 2 of 9 females (Table 4-1) displayed minimal histopathological evidence of cerebellar degeneration (foci of internal granular layer cells having pyknotic nuclei with distended, hydropic perikarya). This study demonstrated a NOAEL and LOAEL for neurotoxicity in F-344 rats following subacute exposure to methyl chloride of 3,500 and 5,000 ppm, respectively.

Jiang et al. (1985) conducted an ultrastructural study of lesions induced in the cerebella of C57BL/6 mice (10 females/concentration) exposed 6 h/day, 5 days/wk for 2 weeks to 0 or 1,500 ppm (0 or 3,098 mg/m^3) methyl chloride. Two mice died during the second week of exposure, and several displayed ataxia; no clinical signs of CNS disturbance were noted in the other animals, including those displaying histological evidence of severe cerebral degeneration, and no gross abnormalities of the brain or other organs were observed. Under light microscopy, two types of lesions were found in inner granular layer cells of the cerebellum (most frequently in

the ventral paraflocculus: a coagulative necrosis (also seen in controls, but in milder form and in substantially fewer cells) involving nuclear and cytoplasmic condensation; and a focal malacia involving edema in groups or extensive areas of cells, with nuclear condensation, karyorrhexis, necrosis, separation of myelinated axons, and microvacuolation. Electron microscopy confirmed the type one lesion, showing pyknotic nuclei without cytoplasmic edema, but with variable disruption of organelles. Areas of malacia exhibited characteristics ranging from perikarya edema of granule cells to near complete destruction of all tissue components with the exception of blood vessels (nuclear pyknosis and condensation, karyorrhexis, organelle remnants, etc.). Few abnormalities were observed in the kidneys of treated females (slight degeneration of proximal tubules with some proteinaceous material in tubular lumina was seen in two animals), leading the researchers to conclude that the brain lesions were most likely not a secondary effect of renal toxicity (these types of brain lesions had been associated with renal insufficiency in humans).

In a study more fully described in Section 4.4.2, Landry et al. (1985, 1983c) exposed female C57BL/6 mice (12/group) continuously (22-22.5 h/day) to 0, 15, 50, 100, 150, 200, or 400 ppm (0, 31, 103, 206, 309, 412 or 824 mg/m^3) or intermittently (5.5 h/day) to 0, 150, 400, 800, 1,600, or 2,400 ppm (0, 309, 824, 1,648, 3,296 or 4,956 mg/m^3) of methyl chloride for 11 days. Continuous exposure was interrupted once in the morning and once in the afternoon, and animals were observed twice daily. Neurofunctional testing was conducted during the course of the study that consisted of monitoring mice (previously trained on the apparatus) for their abilities to stay on an accelerating rod (acceleration = 1 rpm/sec, from 10 rpm up to 70 rpm) 2 to 2.5 h post-exposure after 4, 8 and 11 days of exposure. Upon termination, all mice were subjected to gross and histopathological examination. Body and organ weights were obtained, as were samples of most major organs and tissues (e.g., areas of the spinal cord), including tissue samples for examination by electron microscopy from the cerebella of three pre-selected mice from each of the 0 and 150 ppm continuously-exposed groups after 1, 2, 4, 6, 8, or 10.5 days of exposure. In addition to other effects described in Section 4.4.2, various indications of neurotoxicity were observed in both the continuously (C) and intermittently (I) exposed groups. Exposure to 400-C or 200-C ppm was lethal after 4 or 5 days, respectively. Mice exposed to 150-C to 400-C ppm developed poor motor coordination and deteriorated to a moribund

condition at a rate that was dose-dependent. After 3 days, 200-C mice were severely affected, displaying loss of appetite and ataxia with frequent falling, but were not yet moribund and retained good muscle tone and responsiveness to stimuli such as touch. In intermittently exposed mice, a transient (i.e., at 0.5 h, but not 3 h, post-exposure) sedation was observed in 1,600-I and 2,400-I ppm groups at 4-7 days of exposure, but not after 8 days. Slow movement was apparent in 2,400-I ppm mice after 5 days of exposure, and some hind-limb extensor rigidity by 8 days; these animals deteriorated and were sacrificed moribund on Days 8-9. Mice exposed to 1,600-I ppm displayed less severe effects, including slightly rigid hind limbs and some tendency to rear on hind legs (2/12) and be more excitable than controls; these effects lessened during overnight periods of nonexposure. No significant decrements in rotating rod performance were noted for the control, 15-C to 100-C ppm, and 150-I and 400-I ppm groups. Substantially diminished performance was seen in the 150-C ppm group after 4 and 8 days, with animals moribund or dead by Day 11. Animals in the 200-C ppm group were essentially nonfunctional after 4 days and moribund or dead at later times, while those in the 400-C ppm group were moribund by Day 4. Significant decrements were observed in the 800-I and 1,600-I ppm groups only on Day 4, and in the 2,400-I ppm group only on Days 4 and 8 (animals had multiple manifestations of toxicity on Day 8 and were moribund or dead by Day 11). Histopathologically, continuous exposure to 100 or more ppm resulted in concentration- and duration-dependent degenerative changes to the cerebellum, principally in the granule cells, that was characterized by nuclear pyknosis and karyorrhexis. Transient intra- and extracellular vacuolation in the purkinje and/or molecular cell layer and in the white matter were also noted. Only slight granule cell degeneration was observed in one-third of the 400-I and two-thirds of the 800-I and 1,600-I ppm animals, whereas all of the 2,400-I ppm animals expressed the lesion in slight-to-moderate degree. Electron microscope observations were consistent with those obtained through light microscopy (Landry et al., 1983c). There was no evidence of damage to spinal or peripheral nerves. This study's neurotoxicity NOAEL and LOAEL for continuous subacute exposure were 50 and 100 ppm, respectively, and for intermittent exposure were 150 and 400 ppm, respectively. These NOAELs were nearly proportionate to the product of concentration times exposure duration, although the dose-response curve for continuous exposure was much steeper than that for intermittent exposure. It is also noteworthy that the cerebellar lesions were observed at lower concentrations than were the decrements in rod running performance.

In a study by Morgan et al. (1982) (detailed in Section 4.4.2), neurotoxic effects were observed to varying degrees in three strains of mice (C3H, C57BL/6, $B6C3F_1$) that were exposed (5/sex/strain/concentration) 6 h/day for 12 consecutive days to 0, 500, 1,000, or 2,000 ppm (0, 1,032, 2,065, or 4,130 mg/m^3) of methyl chloride. At the 2,000 ppm concentration, all male $B6C3F_1$ mice were moribund or dead by Day 2, a single C57BL/6 male died on Day 2, and all remaining mice were moribund by Day 5. All mice in the remaining groups survived the 12 day exposure regimen, with the exception of one 1,000 ppm C3H male that died on Day 11. Prior to death, some mice were described as exhibiting moderate to severe ataxia. In addition to renal and hepatic effects, Table 4-1 (see page 84) summarizes the occurrence of histopathological changes that were observed in the brains of exposed mice. The lesion was described as focal degeneration of the cerebellar internal granular layer, consisting of small foci of granule cells having dense pyknotic nuclei that were surrounded by what appeared to be distended, hydropic perikarya; the effect was observed only in granule cells, and most frequently in the dorso-medial cerebellar folia. The authors speculated on the possible involvement of hepatic or renal toxicity in the formation of this lesion, although the literature did not strongly support such an association, nor did the findings of Jiang et al. (1985) discussed above. From Table 4-1, it is readily apparent that the lesion demonstrated a marked strain and sex specificity, occurring principally in C57BL/6 females. Based on this lesion and the reported ataxia observed in some animals, this study suggests a NOAEL and LOAEL for neurotoxic effects in mice after intermittent subacute exposure to methyl chloride of 500 and 1,000 ppm, respectively. Note that hepatocellular (and minimal renal) toxicity was observed in these same groups of mice at 500 ppm (see Section 4.4.2).

Similar neurotoxic effects have been reported in guinea pigs (Kolkmann and Volk, 1975). The English abstract of this German study indicates that 19 guinea pigs of both sexes were exposed 6 times per week for 10 min to 2% (v/v) methyl chloride in air. Total exposure ranged from 6 to 61 inhalations over the course of 7 to 70 days. Normal behavior was observed for 9 animals after 32 exposures in 37 days, while 6 animals displayed staggering, ataxia of the head, and retarded spontaneous reactions and mobility, with ataxia and paresis of the hind legs first occurring after 17 exposures. These symptoms were reported to have occurred in 4 animals (presumably the remaining ones) only after 25 days. Gross pathological examination revealed

necrosis in the cerebellum (mainly in the lower vermis) that was accompanied by tissue swelling in several regions. Light and electron microscopy observations indicated histopathological changes in the cerebella; these were progressive in nature (first noted after 10 days of exposure), were confined principally to granular layer cells, and included pyknotic nuclei, cytoplasmic edema and homogenization, necrosis, vacuolar degeneration, chromatin fragmentation, organelle disorganization with cellular debris, and cell membrane rupture. These neurotoxic findings in guinea pigs after repeated, acute exposure to a high concentration of methyl chloride substantially agree with the available data for rats and mice.

In the Dow Chemical Company 90-day inhalation study detailed in Section 4.2.2, McKenna et al. (1981a) exposed groups of CD-1 mice (10/sex/concentration), Sprague-Dawley rats (10/sex/concentration), and male Beagle dogs (4/concentration) for 6 h/day, 5 days/wk during a 93-95 day period (a total of 64-66 exposures) to methyl chloride concentrations of 0, 50, 150, or 400 ppm (0, 103, 310 or 824 mg/m^3). Weekly for the first 4 weeks, then bi-weekly thereafter (for a total of 9 intervals), rodents (5/sex/species/concentration) were administered a simple battery of tests to evaluate sensory and motor function. These included tests for body position, respiration, piloerection, exophthalmos/palpebral closure, tremor, corneal reflex, pinna reflex, tail pinch, toe pinch, righting reflex, visual placing, wire maneuver, hind limb clamping, and grasp irritability. To simplify data analysis, the exposure period was divided into three testing periods, each consisting of 3 intervals, with scores averaged for each period and then compared with control values using the Mann-Whitney U-test ($p < 0.05$). Routine clinical observations failed to reveal any signs or symptoms related to methyl chloride exposure in any of the species, including CNS effects in the dogs. Statistically significant differences from control values were noted for pinna reflex (400 ppm male mice, Days 16-39) and hind limb clamping (150 and 400 ppm male rats, Days 16-39; 400 ppm female rats, Days 40-66), but because these were not convincingly concentration- or exposure duration-related in nature, they were considered by the researchers to be sporadic and/or incidental effects unrelated to methyl chloride exposure. The only other statistically significant decrement observed was in the wire maneuver test, which demonstrated some dependence on methyl chloride concentration and exposure duration (50 ppm male mice, Days 40-66; 150 ppm female rats, Days 40-56; 400 ppm female rats, days 16-39 and 40-66). However, this performance decline was not associated with any discernible neuromuscular

incoordination or other deficit, was interpreted as a general muscular weakness characterized by the animals' inability to raise their hind quarters to the wire while gripping it with their forelimbs, and was complicated by a general decline in performance of this test over time in all groups, perhaps as a result of increasing body weight. Hence, the authors felt the toxicological significance of this finding was suspect, and identification of an unequivocal NOAEL/LOAEL for neurotoxicity from this study is questionable, although the LOAEL may have been in the vicinity of 400 ppm or somewhat higher. However, it is noteworthy that the 90-day inhalation study conducted by Battelle (Mitchell et al., 1979a), summarized in Section 4.2.2, did not report neurotoxic effects in F-344 rats or $B6C3F_1$ mice at methyl chloride concentrations of 375, 750, or 1,500 ppm. The subsequent 24-month chronic inhalation study (CIIT, 1981) noted clinical signs of CNS toxicity, with associated neurofunctional impairment and cerebellar lesions, only in mice at the highest concentration of 1,000 ppm (Section 4.2.2) and histopathology indicated axonal swelling and degeneration occurred in areas of the spinal cord. The spinal cord lesions in exposed animals were similar in incidence to controls at terminal necropsy. At earlier time points, incidence over controls was elevated in lower dose groups, but was not dose-related.

4.4.2 Acute and Subacute Inhalation Studies

As described in Sections 3.2 and 3.3, two studies (Dodd et al., 1982; Landry et al., 1983a) reported an effect on tissue levels of NPSH following acute inhalation exposure to methyl chloride. Dodd et al. (1982) exposed male F-344 rats for 6 h to 0, 100, 500, or 1,500 ppm (0, 206, 1,032, or 3,098 mg/m^3) observing time- and concentration-dependent depletions of NPSH relative to controls. After 6 h of exposure, NPSH levels in liver, kidney and lung were reduced in the 500 ppm group to 41, 59, and 55%, respectively, and in the 1,500 ppm group to 17, 27, and 30%, respectively. Levels were not significantly affected in any tissue by exposure to 100 ppm, nor in the blood by any concentration, and by 8 h after exposure NPSH levels had recovered to 80-95% of control values in the liver, kidney, and lungs. When Landry et al. (1983a) exposed male F-344 rats for 6 h to methyl chloride concentrations of 0, 50, 225, 600, or 1,000 ppm, they also observed concentration-related depletions of NPSH in the liver, kidney, testis and epididymis (but not in the blood, brain or lung) at 225-1,000 ppm. After exposure to 1,000 ppm, residual NPSH levels relative to controls were lowest in the liver (13%) and highest in the testis

(70%). The potentially toxic consequences of NPSH (i.e., principally GSH) depletion are not precisely known, but may include enhancement of the toxicity of other chemicals that are normally detoxified by conjugation with GSH, as well as reductions in the capacities of GSH to buffer against excessive lipid peroxidation, free radical generation, and thiol oxidation, to transport amino acids, and to serve as a cofactor in enzymatic reactions (e.g., with formaldehyde dehydrogenase).

Chellman et al. (1986b), using male $B6C3F_1$ mice, examined the role of GSH in mediating the acute toxicity of methyl chloride exposure in brain, liver, and kidney target tissues. Groups of mice (3-5/group) were exposed for 6 h to 2,500 ppm methyl chloride, ± pretreatment (at -1.5 h) with 4 mmol buthionine-S,R-sulfoximine (BSO), a potent and specific inhibitor of γ-glutamylcysteine synthetase. To determine the LC50, groups of 5 mice were exposed to concentrations of methyl chloride which were increased in 500 ppm increments from nonlethal concentrations to a concentration that caused 100% mortality. Relative to control (-BSO) values at 0, 3, and 6 h of methyl chloride treatment, GSH (measured as NPSH) in +BSO mice was substantially depleted in kidney (to 25, 28, and 32%) and liver (to 19, 35, and 65%), and was steadily declining in the more BSO-resistant brain tissue (to approximately 90, 70, and 58%). This BSO pretreatment reduced the methyl chloride exposure's 18-h lethality from 93% (14/15) to 0 (0/10), and increased the approximate LC_{50} from 2,200 to 3,200 ppm. Male mice were also exposed to 1,500 ppm methyl chloride 6 h/day, 5 days/wk for 2 weeks, ± daily pretreatment with 2 mmol BSO (dose reduced to eliminate toxic side-effects seen during the second week with 4 mmol; NPSH depletion was similar to higher dose, but somewhat less in magnitude at 0, 3, and 6 h for kidney [approximately 40, 30, and 35%] and liver [approximately 72, 61, and 83%]). It was observed that BSO protected against both methyl chloride-induced lethality (28% [10/36] and 11% [5/45] without BSO in two trials, versus 0 deaths in BSO pretreated animals) and the induction of characteristic histopathological lesions in the brain (multiple degenerative/necrotic foci in the granular cell layer).

Hepatic toxicity in male mice exposed for 6 h to 1,500 ppm methyl chloride was reflected in hepatocellular necrosis and cytoplasmic vacuolation, as well as nearly a 50-fold increase in serum ALT activity (from 46 IU/liter in controls to 2,147 IU/liter in 1,500 ppm animals).

Pretreatment of the animals with 8 mmol BSO, 0.25 mL/kg DEM or fasting for 18 h was found both to substantially deplete hepatic NPSH (to 26, 40, or 50% of controls, respectively) and to virtually eliminate hepatotoxicity as measured by serum ALT levels (43, 42, and 100 IU/liter, respectively). With respect to kidney toxicity in animals treated 6 h/day, 5 days/wk for 2 weeks to 1,500 ppm methyl chloride, a statistically significant drop in BUN level (from 39.4 to 28.0%) and a slight increase in number of basophilic cortical tubules were observed relative to controls, but no significant changes were seen in absolute or relative kidney weights, glomerular filtration rate (creatinine clearance), urinary excretion rates of glucose or protein, or urine osmolality. However, incorporation of tritiated thymidine into kidney DNA was elevated 3-fold in male mice and 8.5-fold in female mice by the methyl chloride exposure, presumably reflecting compensatory cell regeneration. In males, pretreatment with 2 mmol BSO completely eliminated this increase, while having no effect on label incorporation when administered alone (the effect of BSO pretreatment in females was not determined). This study demonstrates that methyl chloride's lethality and target organ toxicity can largely be prevented by conditions that lower tissue NPSH levels, again implicating GSH as a pivotal factor in methyl chloride's mode of toxic action.

Studies previously discussed in Sections 4.3 (Chellman et al., 1986a, 1987) described the mitigating effects of the anti-inflammatory agent BW755C on epididymal inflammation and dominant lethality (postimplantation loss) in F-344 rats. In another report from this laboratory (Chellman et al., 1986c), the inhibitory effects of BW755C on methyl chloride toxicity in male F-344 rats were also explored. In animals exposed to 7,500 ppm methyl chloride 6 h/day for 2 days, BW755C (10 mg/kg ip, 1 h pre- and postexposure) prevented both lethality (0/6 treated versus 8/12 control animals) and epididymal granuloma formation (in 0/6 treated versus 4/4 control animals). Rats exposed 6 h/day for 5 days to 5,000 ppm methyl chloride without BW755C exhibited hepatocellular cloudy swelling, renal tubular degeneration, adrenal cortex vacuolar degeneration, cerebellar necrosis of the granule cell layer, and degenerative lesions in the testis and epididymis (including epididymal sperm granulomas). With the exception of adrenal gland vacuolation, BW755C co-treatment prevented the detectable occurrence of all these methyl chloride-induced lesions. Subsequent experimentation (Chellman et al., 1987), utilizing a larger number of animals examined at more and later time points, provided evidence

that testicular toxicity was not, in fact, inhibited by BW755C. It was further demonstrated that BW755C treatment did not significantly alter the amounts of $^{14}CO_2$ expired or ^{14}C in urine after exposure to radiolabeled methyl chloride, nor the distribution of radioactivity 4 h post-exposure among liver, kidney, testis, epididymis, and brain tissues, nor hepatic levels of GSH (measured as NPSH). It was thus concluded that BW755C's inhibition of methyl chloride toxicity in the male F-344 rat was likely a consequence of its pharmacological anti-inflammatory properties as an inhibitor of prostaglandin and leukotriene synthesis (arachidonic acid derivatives known to mediate inflammatory reactions). In tissues (e.g., brain) where toxicity is not accompanied by an apparent inflammatory response, the authors speculated that other leukotriene/prostaglandin effects could be involved, such as increased capillary permeability and edema, or those implicated in the pathophysiological mediation of tissue injury.

In a subacute inhalation study reported by Dow Chemical Company (Burek et al., 1981), Sprague Dawley rats (40/sex/concentration) were exposed for 48 h to 0, 200, 500, 1,000, or 2,000 ppm (0, 413, 1,032, 2,065, or 4,130 mg/m^3) of methyl chloride (for logistical reasons, exposure was divided into two separate periods: Part I included 0, 200, and 500 ppm exposures; Part II included 0, 1,000, and 2,000 ppm exposures). Exposures were temporarily halted in order to remove from the chamber 20 rats/sex/concentration, 10 of which were sacrificed shortly thereafter for blood, urine, and pathology evaluation, and 10 of which were held for a recovery period of up to 12 days, followed by sacrifice for blood, urine, and pathology evaluation. Exposures were restarted, exposing the remaining 20 rats/sex/ concentration for an additional 24 h (for a total exposure of 72 h). Rats were periodically observed both during and after exposure for signs of overt toxicity. Hematologic parameters examined included PCV, RBC, WBC, and Hgb. Levels of BUN, total bilirubin, serum AP, ALT, and AST were also determined. Urine samples were analyzed for glucose, protein, ketones, bilirubin, occult blood, and urobilinogen. Gross necropsies were conducted on all animals, with fasting body weights and organ weights (liver, kidneys, brain, heart, and testes) obtained from all those in recovery groups; organ-body weight ratios were subsequently calculated. All rats were given ophthalmologic and gross pathologic exams, the latter on representative sections of all major organs and tissues. Histopathological examination of significant gross lesions, liver, kidney, lung and brain was conducted on all rats, as well as on testes and epididymides of male rats.

No overt signs of toxicity were noted in any rats exposed to 0, 200, or 500 ppm of methyl chloride. From 24 through 40 h of exposure, animals exposed to 1,000 or 2,000 ppm appeared progressively less alert, and by 48 h the 1,000 ppm rats appeared lethargic, while the 2,000 ppm rats were either lethargic, moribund, or dead (mortality was 14/20 males, 10/20 females). After 72 h of exposure, the 1,000 ppm rats were either sick or moribund while all those in the 2,000 ppm group were dead. After 48 h of exposure, mean body weight changes of 200 ppm rats were not significantly different from controls; mean body weights were significantly decreased for rats exposed to 500 ppm (5-6%), 1,000 ppm (10-14%), or 2,000 ppm (17-20% based upon the 3 survivors/sex). After 72 h of exposure, body weight gain of 200 ppm males was only 1% compared with 6% for controls, whereas 500 and 1,000 ppm rats of both sexes experienced 6-8% and 28-30% body weight declines, respectively. During the 11-12 day post-exposure periods, the rate and degree of body weight recovery were generally inversely related to methyl chloride concentration. After 11-12 days of recovery, mean fasted body weights were significantly reduced in the 72 h-500 ppm, 48 h-1,000 ppm, and 72 h-1,000 ppm male groups, but not in any female groups. Most statistically significant alterations of absolute and relative organs weights were judged secondary to decreased body weight. However, exposure-related decreases of approximately 50% in the absolute and relative testicular weights were observed in males of the 72 h-1,000 ppm group (although not of males in the 48 h-1,000 ppm, or in any of the 500 or 200 ppm, groups), an effect considered secondary to concomitant epididymal lesions. Statistically significant, exposure-related decreases in mean absolute and/or relative liver weights were observed after 11-12 days of recovery for males of all surviving methyl chloride-exposed groups (200, 500, or 1,000 ppm for 48 or 72 h); only the mean relative liver weight of the 72 h-1,000 ppm group was decreased among females.

Most alterations in hematological parameters appeared to be within the range of normal variability and were judged toxicologically insignificant. Increased hematocrit, RBC, and Hgb values observed in the 48 h-1,000 and-2,000 ppm and the 72-1,000 ppm groups were considered the likely result of dehydration and hemoconcentration in lethargic or moribund animals. Treatment-related alterations in all clinical chemistry parameters were observed in the 2,000 ppm rats that were sacrificed at 48 h: increased BUN values reflected kidney toxicity and renal failure; increased ALT, AST, and total bilirubin values indicated liver toxicity; and decreased AP

values were considered likely to be secondary to the rats not eating. Similar but less severe effects were noted in the 72 h-1,000 ppm group, all of which returned to normal by the end of the recovery period. Decreases in serum AP levels were also observed in all 500 and 200 ppm groups (48 or 72 h exposures), were statistically significant for 48 and 72 h-500 ppm males and for 72 h-200 ppm females, and were considered treatment-related, probably the result of decreased food intake. Altered urinalysis values in the 48 and 72 h-1,000 or -2,000 ppm groups appeared exposure-related, and were consistent with the kidney lesions and renal failure observed in these animals.

No exposure-related gross or histopathological alterations were observed in any rats exposed to 200 ppm methyl chloride for 48 h. The primary cause of death in rats exposed to 1,000 or 2,000 ppm for 48 or 72 h was kidney toxicity and subsequent renal failure. Kidneys were frequently dark and displayed varying degrees of renal tubular necrosis, degeneration, cytoplasmic heterogeneity, regeneration, and epithelial cell lipid accumulation. Evidence of renal toxicity in other exposure groups was not reported Liver toxicity, although apparently less severe, was noted in all exposure groups. This ranged from dark livers, necrosis, inflammation, and degenerative changes (increased lipid, variable-sized nuclei, altered tinctorial properties) seen to varying extents in 1,000 and 2,000 ppm rats, to altered tinctorial appearance of hepatocytes and slightly elevated lipid content in 72 h-200 ppm males and females, respectively; these minimal effects appeared to be reversible. Toxic effects in the epididymides (degeneration, inflammation, interstitial edema, decreased sperm and/or the presence of proteinaceous debris in tubular lumina, sperm granulomas, scarring, and obstructive changes) were observed in male rats exposed for 48 or 72 h to 500, 1,000 or 2,000 ppm. These effects tended to be more severe at higher doses, and many were still present in 500 and 1,000 ppm males after 12 days of recovery. Testicular atrophy and degenerative changes were also observed in some males, probably as a result of the obstructive epididymal lesions. In summary, this study suggests a subacute LOAEL of 200 ppm methyl chloride, based upon minimal and reversible liver effects seen in rats after 48 and 72 h of continuous exposure, followed by 11-12 days of recovery.

In another Dow Chemical Company study (McKenna et al., 1981b), three groups of 3 male Beagle dogs (ages 7-8 mo) and 3 male cats (ages 8-9 mo) were exposed for approximately

23.5 h/day for 3 days (i.e., 72 h treatment regimen) to methyl chloride concentrations of 0, 200, or 500 ppm (0, 413 or 1,031 mg/m^3). For approximately 0.5 h/day, exposure chambers were exhausted to <50 ppm, and cages were removed for cleaning and replenishment of food and water after the animals had been taken out for observation of any signs of toxicity. Animals were observed during an acclimation period of >2 mo (19 days for one replacement dog), and were handled frequently so that staff could become familiar with them and their personalities. Each work day, animals were observed during and after exposure for signs of toxicity, and on post-exposure Days 4, 12, 19, and 26, video tapes were made of one 0 ppm dog, one 200 ppm dog, and all three 500 ppm dogs. Eyes were examined 1 mo (15 days for the replacement dog) prior to and 4 days after exposure, and body weights were recorded from 3-10 weeks prior to exposure to 2-4 weeks after exposure. Monitored hematology parameters included RBC, WBC, differential WBC, PCV, and Hgb; clinical chemistry included BUN, ALT, AST, AP, bilirubin, and glucose; and urinalysis included pH, glucose, ketones, protein, bilirubin, occult blood, urobilinogen, specific gravity, color and appearance, and microscopic examination for RBC, WBC, casts, crystals, bacteria, mucus, epithelial cells, etc. Neurological examinations were performed on all dogs on post-exposure Day 4, and again on post-exposure Day 26 on the 500 ppm dogs. These consisted of observing each dog's gait, posture, demeanor, and general appearance, and evaluating cranial nerves, spinal reflexes, pain sensation, and attitudinal and postural reactions. Gross necropsy and pathology examinations were conducted on all dogs and cats, as was microscopic histopathology on most major organs and tissues of each. Limited statistical analysis was performed with the level of significance set at $p < 0.05$ (two-sided).

During the first 24 h of treatment, no differences in demeanor or condition were observed between control and methyl chloride-exposed dogs. After 48 h of treatment, 500 ppm dogs appeared more tranquil with one exhibiting intermittent tremor and slight excess salivation, but all were judged alert and responsive. Immediately after 72 h of treatment, control and 200 ppm dogs were comparable, except that one dog displayed possible hind limb stiffness that disappeared by the next day, and likely resulted from confinement. All 500 ppm dogs appeared weak, but alert and responsive, and displayed a range of adverse effects that varied in severity from animal to animal. These included hind and fore limb stiffness and incoordination, occasional slipping and falling, inability to sit up or walk, limb tremor, and excessive salivation.

No visible changes of condition were observed in the 500 ppm dogs at 24 h post-exposure, except that the dog unable to sit up displayed decreased appetite and difficulty in eating; however, this dog readily consumed soft, moist canned dog food when offered and gradually returned to the normal, solid diet by post-exposure Day 14. Improvement was noted in all 500 ppm dogs by post-exposure Day 10 that continued until termination on Day 27. The most severely affected dog was able to get up and take several steps by post-exposure Day 11, and by the study's end was able to frequently walk about and appeared alert and in good spirits, despite continued limb tremor and intermittent ataxia. During the first 48 h of exposure, the 200 and 500 ppm cats evidenced a decline in appetite that then recovered, and after 24 h they appeared less active than controls, but always were alert and displayed no signs of inactivity or sluggishness upon removal from the exposure chamber. Throughout the two week recovery period, 200 and 500 ppm cats were comparable to controls.

Ophthalmoscopic examination revealed no treatment-related eye lesions or abnormalities in any of the dogs or cats. Body weight loss was slight and temporary in the 200 ppm dogs (attributed by the study authors to prolonged residence time in the exposure chambers). Dogs in the 500 ppm group exhibited 5-8% body weight losses that persisted for several days, then slowly recovered to pretreatment values by post-exposure days 19-25. In cats, the 500 ppm group experienced a transient loss in body weight that was statistically non-significant, but may have been treatment-related. No statistically significant alterations in mean organ weights were observed in either dogs or cats, with the exception of a decrease in relative mean heart weight in 200 ppm cats. Because of animal variability, small sample size and lack of dose-response, this finding was not judged to be toxicologically significant. Only a few hematological findings achieved statistical significance; altered mean PCVs in 500 ppm dogs and cats were not viewed as exposure-related because of lack of dose-response, consistency across post-exposure days and/or other data abnormalities. Alterations in differential WBCs (increased segmented neutrophils, decreased lymphocytes) were observed in 500 ppm dogs immediately after exposure, but were not seen at the next sampling time (6 days post-exposure). Also, just prior to necropsy, a possible decline in WBC ($p < 0.10$) was observed for 500 ppm dogs. No statistically significant differences in clinical chemistry values were found in cats, and those that were in dogs (e.g., ALT, AST) were inconsistent, difficult to interpret, and were not judged treatment-

related or toxicologically important. Similarly, no significant alterations in urinalysis parameters were observed in either dogs or cats.

Neurological evaluations revealed no abnormalities in control or 200 ppm dogs, whereas each of the three 500 ppm dogs exhibited various clinical deficiencies. The most severely affected dog was alert and good-natured during the first examination (4 days post-exposure), but was unable to walk, lay in lateral recumbency, and exhibited posterior paresis, extensor tonus of all four limbs, and when excited or attempting to move, opisthotonus and intention tremors. By 26 days post-exposure, spinal reflexes and postural reactions were normal, balance was maintained normally, and walking with intermittent ataxia was observed. Thus, most neurological abnormalities had partially or fully resolved The other two dogs were similarly but less severely affected on post-exposure Day 4, and appeared to be fully or nearly completely recovered on post-exposure Day 26.

No treatment-related alterations in gross or histopathology were observed in any 200 ppm dog. All three 500 ppm dogs displayed lesions in the brain and spinal cord (vacuolization, swollen eosinophilic axons, axon loss, demyelinization, and microglial cells that contained phagocytosed debris), which were characterized as generally very slight-to-slight and multifocal in nature. They were localized to the brain stem and the lateral and ventral funiculi of the spinal column, and were not observed in the cerebrum, cerebellum, or peripheral nerves. Brain and/or spinal cord lesions were also found in control (1/3), 200 ppm (1/3), and 500 (3/3) ppm cats. Several characteristics of these lesions (perivascular aggregates of mononuclear cells, location in the cerebrum and cerebellum as well as the midbrain, presence in 1 of 3 cats in the control and 200 ppm groups) led the authors to speculate that they were likely the result of either a post-vaccinal reaction or a viral infection, or both; however, it was recognized that exposure to 500 ppm methyl chloride could possibly have exacerbated such a disease process. The findings of this study indicate a NOAEL of 200 ppm for a continuous (nearly) 72 h exposure to methyl chloride, and a LOAEL of 500 ppm based principally upon a spectrum of clinically and histopathologically observable neurological effects seen in male Beagle dogs.

Landry et al. (1985; this Dow Chemical Company study was also submitted in somewhat greater detail to the U.S. EPA, cited herein as Landry et al., 1983c) exposed female C57BL/6 mice (12/group) continuously (20-20.5 h/day) to 0, 15, 50, 100, 150, 200, or 400 ppm (0, 31, 103, 206, 309, 412, or 824 mg/m^3), or intermittently (5.5 h/day) to 0, 150, 400, 800, 1,600, or 2,400 ppm (0, 309, 824, 1,648, 3,296, or 4,956 mg/m^3), of methyl chloride for 11 days. Continuous exposure was interrupted once in the morning and once in the afternoon in order to move intermittently exposed mice in and out of the exposure chambers, observe all animals, train, or test animals, etc. All animals were observed twice daily after venting exposure chambers, and body weights were recorded prior to initiating the exposure regimen and after 4 and 8 days of exposure. Neurofunctional testing was conducted during the course of the study; methods and results are described later in Section 4.4.1. Upon termination, the non-fasted mice were subjected to gross and histopathological examination. Body and organ weights were obtained, as were samples of most major organs and tissues, including tissue samples for examination by electron microscopy from the cerebella of three pre-selected mice from each of the 0 and 150 ppm continuously-exposed groups after 1, 2, 4, 6, 8, or 10.5 days of exposure.

No exposure-related effects were observed in mice exposed continuously or intermittently to the lower concentrations of methyl chloride (15-C and 50-C ppm, or 150-I ppm, respectively), whereas exposure to 400-C or 200-C ppm was lethal after 4 or 5 days, respectively. Mice exposed to 150-C to 400-C ppm developed poor motor coordination and deteriorated to a moribund condition with accompanying inanition at a rate that was dose-dependent. After 3 days, 200-C mice were severely affected, displaying loss of appetite and ataxia with frequent falling, but were not yet moribund and retained good muscle tone and responsiveness to stimuli such as touch. In intermittently exposed mice, a transient (i.e., at 0.5 h, but not 3 h, post-exposure) sedation was observed in 1,600-I and 2,400-I ppm groups at 4-7 days of exposure, but not after 8 days. Slow movement and roughened haircoats were apparent in 2,400-I ppm mice after 5 days of exposure, and some hind-limb extensor rigidity by 8 days; these animals deteriorated and were sacrificed moribund on Days 8-9. Mice exposed to 1,600-I ppm displayed less severe effects, including slightly rigid hind limbs and some tendency to rear on hind legs (2/12) and be more excitable than controls; these effects tended to mitigate during overnight periods of nonexposure. Mean body weights were significantly diminished in the 2,400-I, 200-C,

and 150-C ppm groups, but were not affected at 1,600-I or 50-C ppm. Body weights were not obtained for 400-C ppm mice, while those of the 100-C ppm group appeared diminished relative to controls, although not to a statistically significant degree. No organ weight data were reported for the 200-C and 400-C ppm groups.

Mean relative (but not absolute) kidney weights were increased in the 150-C and 2,400-I ppm groups by 9 and 19% relative to controls, respectively, but not in the 50-C, 15-C, or 150-I ppm groups (kidney weight data were not obtained for the 100-C, 1600-I, 800-I, or 400-I ppm groups). Furthermore, a statistically significant increase in mean relative kidney weight was not seen in 150-C mice sacrificed after only 1, 2, 4, 6, or 8 days of exposure (but small increases were reported at 2, 4, and 6 days). Statistically significant increases in mean absolute and relative liver weights were reported at the end of exposure for the 1,600-I ppm group, whereas statistically significant decreases in one or both of these values were reported for the 150-C ppm group after 2, 4, 6, and 10.5 (but not 1 or 8) days of exposure. No other liver weight changes were statistically significant. Decreases in mean absolute and relative thymus weights were statistically significant and considered exposure-related (reflecting decreased body weights and stress) for the 2,400-I, 1,600-I, and 150-C ppm groups; similarly significant decreases in the 50-C and 15-C ppm groups were judged spurious by the study authors because corroborating histopathology data were not seen, nor was a decrease at the next higher dose, 100-C ppm.

Neurological gross and histopathological effects were present in many of the exposure groups, and are detailed in Section 4.4.1. Other gross pathology observations included significant inanition (i.e., marked weakness) in 400-C and 200-C ppm mice prior to death or sacrifice, and in some 150-C ppm mice after 4 or more days of exposure. No changes considered exposure-related were grossly recognizable in 15-C, 50-C, or 100-C ppm mice. Inanition was also apparent in the 2,400-I ppm group, as was thin, watery blood from the heart, a finding supported by low blood PCV values. The spleens of this group were considerably enlarged, suggestive of extramedullary hematopoiesis. The in-life observation of red urine in this group was determined to result from hemoglobinuria consistent with intravascular hemolysis (hemoglobinemia), rather than from hematuria. The 1,600-I ppm group had decreased ingesta (suggesting diminished food consumption) and a decrease in size of the thymus. No gross

exposure-related effects were noted in the 400-I or 800-I ppm groups. Microscopically, evidence of kidney toxicity was found only in the 2,400-I ppm group, and consisted of slight multifocal tubular degeneration and regeneration, and eosinophilic-staining tubular casts. Liver toxicity in 100-C, 150-C, 200-C, and 400-C ppm mice was reflected microscopically as decreased hepatocyte size resulting from reduced glycogen content, as well as focal periportal hepatocellular degeneration and/or necrosis in the 400-C ppm group. Two of the six mice examined histopathologically in the 100-C ppm group had decreased hepatocyte size, one lesion of which was characterized as severe. No exposure-related effects were observed in the 15-C and 50-C ppm groups. Similar hepatic effects, but without degeneration or necrosis, were variably seen in mice from the 400-I through 2,400-I pm groups. Extramedullary hematopoiesis was microscopically confirmed in the enlarged spleens of the 2,400-I ppm mice. Based upon the liver effects described here and the neurological effects described in Section 4.4.1, this study defines methyl chloride NOAELs and LOAELs of 50 and 100 ppm for 11 days of continuous (22 h/day) exposure, or 150 and 400 ppm for 11 days of intermittent (5.5 h/day) exposure.

The histopathology of subacute methyl chloride exposure in one strain of rat (F-344) and three strains of mice (C3H, C57BL/6, B6C3F_1) was investigated by Morgan et al. (1982). The histopathology findings of this study are summarized in Table 4-1. Groups of rats (10/sex/concentration) were exposed 6 h/day to 0, 2,000, 3,500, or 5,000 ppm for 5 days, then for another 4 days after a 2-day break (i.e., on Days 1-5 and 8-11). Groups of mice (5/sex/strain/concentration) were exposed 6 h/day to 0, 500, 1,000, or 2,000 ppm for 12 consecutive days. At 2,000 ppm, all male B6C3F_1 mice were moribund or died by Day 2; one male C57BL/6 died on Day 2, and all remaining mice were moribund by Day 5. One 1,000 ppm C3H male died on Day 11, while all remaining 0-1,000 ppm mice survived the entire 12 day exposure. Moderate to severe ataxia was exhibited by some mice prior to death. Hematuria developed in all 2,000 ppm females by Day 4, in all 1,000 ppm females by Day 8, and in 2,000 ppm C3H males on Days 4-5. In the 5,000 ppm rat group, all males and 3 females developed foul-smelling diarrhea by Day 3, several exhibited perineal urine staining, forelimb ataxia was observed in most females and about half the males on Day 3, hindlimb paralysis developed in 2 males and 1 female on Day 5, and 1 female suffered convulsions during the Day 11 exposure. In the 3,500 ppm rat group, 1 male developed diarrhea and 3 females

Table 4-1[a]. Histopathology of mice and rats exposed to methyl chloride for 9-12 days: Severity[b] (Incidence)[c]

		C3H Mouse		C57BL/6 Mouse		$B6C3F_1$ Mouse		F-344 Rat	
Lesion	ppm	M	F	M	F	M	F	M	F
Cerebellar degeneration	5000							0.3	0.22222222
	3500	- (0/5)	- (0/5)	- (0/5)	+++ (4/4)	- (0/5)	0.4		
	2000	- (0/4)	- (0/5)	+ (3/5)	++ (5/5)	- (0/5)			
	1000	- (0/5)	- (0/5)	- (0/5)	- (0/5)	- (0/5)			
	500								
Degeneration and necrosis of renal proximal convoluted tubules	5000							+++ (10/10)	++ (10/10)
	3500							+ (10/10)	+ (5/10)
	2000	+++ (5/5)	+++ (5/5)	++ (3/5)	++ (5/5)	0.2	+++ (5/5)	+ (8/10)	- (0/10)
	1000	+ (1/5)	- (0/5)	- (0/5)	- (0/5)		- (0/5)		
	500	- 0/5)	- 0/5)	- (0/5)	- (0/5)		- 0/5)		
Basophilic renal tubules	5000							- (0/10)	- (0/10)
	3500							- (0/10)	- (0/10)
	2000	- (0/5)	- (0/5)	- (0/5)	- (0/5)	- (0/5)	- (0/5)	- (0/10)	- (0/10)
	1000	+ (2/5)	++ (5/5)	+ (2/5)	- (0/5)	++ (3/5)	+ (2/5)		
	500	- (0/5)	- (0/5)	- (0/5)	- (0/5)	+ (1/5)	- (0/5)		
Hepatocellular degeneration	5000							1.9	+ (9/10)
	3500								+ (9/10)
	2000	1.2	- (0/5)	+++ (5/5)	- (0/5)	++ (5/5)	0.8		+ (8/10)
	1000		- (0/5)	+ (3/5)	+ (3/5)	- (0/5)			
	500		- (0/5)	+ (3/5)	+ (2/5)	- (0/5)			
Testicular degeneration	5000							+++ (10/10)	
	3500							+ (10/10)	
	2000							+ (10/10)	
Adrenal fatty degeneration	5000							+++ (10/10)	+++ (10/10)
	3500							+ (4/10)	++ (10/10)
	2000							- (0/10)	- (0/10)

[a] Modified from Morgan et al. (1982)
[b] Severity grades (overall for the group): (-) = not observed, (+) = minimal, (++) = moderate, (+++) = severe
[c] Incidence = (number affected/number examined)

displayed perineal staining. Following the Day 5 exposure, 13 rats were sacrificed in extremis (5,000 ppm: 6 males and 5 females; 3,500 ppm: 2 females). It was noted that the rats seemed to tolerate the methyl chloride exposure much better during the second week (Days 8-11).

In mice, focal degeneration of the cerebellar internal granular layer was observed primarily in C57BL/6 females at 1,000 and 2,000 ppm. Kidney toxicity was manifested as degeneration and necrosis of the proximal convoluted tubules in all mouse groups at 2,000 ppm (and in one C3H male at 1,000 ppm), and as renal tubular basophilia in all groups (except C57BL/6 females) at 1,000 ppm. Moderate to severe hepatotoxicity was confined to C57BL/6 and $B6C3F_1$ males at 2,000 ppm, and included hepatocellular vacuolation, degeneration, and necrosis, as well as accumulation of proteinaceous material in the small and large bile ducts. Many mice in the remaining groups were reported to have displayed glycogen depletion, cytoplasmic vacuolation, and hepatocytic hydropic degeneration and loss of basophilia. In general, rats responded similarly, but appeared more resistant to methyl chloride's toxicity. The identical brain lesion found in mice was also observed in rats, but only at the highest concentration (5,000 ppm). Similar and clearly dose-dependent degeneration of renal proximal convoluted tubules was observed, but not tubular basophilia. Liver toxicity was limited to minimal hepatocellular degeneration without necrosis and some loss of normal cytoplasmic basophilia; this response was evident in all rat groups except the low-dose (2,000 ppm) males. Rat adrenals, testes and epididymides were also examined for signs of exposure-related toxicity. The adrenals were observed to have a dose-dependent accumulation of clear cytoplasmic droplets that were assumed to represent lipid deposits. Clearly dose-dependent testicular degeneration was also found, which included reduced numbers of late-stage spermatids, separation and luminal sloughing of spermatocytes and early-stage spermatids, irregular and apparently membrane-bound vacuoles in the germinal epithelium, and variable formation of giant multi-nucleate cells. Accompanying these testicular lesions were epididymal tubules that contained reduced numbers of sperm, sloughed spermatocytes, giant cells and cellular debris, as well as some epithelial areas having eosinophilic hyaline droplets and degenerating cells of unknown type. For intermittent, subacute exposure of 9-12 days duration, this study defines a LOAEL in mice of 500 ppm methyl chloride based principally upon hepatotoxic effects, and a LOAEL in rats of 2,000 ppm based upon renal, hepato- and testicular-related toxicity. NOAELs were not established in this study, although it

serves to highlight species, strain, and sex differences in susceptibility to methyl chloride toxicity that complicate extrapolation of animal results to humans.

4.4.3 Genotoxicity Studies

A number of studies have explored methyl chloride's potential for genotoxicity, including those briefly summarized in this section. In the *Salmonella typhimurium*/microsome reverse mutation assay (the Ames Test), methyl chloride was tested at concentrations of 1, 4, and 7% in tester strains TA1535 and TA100, which detect base-pair substitution mutations, and in TA1537 and TA98, which detect frame-shift mutations (du Pont, 1977). Both in the absence and presence of exogenous metabolic activation (rat liver S9 mix), methyl chloride demonstrated dose-dependent mutagenicity in both base-pair substitution strains, being clearly mutagenic in TA1535 at 4 and 7% (41-49-fold increase in revertants, 7% +S9), and in TA100 at all three concentrations (16-fold increase in revertants, 7% +S9). No mutagenicity was observed in TA1537 or TA98. Longstaff et al. (1984) reported finding methyl chloride mutagenic in strains TA1535 (maximum response at 5%, a 6.2-fold revertant increase) and TA100 (maximum response at 10%, a 7.3-fold revertant increase), but not in strains TA1538 or TA98. Similarly, 5% methyl chloride (apparently -S9) was reported mutagenic in TA100 (Simmon, 1981), and dose-dependent increases in revertants (maximum 55 [-S9] and 137 [+S9] fold increases) were observed in TA1535 over a concentration range of 0.5-0.8 to 20.7% (Andrews et al., 1976). Monitoring forward mutation to 8-azaguanine resistance in *Salmonella* strain TM677, Fostel et al. (1985) reported a concentration-dependent, 7-10-fold increase in number of mutant colonies over a methyl chloride concentration range in air of 5-30%.

Using the established human lymphoblast cell line, TK6, these authors also demonstrated that a 3-h exposure to 0.3-5% methyl chloride induced concentration-dependent increases in the number of cells resistant to trifluorothymidine (i.e., presumptive mutations at the thymidine kinase locus) and in the frequencies of sister chromatid exchanges (SCE); approximate increases were 14- and 3-fold, respectively (Fostel et al., 1985). However, even at concentrations that induced mutation and SCE, no DNA damage was detectable by the alkaline elution assay (i.e., no significant increase in DNA single-strand, alkaline-labile sites was observed).

Alkaline elution was also used to analyze kidney cell DNA from male $B6C3F_1$ mice that had been exposed 6 h/day for 4 consecutive days to 1,000 ppm of methyl chloride (Jäger et al., 1988); evidence for DNA-DNA (DDC) or DNA-protein crosslinks (DPC) was not observed, but there was some indication of DNA single-strand breaks (SSB). The same technique was applied to liver and kidney cell DNA from $B6C3F_1$ mice (6/sex) exposed for a single 8-h period to 1,000 ppm of methyl chloride (Ristau et al., 1989). To minimize repair of DNA lesions, these animals were sacrificed immediately after exposure, as opposed to 5-6 h post-exposure in the Jäger et al. (1988) study. Under these conditions, alkaline elution profiles revealed no evidence of DNA damage to liver tissue from either sex, or to kidney tissue from females. However, while DDC and SSB were similarly lacking in kidney tissue from males, there was evidence of methyl chloride-induced DPC. These authors conducted a subsequent time-course study of renal DNA lesions in male $B6C3F_1$ mice exposed for 8 h to 1,000 ppm of methyl chloride and sacrificed either at 0, 5 or 48 h post-exposure, or exposed 6 h/day for 4 days and sacrificed 0 or 5 h post-exposure (Ristau et al., 1990). In the single exposure groups, evidence for DPC, but not SSB, was again observed immediately after exposure; at 5 h post-exposure, DPC were no longer apparent, but there was evidence of SSB; by 48 h, no significant indications of either DPC or SSB were found. In the 4-day exposure groups, only a slight indication of DPC at 0 h post-exposure and low levels of SSB 5 h later were reported. Thus, methyl chloride appeared to induce DPC that were rapidly repaired, as well as retard the resealing of SSB that were presumed to occur during DPC excision repair, or perhaps though free-radical mechanisms as a result of methyl chloride-induced GSH depletion and associated lipid peroxidation. Repeated exposure may have enhanced the ability of renal tissue in male mice to repair DPC, but SSB resealing was apparently still retarded. The authors speculated that these effects may result from endogenous formaldehyde formation during methyl chloride exposure, and that they could contribute to the renal tumorigenicity of methyl chloride that has been observed only in male mice.

Although exposure of rodents to ^{14}C-labelled methyl chloride results in significant levels of DNA-associated radioactivity, such DNA isolated from the kidneys of male and female $B6C3F_1$ mice (Peter et al., 1985) and from the liver, kidney, lung and testes of male F-344 rats (Kornbrust et al., 1982) indicated that the radioactivity was associated only with normal purine bases.
In conjunction with the macromolecular synthesis inhibitor studies described under the

toxicokinetic sections (e.g., Kornbrust et al., 1982), these results demonstrated that methyl chloride does not directly methylate DNA to any significant degree, but rather becomes incorporated into cellular macromolecules through normal one-carbon pool anabolic pathways.

Studies investigating methyl chloride's ability to induce unscheduled DNA synthesis (UDS) have reported somewhat mixed results. In a review of their own work and that of others, Furihata and Matsushima (1987) reported methyl chloride negative for in vivo induction of UDS in rat liver (concentration not specified), whereas Working et al. (1986) found it negative after 1-5 days of 6 h/day exposure to 3,500 ppm, but weakly positive after a single 3-h exposure to 15,000 ppm. In the in vitro rat hepatocyte UDS assay, methyl chloride was positive in two studies: at 3 and 5% (Working et al., 1986; negative at 1%), and at 1% (Butterworth et al., 1989; negative at 0.1 and 0.3%, toxic at 3%). In vitro exposure of pachytene spermatocytes induced UDS at 3, 5 and 10%, but not 1% (Working et al., 1986; Working and Butterworth, 1984); on the other hand, in vivo exposure 6 h/day to 3,500 ppm for 1, 3, 5 or 9 days, or for a single 3 h period to 15,000 ppm, failed to induce UDS in these cells. Tracheal epithelial cells failed to demonstrate UDS under either the in vitro or in vivo exposure regimens, although there was a suggestive positive in vitro response at 3%; 5 and 10% were toxic to the cells (Working et al., 1986). Finally, using primary cultures of human hepatocytes from three individuals, methyl chloride was negative at 0.1-0.3%, weakly positive in one and negative in another at 1%, and toxic at 2-10% (Butterworth et al., 1989). Thus, in rodents and humans, methyl chloride may be capable of causing DNA damage that induces UDS, but only at high concentrations (especially *in vivo*) and perhaps only in liver.

Other genotoxicity-related endpoints reported include the induction of sex-linked recessive lethals in *Drosophila melanogaster*, where 20% methyl chloride was found to be a potent mutagen in all post-mitotic germ cell stages (University of Wisconsin, 1982; Bioassay Systems Corporation, 1984); the in vitro induction of BHK21 cell transformation (Longstaff et al., 1984; methyl chloride concentration not specified) and the enhancement of viral transformation in cultured Syrian hamster embryo cells by a 20-h exposure to 6,000-25,000 ppm (but not 3,000 ppm) of methyl chloride (Hatch et al., 1983); and dominant lethal assays conducted in Sprague-Dawley rats (SRI, 1984) or F-344 rats (Chellman et al., 1986a; Working et al., 1985a).

In the Sprague-Dawley rat study, dominant lethal effects were seen at 2,000 and 3,000 ppm (1,000 ppm was comparable to controls), but were generally not seen by 8 weeks after the termination of exposure, indicating that spermatogonial stem cells were probably not damaged. The dominant lethal assays performed in F-344 rats, along with associated studies on reproductive toxicity and mechanisms (detailed in Section 4.3), indicated: (1) methyl chloride at 3,000 ppm (1,000 ppm was again comparable to controls) induced a modest increase in postimplantation fetal loss during Week 1 post-exposure, and a significant increase in preimplantation loss throughout 8 weeks of post-exposure mating; (2) the pattern of pre- and postimplantation loss was not typical of that seen for agents known to act by direct genotoxic mechanisms; (3) elevated preimplantation loss was the result of failure of fertilization due to the cytotoxic effects of methyl chloride on sperm, principally those in the testes at the time of treatment, rather than direct genotoxicity; and (4), even the elevated postimplantation loss (generally considered the most reliable indicator of genotoxic effects) appeared to result not from any intrinsic genotoxic potential of methyl chloride, but rather from its induction of an epididymal inflammatory response (Chellman et al, 1986a,c, 1987; Working et al., 1985b; Working and Bus, 1986; Working and Chellman, 1989).

In summary, while methyl chloride appears to possess direct genotoxic activity at relatively high concentrations in a variety of in vitro test systems, its in vivo genotoxicity, at least in rodents, appears to result as a secondary effect of induced cytotoxicity. Furthermore, any genotoxic response in humans may be marginal in magnitude if present at all, and would appear to require exposure to highly toxic levels of methyl chloride, much higher than those likely to be encountered by the general population, or even industrial workers under most scenarios.

4.4.4 Mode of Action in Relation to Toxicity

While the mode of action of methyl chloride's toxicity with respect to multiple endpoints across various organisms (including humans) has not yet been firmly described in complete detail or to the exclusion of apparent inconsistencies, the three studies discussed above and those presented previously (especially in Sections 3.2, 3.3, 4.3 and 4.4.3) suggest that a number of generalizations can be made. First, it seems apparent that methyl chloride does not directly

methylate cellular macromolecules (especially DNA) to any appreciable extent, at least in vivo in mammals. Thus, while demonstrating weak direct-acting mutagenicity in several in vitro test systems, evidence of DNA damage (SSB, DDC, DPC) or repair (UDS) induced by methyl chloride has been inconsistently observed in several in vitro and in vivo systems, and when found has been associated with relatively high, toxic doses.

Second, this toxicity, in some cases mediated through inflammatory responses, has convincingly been demonstrated to be responsible for methyl chloride's dominant lethal effects, both in pre- and postimplantation embryonic loss (via reduced fertilization resulting from toxic effects on sperm, and the genotoxic effects of reactive oxygen species potentially associated with induced epididymal inflammation, respectively). Third, although the toxicities observed in the other principal target organs (brain, kidney, liver, and testis) apparently do not exhibit significant manifestations of inflammation, toxicity reduction in the first three organs (not in the testis) by the anti-inflammatory agent BW755C suggests that perturbation of normal prostaglandin and/or leukotriene metabolism by methyl chloride could be involved in its mechanism(s) of action in certain target tissues.

Fourth, methyl chloride's substantial capacity to deplete tissue GSH is clearly a significant aspect of its toxicity, probably as a reflection both of its conjugation to GSH and postulated subsequent metabolism to toxic intermediates and end-products (e.g., methanethiol, formaldehyde, formate; see Figure 1), as well as of the possible impairment of GSH-dependent cellular functions, e.g., detoxication of free radicals and other electrophilic compounds via conjugation, glutathione peroxidase suppression of lipid peroxidation, amino acid transport, activities of GSH-requiring enzymes such as FDH, etc. Depletion of GSH coupled with known polymorphisms in the GSST1 genotype that characterize two groups of humans on the basis of methyl chloride metabolism in erythrocytes suggest that these groups may also be distinguished in their toxicological outcomes although there are no data at present.

Finally, although its proposed scheme of metabolism suggests that the frequently observed roles of GSH conjugation (detoxication) and P-450 oxidation (metabolic activation enhancing toxicity) are largely reversed for methyl chloride, data from several studies (Jäger et al., 1988;

Hallier et al., 1990; Dekant et al., 1995) collectively illustrate the possibility that P-450 metabolism of methyl chloride (presumably to formaldehyde) might be significantly involved in at least some target organ toxicity in certain species. Oxidation of methyl chloride to formaldehyde by CYP2E1 was found to be significantly higher in kidney microsomal preparations from male mice than from female mice, and was negligible in preparations from either sex of rat. Although this biotransformation activity appeared more than twice as great in male and female mouse liver preparations than in those of the male mouse kidney, it was noted both that the kidney contains a variety of cell types, and that among them the target cell of interest, the proximal tubular epithelial cell, contains most of the renal CYP2E1. As formaldehyde is genotoxic and is known to produce toxic effects in proximal tubule cells, it was hypothesized that these observations could provide a basis for the occurrence of kidney tumorigenicity seen in male mice, but not in female mice or rats of either sex. Furthermore, as CYP2E1 has not been found in the kidneys of humans of either sex, this proposed mechanism of methyl chloride's tumorigenicity would not support extrapolation of the chronic mouse study findings to humans.

4.5 SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN)—ORAL AND INHALATION

No epidemiological or occupational studies, nor any case or anecdotal reports, were located that described or suggested significant exposure via the oral route. Similarly, insufficient data exist for evaluating the effects in animals of oral exposure to methyl chloride. As noted earlier, primarily because of its chemical properties and current patterns of use, inhalation is almost certain to be the only meaningful route of exposure to methyl chloride for the general population, and most probably for occupational scenarios as well (although dermal exposure could be possible).

Following acute inhalation exposure, the most prominent effects in humans appear related to a general depression of the central nervous system and include headache, nausea, vomiting, dizziness, blurred or double vision, slurred speech, lack of coordination, muscle spasms, convulsions, respiratory depression, unconsciousness, coma, and death. Case reports indicate

that acute exposures sufficient to cause severe CNS problems in humans may also affect the liver, kidney, lung, heart (tachycardia, low blood pressure, ECG abnormalities) and gastrointestinal tract, although effects on the latter two organ systems may be secondary to CNS toxicity. Fatalities have resulted from only a single or very few exposures to very high methyl chloride concentrations (≥30,000 ppm). Signs and symptoms typically appeared within hours or a day or two of exposure and generally resolved within days to several months.

The CNS effects (e.g., fatigue, loss of appetite, headache, disequilibrium, blurred vision, loss of coordination, confusion, anxiety, personality changes, short-term memory loss, nausea, and vomiting) of longer-term, lower-level exposures are considered generally to be relatively mild. Exposure of volunteers to 20-150 ppm for up to 7.5 h/day over 2-5 consecutive days revealed no detrimental effects on a wide range of physiological and clinical parameters nor were there seen any neurological abnormalities or biologically significant decrements in cognitive functioning, including arithmetic task performance. In another exposure study, however, exposure of volunteers for 3 h to 200 ppm did produce marginally significant decrements in three performance tasks in which visual vigilance was the most sensitive indicator for methyl chloride impairment.

Adverse effects observed in acute, subchronic, and chronic studies of rodents include CNS functioning, degenerative brain changes, axonal degeneration in the spinal cord, and effects in liver, kidney, and testes. Spinal cord and brain lesions were also observed after subacute exposure of dogs (McKenna et al., 1981b). Four subacute studies (continuous or intermittent exposures over 2-11 days, at 200-5,000 ppm for rats and 15-2,400 ppm for three strains of mice: C57BL/6 females, C3H, and B6C3F1) demonstrate that methyl chloride's toxic effects can vary qualitatively or quantitatively by species, strain, and sex, even among rodents (Burek et al., 1981; Jiang et al., 1985; Landry et al., 1985; Morgan et al., 1982).

Continuous exposure to 100 ppm caused degenerative changes in granule cells of the cerebellum as well as liver toxicity in female C57BL/6 mice (Landry et al., 1983c, 1985). At 100 ppm and higher, mice exhibited various stages of glycogen depletion without hepatocellular degeneration. No treatment-related lesions were noted in the spinal cord. Exposure to 150-

400 ppm (continuous) or 1,600 and 2,400 ppm (intermittent) produced gross signs of neurotoxicity (ataxia, transient sedation, hind-limb extensor rigidity, etc.) and led to a moribund condition. After intermittent exposure, slight-to-moderate degenerative lesions of the cerebellum began to appear at 400-1,000 ppm, becoming severe at 1500-2,000 ppm. Such cerebellar lesions were not seen in C3H mice or in $B6C3F_1$ males, and were moderate to severe in $B6C3F_1$ females at 1,000-2,000 ppm. (Morgan et al., 1982). Evidence from metabolism studies that could explain any particular susceptibility of the female B57BL/6 is lacking. In vitro studies with either a liver or kidney cytosol preparation showed that the female C57BL/6 did not differ from the C3H and B6C3F1 in their ability to metabolize methyl chloride (Hallier et al., 1990). Neurofunctional testing of C57BL/6 females demonstrated significant decrements in performance at concentrations as low as 800 ppm (intermittent) and 150 ppm (continuous), thus indicating the cerebellar lesions to be a useful indicator of neurotoxicity. (Landry et al., 1985, 1983c). Similar brain lesions in humans, unrelated to methyl chloride exposure, have been associated with renal insufficiency. However, the lack of significant kidney abnormalities in mice at methyl chloride concentrations that induce severe cerebellar granule cell necrosis and focal malacia suggest that they probably are not a secondary effect of renal toxicity (Jiang et al., 1985).

Histopathological evidence of brain lesions was also seen in rats. Slight degeneration of the cerebellar internal granular layer was seen at 5,000 ppm (Morgan et al., 1982) in several F344 rats exposed intermittently for 5 days, as were forelimb ataxia, hindlimb paralysis, and convulsions (one female). In Sprague-Dawley rats exposed continuously for 48 and 72 h to 200 to 2,000 ppm (Burek et al., 1981), the brain was cited as being analyzed, but no histopathological evidence of lesions were reported. There was substantial mortality after 48 continuous hours of exposure at 2,000 ppm, but not at 1,000 ppm. The primary cause of death after was kidney toxicity (renal tubular necrosis, degeneration and regeneration, lipid accumulation, etc.) followed by renal failure. Dose-dependent kidney toxicity was somewhat greater in males than in females. Liver toxicity in the Sprague-Dawley ranged in severity from necrosis, inflammation and assorted degenerative changes at 2,000 and 1,000 ppm, down to minimal and reversible effects in 200 ppm males (altered tinctorial properties) and females (slightly elevated lipid content). In the F344, liver toxicity was found at 3,500-5,000 ppm in both sexes, but at 2,000 ppm only in females (Morgan et al., 1982). Various degenerative and inflammatory toxic effects were also

noted in the epididymides of most Sprague-Dawley males exposed to levels at 500 ppm and above, along with some testicular atrophy and degeneration. Testicular degeneration in F344 males was minimal at 2,000 and 3,500 ppm, but severe at 5,000 ppm.

Clinical, behavioral and histopathological evidence of neurotoxicity were the only significant effects observed in dogs at 500 ppm, but not at 200 ppm, following 3 days of continuous exposure. Cats were unaffected at either concentration. Repeated, short exposures to a high concentration of methyl chloride have also produced neurotoxicity in guinea pigs (Kolkmann and Volk, 1975).

Subchronic exposure (6 h/day, 5 d/wk for 13 weeks) of male Beagle dogs, two strains (Sprague-Dawley and F344) of rats and two strains (B6C3F1 and CD-1) of mice at or below 400 ppm failed to demonstrate any unequivocal evidence of significant toxicity, including histopathological or functional neurotoxicity (McKenna et al., 1981a; Mitchell et al., 1979a,b). Hepatotoxicity was the only organ-specific effect noted in rodents exposed to 750 ppm (increased relative liver weight, mice) and higher.

In a chronic study (CIIT, 1981) at 0, 50, 225 or 1,000 ppm, the mouse was more sensitive than the rat. The following adverse effects were observed in mice: cerebellar degeneration and neurofunctional impairment (1,000 ppm), axonal swelling and degeneration in the spinal cord (50 ppm and higher), hepatocellular lesions (1,000 ppm), renal toxicity (tubuloepithelial hyperplasia and karyomegaly and cortical cysts, (males at 1,000 ppm), splenic atrophy and/or lymphoid depletion (1,000 ppm), and testicular toxicity (degeneration/atrophy of the seminiferous tubules, male mice and rats at 1,000 ppm). The increased incidence of axonal swelling and degeneration at two areas of the spinal cord of the mouse compared to controls indicates a LOAEL of 50 ppm with no NOAEL. It is noted that lesions described as renal cortical microcysts were observed in 6 male mice at 50 ppm after 24 months, but not in any at 0, 225, or 1,000 ppm (at any time of sacrifice or death), and were considered by the study authors "to be related to methyl chloride exposure, although their relationship to the other renal lesions is not clear." These microcysts (and even the cysts) could represent background lesions that occur principally in aged mice. Although these lesions were previously used to define a chronic

inhalation LOAEL in male mice of 50 ppm (U.S. EPA, 1989), they appear to be associated with significant uncertainty (Johnson, 1988; see also Farber and Torkelson, 1989).

No human or animal studies were located concerning the reproductive or developmental effects of methyl chloride by the oral route of exposure, and the minimal information available for inhalation exposure in humans is inadequate to permit any significant conclusions. Several studies have characterized in detail the effects of 5-9 days of intermittent exposure (6 h/day) to methyl chloride on the reproductive performance and physiology of male F-344 rats (Chapin et al., 1984; Working et al., 1985a,b; Working and Bus, 1986; Chellman et al., 1986a,c, 1987). Reduced fertility, testicular toxicity (seminiferous epithelium degeneration, delayed spermiation, reduced testicular weight and numbers of sperm and spermatids, sperm abnormalities and reduced motility, abnormal histopathology, reduced levels of NPSH and circulating testosterone), epididymal toxicity (inflammation, sperm granulomas, reduced NPSH levels), and dominant lethal effects were observed at 3,000-3,500 ppm, but not at 1,000 ppm. Dominant lethality results primarily from an increase in preimplantation loss, although some postimplantation loss is also observed. The collective data, including studies with the drug BW755C, which inhibits methyl chloride-induced epididymal inflammation and postimplantation loss, but not testicular toxicity or preimplantation loss, strongly suggest that the preimplantation loss results from methyl chloride's cytotoxic effects on sperm located in the testes, with consequent failure of fertilization due to low sperm number and poor sperm quality. Postimplantation loss is thought to result from indirect genotoxicity caused by methyl chloride-induced epididymal inflammation, possibly via the generation of reactive oxygen intermediates that accompany inflammation.

A two-generation reproduction study in F-344 rats demonstrated significant adverse reproductive effects at 1,500 ppm, but not at 150 ppm (Hamm et al., 1985). Only marginal effects (small, reversible reduction in F_0 male fertility; decreased percentage of males and temporary pup weight reductions in F_1 litters) were observed at the study's LOAEL of 475 ppm, suggesting that it may approximate or be only somewhat higher than the true reproductive NOAEL. Finally, it is known that methyl chloride can cross the rat placenta and cause reductions in fetal NPSH levels (Bus et al., 1980), and that it may be teratogenic under certain conditions in mice (but not rats), causing heart malformations at maternally toxic (750 ppm) and nontoxic

(500 ppm) concentrations, but not at 250 ppm (Wolkowski-Tyl et al., 1983a,b). The significance of these mouse heart malformations has been the subject of some controversy (John-Greene et al., 1985; Tyl, 1985), and a more definitive assessment of methyl chloride's teratogenic potential awaits further study.

The data summarized above strongly implicate cytotoxicity rather than direct genotoxicity as the general mode of action for methyl chloride's dominant lethal effects. Although not without some data gaps and inconsistencies, the evidence presented by many of the studies discussed in Sections 3.3, 4.3, 4.4.3, and 4.4.4 suggests that methyl chloride's in vivo modes of action are intimately related to its metabolism. Both in vitro and in vivo data indicate that methyl chloride's primary metabolic pathway involves conjugation with glutathione (Figure 1) and the likely formation of the intermediate methanethiol, which has neurotoxic effects similar to those described for methyl chloride. Consistent with this pathway, methyl chloride exposure has been shown to substantially reduce GSH levels in target tissues. Methyl chloride does not appear to methylate cellular macromolecules, but rather to have its carbon atom incorporated into them via metabolism to formaldehyde and formate, with subsequent entry into the one-carbon pool utilized for normal anabolic reactions. It should be noted that P-450 may to some extent directly oxidize methyl chloride to formaldehyde, and may help mediate the conversion of methanethiol to formaldehyde as well. To the extent that methyl chloride toxicity is a function of methanethiol formation and GSH depletion, P-450 reactions may generally represent a detoxication process, although in specific instances (mouse renal tumors) the reverse could be true (Dekant et al., 1995; see Section 4.6).

Both formaldehyde and formate could contribute to methyl chloride's toxicity, although increases in formate have not specifically been observed following methyl chloride exposure. This fact, coupled with methyl chloride's reduction of GSH, a cofactor required for the FDH-catalyzed conversion of formaldehyde to formate, has led to the suggestion that accumulation of formaldehyde could be a factor in methyl chloride's toxicity (Kornbrust and Bus, 1982, 1983). Target tissue depletion of GSH by methyl chloride has been associated with lipid peroxidation, each found more extensively in mouse liver than in rat liver, paralleling the species' sensitivities to methyl chloride-induced hepatotoxicity (Kornbrust and Bus, 1984). Subjecting mice to

pretreatment conditions that substantially depleted NPSH levels in the brain, liver and kidney protected against lethality and target organ toxicity from subsequent methyl chloride exposure, further implicating a pivotal role for GSH in methyl chloride's mode(s) of action (Chellman et al., 1986b). While GSH depletion may contribute to toxicity through impairment of critical GSH-dependent cellular functions (e.g., free radical detoxication, suppression of lipid peroxidation, amino acid transport, FDH and other GSH-requiring enzyme activities), these latter results suggest that much of methyl chloride's toxicity depends upon the generation of toxic metabolites from the conjugation product, S-methylglutathione. Finally, as noted above, studies involving the anti-inflammatory agent BW755C suggest that some of methyl chloride's toxic effects could be mediated by a perturbation of normal prostaglandin and/or leukotriene metabolism (Chellman et al., 1986a,b, 1987).

4.6 WEIGHT-OF-EVIDENCE VALUATION AND CANCER CHARACTERIZATION—SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

The few studies that have examined methyl chloride's potential carcinogenicity in humans have failed to convincingly demonstrate any association, and in one instance even indicated a lower cancer incidence than expected in workers chronically exposed to methyl chloride (Section 4.1.2). In animals, the only evidence of carcinogenicity comes from a single 2-year bioassay, which found a statistically significant increased incidence of renal benign and malignant tumors only in male B6C3F_1 mice at the high concentration (1,000 ppm), a level at which significant mortality due to neurotoxicity was observed. Two renal adenomas occurred in 225 ppm males and were also considered by the investigators to be related to methyl chloride exposure (CIIT, 1981). Neoplasia were not found at lower concentrations or at any other site in the male mouse, nor at any site or concentration in female mice or F-344 rats of either sex. Renal cortical tubuloepithelial hyperplasia and karyomegaly were also confined to 1,000 ppm male mice.

With respect to likely mode of action, methyl chloride demonstrated weak-to-moderate direct mutagenic activity at high concentrations (0.5-30%) in several reverse or forward mutation

detecting tester strains of *Salmonella* (Andrews et al., 1976; du Pont, 1977; Fostel et al., 1985; Longstaff et al., 1984; Simmon, 1981). At concentrations of 0.3-5%, it was reported to induce mutation and SCE, but not alkaline-labile DNA damage in an established human lymphoblast cell line (Fostel et al., 1985). Exposure of $B6C3F_1$ mice to 1,000 ppm for 8 h, or 6 h/day for 4 days, revealed no evidence of alkaline elution-detectable DNA damage in liver tissue from either sex, or in kidney tissue from females; however, in male kidney tissue, there was evidence of DNA-protein crosslinks (DPC) and DNA single-strand breaks (Jäger et al., 1988; Ristau et al., 1989, 1990). The latter were speculated to result from either excision repair of the DPC, or from free radical damage consequent to methyl chloride-induced GSH depletion and associated lipid peroxidation. Endogenous formation of formaldehyde was suggested as a cause of this DNA damage, that turn was offered as a possible contributor to the renal tumors found only in male mice. Methyl chloride does not directly methylate the DNA from various tissues of rats and mice, including that of the male $B6C3F_1$ kidney (Kornbrust et al., 1982; Peter et al., 1985).

Methyl chloride was weakly positive for the in vivo induction of unscheduled DNA synthesis (UDS) in rat liver at 15,000 ppm, but not at 3,500 ppm, nor in pachytene spermatocytes or tracheal epithelial cells at either concentration (Working et al., 1986). In vitro exposure of the spermatocytes induced UDS at 3-10%, but not 1%, while in the tracheal cells the response was negative at 1%, negative but suggestively positive at 3%, and toxic at 5 and 10%. Primary cultures of human hepatocytes from three individuals were collectively negative at 0.1-0.3%, negative or weakly positive at 1%, and toxic at 2-10% (Butterworth et al., 1989). A high concentration (20%) of methyl chloride was found to be a potent inducer of sex-linked recessive lethal (SLRL) mutations in *Drosophila* (University of Wisconsin, 1982), and 6,000-25,000 ppm (but not 3,000 ppm) enhanced viral transformation in cultured SHE cells (Hatch et al., 1983). Finally, 2,000-3,000 ppm (but not 1,000 ppm) produced dominant lethal effects in Sprague-Dawley rats (SRI International, 1984) and F-344 rats (Working et al., 1985a). However, rather than direct genotoxicity, this dominant lethality appears attributable to cytotoxic effects on sperm in the testes, and to the effects of genotoxic oxidative metabolites resulting from an induced inflammatory response in the epididymides (Chellman et al., 1986a,c, 1987; Working et al., 1985b; Working and Bus, 1986; Working and Chellman, 1989).

These data collectively indicate that methyl chloride is a relatively weak, direct-acting in vitro genotoxicant at high concentrations, and that its weak DNA-damaging effects in vivo either are or are likely to be primarily the result of various cytotoxicity-mediated mechanisms. Furthermore, experiments have demonstrated in $B6C3F_1$ mice that 1,000 ppm of methyl chloride depleted GSH levels more rapidly in liver than in kidney, and to a slightly greater extent in females than males; microsomal GST activities with methyl chloride as substrate were higher in liver than in kidney, and higher in female mice than in male mice and much higher than in male F-344 rats; and microsomal cytochrome P-450 content and/or the oxidation of methyl chloride to formaldehyde by cytochrome CYP2E1 was higher in mouse liver than in rat liver or mouse kidney, and was four- to five-fold higher in male kidney than in female kidney, while being undetectable in rat kidney of either sex (Dekant et al., 1995; Hallier et al., 1990; Jäger, 1988). Although CYP2E1 activity was lower in kidney homogenates than in those from liver by a factor of two, it was noted that the kidney is composed of a variety of cell types and that most of the renal CYP2E1 is contained in the proximal tubular epithelial cell. Thus, the target cell for methyl chloride's observed tumorigenicity is likely to have both high exposure to methyl chloride and high CYP2E1-mediated levels of formaldehyde.

Based upon: (1) reported species, sex and organ differences for DNA damage (DPC and single-strand breaks), GST activity, and GSH depletion; (2) CYP2E1 conversion of methyl chloride to formaldehyde, and the occurrence of renal cortical tubuloepithelial hyperplasia and karyomegaly, as well as; (3) methyl chloride's relatively weak genotoxicity profile, and; (4) elucidated mechanisms of its dominant lethality, at least one plausible mode of action may be suggested for its observed pattern of carcinogenicity. At relatively high concentrations in the male mouse kidney, less methyl chloride may be metabolized via the generally dominant GSH-conjugation pathway, instead being oxidized directly to formaldehyde by relatively high levels of endogenous CYP2E1. Formaldehyde's cytotoxic and genotoxic effects, coupled with the kidney's high capacity for regenerative cell proliferation, may then result in some level of DNA damage and hyperplasia that eventually progress to the formation of tumors. With respect to the applicability of this postulated mode of action to the assessment of cancer risk in humans, it should be noted that CYP2E1 in humans is considered to be present only at very low levels in human kidney samples [there are no peer-reviewed data]; it has been found extrahepatically at

higher levels only in association with diabetes or chemical induction (Dekant et al., 1995; Gonzalez and Gelboin, 1994; Guengerich and Shimada, 1991; Guengerich et al., 1991).

Based upon inadequate evidence of carcinogenicity to both humans and animals, IARC has classified methyl chloride as a Group 3 chemical—"not classifiable as to its carcinogenicity to humans" (IARC, 1987). Under the Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1986a), the weight of evidence for carcinogenicity from human studies would be categorized as "inadequate" and that from animal studies as "limited," therefore resulting in classifying methyl chloride as a "Group C—Possible Human Carcinogen" (U.S. EPA, 1989). When the totality of available data, summarized in the narrative above, are assessed according to the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), methyl chloride's potential for human carcinogenicity may described as "not likely," especially at the lower concentrations to which humans would typically be exposed. This conclusion rests on the lack of evidence for carcinogenicity from limited human studies, the occurrence of tumors in only one organ of one sex of a single rodent species at the highest tested concentration, and mechanistic considerations that suggest the most probable mode of action responsible for the induction of male mouse kidney tumors may not be relevant to humans under normal exposure conditions.

4.7 SUSCEPTIBLE POPULATIONS

There is little information in the reviewed literature that speaks directly to the issue of whether there exist specific human subpopulations that might be particularly susceptible to the toxicity of methyl chloride. As described in Sections 3.1-3.4, a minority subpopulation, the size of which appears to vary by geographical region, has been identified that maintains higher steady-state breath and blood levels of methyl chloride, levels that decline more slowly following termination of exposure than those of the majority population (Putz-Anderson et al., 1981; Stewart et al., 1980; Nolan et al., 1985). This apparent slower metabolism of methyl chloride is also reflected in a report that in 2 of 6 individuals tested, much lower levels of the urinary metabolite S-methylcysteine were detected (van Doorn et al., 1980). Other studies have identified a minority subpopulation of "nonconjugators," individuals displaying little or no detectable conjugation of methyl chloride with the erythrocyte GST(θ) isozyme (GSTT1-1),

apparently as a result of the homozygous loss of a functional GSTT1-1 allele (Peter et al., 1989a,b; Kempkes et al., 1996; Warholm et al., 1994). However, due to the complexities and uncertainties of methyl chloride's metabolism, especially with respect to mechanisms of toxicity, it is not clear whether this (these) subpopulation (s) would have significantly altered susceptibility to methyl chloride, and if so, whether it would be lesser or greater (e.g., Nolan et al., 1985; Warholm et al., 1994). To the extent that P-450 enzymes play a role in mediating methyl chloride toxicity, it should be noted that CYP2E1 is expressed constitutively in human liver, but has been found extra-hepatically only under conditions of induction (e.g., heavy alcohol consumption) or diabetes (Gonzales and Gelboin, 1994). Also, individuals taking prescription CNS depressants (e.g., diazepam) or exposed to other CNS depressants (e.g., alcohol) may be at an increased risk for experiencing some of the CNS effects attributable to methyl chloride (Putz-Anderson et al., 1981).

4.7.1 Possible Childhood Susceptibility

Although factors such as maturation state of physiological and biochemical process in children might possibly influence their susceptibility to methyl chloride, no specific information was retrieved on methyl chloride exposure in infants or children. Animal data indicate that methyl chloride can cross the placental barrier and cause fetal effects, and than it may be capable of causing fetal malformations at concentrations that are maternally toxic or somewhat less. This latter finding remains controversial to some degree, and in any case, the relevance of these observations to humans is uncertain.

4.7.2 Possible Gender Differences

In the various studies referenced above concerning the metabolic subpopulation(s), no gender differences were noted, nor were any discernable in any other of the retrieved experimental, case report, or epidemiological studies. Some gender-related differences were apparent in a number of the acute and chronic animal studies that were reviewed (e.g., Morgan et al., 1982; CIIT, 1981), but these were typically species-, strain- and/or endpoint-specific, making generalizations difficult and extrapolation to humans problematic. As examples, female

C57BL/6 mice were more sensitive to the cerebellar degenerative effects of methyl chloride than were males, but this gender sensitivity was barely detectable in $B6C3F_1$ mice, and was not evident at all in C3H mice or F-344 rats. Similarly, $B6C3F_1$ females appeared more sensitive to acute renal toxicity than males, a gender difference not apparent in the other two mouse strains or in rats, but $B6C3F_1$ males (not females or rats of either sex) were the animals susceptible to the renal tumorigenic effects of chronic methyl chloride exposure. A mechanistic study designed to investigate possible reasons for this effect in male but not female B6C3F1 mice found that sex differences in P-450 metabolism or glutathione depletion were not sufficient to explain the male-specific renal tumors (Hallier et al., 1990). Therefore, the currently available data do not permit the reliable identification of any human gender differences (other than potentially in male reproductive organs) in susceptibility to methyl chloride.

5. DOSE-RESPONSE ASSESSMENTS

5.1 ORAL REFERENCE DOSE (RfD)

No oral dose-response assessment was performed for methyl chloride due to a lack of data.

5.2 INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1 Choice of Principal Study and Critical Effect—with Rationale and Justification

The 2-year study of CIIT (1981) is the only long-term repeated inhalation study currently available. In this study, F-344 rats and $B6C3F_1$ mice were exposed 6 h/day, 5 days/wk, for up to 24 months to concentrations of 0, 50, 225, or 1,000 ppm of 99.97% pure methyl chloride (120/sex/species/concentration). The LOAEL and NOAEL in rats for chronic inhalation exposure to methyl chloride were 1,000 ppm and 225 ppm, respectively, based principally on the occurrence of seminiferous tubule degeneration and atrophy. In mice, a LOAEL of 50 ppm, the lowest concentration tested, was based upon the occurrence of axonal swelling and degeneration in areas of the spinal cord. There was no NOAEL. Spinal cord and brain lesions have also been observed in dogs (McKenna et al., 1981b) exposed subacutely.

In addition to the effects on the rat male reproductive system, effects were observed in liver, brain, kidney, and spleen of mice at 1,000 ppm. Cerebellar lesions and neurobehavioral effects, as well as liver histopathology and significantly elevated levels of the enzyme ALT, kidney lesions (tubuloepithelial hyperplasia and karyomegaly), and spenic lesions (atrophy and lymphoid depletion) were noted in mice at this concentration level. These effects were not apparent in exposed rats.

5.2.2 Methods of Analysis—No-Observed-Adverse-Effect Level/Lowest-Observed-Adverse-Effect Level

Derivation of the RfC was not based on Benchmark Dose Analysis because the critical effect (axonal swelling and degeneration of the spinal cord) did not have a defined dose-response incidence trend. Therefore, the NOAEL/LOAEL approach was used in all further analysis.

The LOAEL of 50 ppm (103.2 mg/m^3) is adjusted to a continuous exposure = LOAEL(ADJ): 103.2 mg/m^3 × (6 h/24 h) × (5/7 days) = 18.4 mg/m^3.

The physico-chemical characteristics of methyl chloride and its distribution in rodents after inhalation exposures indicate that it would be identified as a Category 2 gas, following guidance for derivation of inhalation RfC values (U.S. EPA, 1994b). The human equivalent concentration (HEC) for methyl chloride was derived by multiplying the LOAEL(ADJ) by a dosimetric adjustment factor for gas: respiratory effects in the region of critical effect. This factor, the regional gas dose ratio (RGDR), was assumed to be a ratio of 1.0 because the blood: gas partition coefficient values are unknown. Thus, the LOAEL, multiplied by the RGDR to yield the LOAEL human equivalent concentration (HEC), is:

$$LOAEL_{HEC} = LOAEL_{ADJ} \times RGDR = 18.4\ mg/m^3 \times 1.0 = 18.4\ mg/m^3.$$

5.2.3 RfC Derivation—Including Application of Uncertainty Factors (UF) and Modifying Factors (MF)

Uncertainty factors were applied for interspecies extrapolation from animals to humans (10), for extrapolating from a LOAEL (10), for intraspecies variability (3), and for database deficiencies (3). Because two factors of 3 ($10^{1/2}$) coalesce to a 10, a total UF of 1,000 was applied. The inhalation toxicity database for methyl chloride is judged to be limited. A two-generation reproductive/developmental study in the mouse is needed to evaluate the effect of methyl chloride on male reproductive parameters. In addition, there were shortcomings (e.g., not all mouse tissues were examined at all time points) in the conduct of the CIIT (1981) study that comprised interpretation of the effects.

A total uncertainty factor of 1,000 (3 for interspecies variability, 10 for intraspecies variability, 10 for extrapolating from a LOAEL, and 3 for database deficiencies) is applied to the $LOAEL_{HEC}$ of 18.4 mg/m^3, yielding an RfC of 2E-2 mg/m^3.

5.3 CANCER ASSESSMENT

The human data are inadequate to judge the carcinogenic potential of methyl chloride. The laboratory animal data are limited to a single 2-year rodent bioassay that found a statistically significant increased incidence of renal tumors only in male $B6C3F_1$ mice at the high concentration (1,000 ppm); two renal adenomas occurring in 225 ppm males were also considered related to methyl chloride exposure by the study authors (CIIT, 1981). Neoplasia were not found at lower concentrations or at any other site in the male mouse, nor at any site or concentration in female mice or F-344 rats of either sex. The weight of evidence suggests that, under the U.S. EPA 1996 Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), the carcinogenic potential of methyl chloride in humans would be classified as "not likely" especially at the lower concentrations to which humans would typically be exposed. Mechanistic considerations and the occurrence of tumors in only one organ of one sex of a single rodent species suggest that the induction of kidney tumors in male mice may not be relevant to humans.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1 HUMAN HAZARD POTENTIAL

Methyl chloride is used primarily in the manufacture of silicones, agrichemicals, methyl cellulose, quaternary amines, and butyl rubber; former uses as a refrigerant and an anesthetic have been largely discontinued. It exists as a gas that is slightly soluble in water; thus the majority of exposures are through inhalation.

Methyl chloride is readily absorbed in the respiratory tract and distributes throughout the body. Its toxicity is thought to lie primarily with its metabolism. The effects noted in the cerebellum of exposed rodents and in the spinal cord of mice, coupled with the known CNS effects in humans suggest that inhalation of methyl chloride poses risks to CNS functioning at levels above the RfC.

6.2 DOSE RESPONSE

The quantitative estimates of human risk as a result of low-level chronic exposure to methyl chloride are based on animal experiments because no epidemiological studies with adequately quantitative methyl chloride exposures have been reported. The RfC is an estimate of an inhalation human exposure that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The RfC for methyl chloride was derived from a 2-year chronic bioassay in mice (CIIT, 1981), in which exhibited exposure-related axonal degeneration and atrophy in the spinal cord at levels below those causing lesions in the cerebellum. Although the incidence of spinal cord lesions at all exposure concentrations was elevated above those of controls, a clear dose-response trend was not apparent. A LOAEL, but not a NOAEL, was identified. The RfC for methyl chloride is 0.02 mg/m^3. An overall uncertainty factor of 1,000 was applied to the LOAEL to account for extrapolation from mouse to human, intraspecies variability, and database insufficiencies. An oral exposure RfD was not derived due to a lack of adequate studies.

The overall confidence in the RfC assessment is low; the confidence in the principal study is medium Although it was well-designed and well-conducted, it was not published in the peer-reviewed literature. The overall confidence in the database is low because there are few supporting inhalation studies. Additional animal and human studies focused specifically on methyl chloride exposure and its potential reproductive and developmental effects will be necessary to increase the confidence in the RfC.

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