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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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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 202-566-1676.

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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. In general, the RfD is 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., 7 years to a lifetime). The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry), and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question, as well as 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 the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µgL drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing 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 were used 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* (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); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (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); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S.

EPA, 1998b, 2000a); Memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Policy for Risk Characterization; *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b).

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; the compound is also commonly referred to as chloromethane or monochloromethane, and is or has been supplied in several purity grades: pure ($\geq$ 99.5%), technical, and two "refrigerator" grades (HSDB, 1999; Lewis, 1993). Selected chemical and physical parameters of methyl chloride are presented in Table 1.

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, 1989; Lewis, 1993). It is moderately soluble in water, in which it decomposes to methanol and hydrogen chloride; it is generally synthesized from these same two chemicals, also to a lesser degree from the chlorination of methane (ATSDR, 1998; Lewis, 1993; U.S. EPA, 1986d).

Table 1. Selected physical and chemical properties of methyl chloride

Parameter	Data	Reference
Empirical formula	CH_3Cl	Lewis (1993)
Molecular weight	50.49	Budavari (1989)
Melting point	–97.6 °C	Lewis (1993)
Boiling point	–23.7 °C	Lewis (1993)
Vapor pressure	3,670 mm Hg at 20 °C 4,310 mm Hg at 25 °C	ATSDR (1998)
Solubility In Water	6,263–7,400 mg/L at 20 °C 4,800–5,325 mg/L at 25 °C	U.S. EPA (1986d) ATSDR (1998)
Log K_{OW}	0.91 (experimental)	ATSDR (1998)
Odor threshold	100 ppm	Sittig (1991)
Blood-air partition coefficient (modeled human data)	2.12–2.49, 10 ppm exposure 1.71–1.83, 50 ppm exposure	Nolan et al. (1985)
Conversion factors (in air at 25° C)	1 ppm (v/v) = 2.064 mg/m^3 1 mg/m^3 = 0.4845 ppm (v/v)	ATSDR (1998)

Methyl chloride is found ubiquitously in nature; the vast majority comes from such natural sources as the ocean, microbial fermentation, and biomass fires. These 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 to 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); the OSHA permissible exposure limit (PEL) values are a 100 ppm TWA, and a 200 ppm ceiling with a 5-minute maximum peak of 300 ppm in any 3-hour 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 in the body (Putz-Anderson et al., 1981; Nolan et al., 1985). 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 (Guengerich et al., 1991) 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 (Redford-Ellis and Gowenlock, 1971; Hallier et al., 1990). 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 the bulk 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 (Kornbrust and Bus, 1983) comprise several sulfur-containing compounds, all thought to be derived from the initial GSH conjugate (S-methylglutathione).

3.1. ABSORPTION

Absorption of methyl chloride in humans is likely to occur almost exclusively through inhalation, although dermal absorption could constitute a minor route of exposure in certain scenarios. Ingestion of the compound seems highly improbable under normal circumstances, although it is moderately soluble in drinking water. Data on the absorption of methyl chloride are available only for the inhalation route of 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. Under current modeling practices, it is a reasonable assumption that the partition coefficient for the mouse would be similar to that for the rat.

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 hours 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, $p < 0.01$). For the 200-ppm exposure group, the mean alveolar breath concentration (± standard deviation) of methyl chloride was 63 ± 23.6 ppm, whereas that in the blood was 11.5 ± 12.3 ppm. Corresponding breath and blood concentrations for the 100-ppm exposure group were 36 ± 12 ppm and 7.7 ± 6.3 ppm, respectively. These data reflect large interindividual 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 levels in the breath of more than 100 ppm, substantially higher than the remainder. Under these conditions, mean alveolar breath levels were proportionate to exposure concentrations, whereas blood levels were somewhat less so.

In another human study, which utilized a complex exposure scheme, groups of 2–4 males were exposed for 7.5 hr, 3 hr, or 1 hr 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 d/wk over 6 weeks (Stewart et al., 1980). Similarly, three groups of women were exposed for 7.5, 3 or 1 hr 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 dropout, data were not always available for every time point for each of the subjects initially in the study, thus somewhat compromising the study’s quality.

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. Of the 11 male and 9 female subjects, 3 males and 1 female were identified as “high responders” (or more appropriately, “slow metabolizers” or “rapid eliminators”) whose alveolar breath levels of methyl chloride were 60%–110% higher than their identically exposed peers immediately after exposure, and three- to six fold higher at 1 hr postexposure. Blood concentrations of methyl chloride were low even just before exiting the exposure chamber, declined rapidly, and were difficult to measure, especially for the 20-ppm exposure and for most time points other than the pre-exit one. Because of interindividual variation and lack of complete data for all individuals for all exposure sessions, 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-hr 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 six male volunteers who were each exposed for 6 hr to 50 ppm and to 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. Somewhat surprisingly, 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 as previously indicated, "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 than those of the remaining four subjects, while their venous blood levels were 3 times higher than the other subjects.

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 hr in a closed, atmosphere-recirculating chamber to 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, composed 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}), whereas 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/hr = 7.7 mg/kg/hr). Thus, at low concentrations, absorption of methyl chloride would be essentially organ perfusion-limited and appear nearly first-order.

Using a dynamic, nonrecirculating exposure chamber, Landry et al. (1983a) exposed male Fischer 344 rats for 6 hr and male beagle dogs for 3 hr 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 rapidly 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. 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. Virtually identical uptake rates at both time points indicated that apparent steady-state conditions had been achieved by 1.5 hr 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, which compared favorably to the 20-fold difference expected from a linear, dose-independent model of uptake. 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 respiratory minute volume (RMV) for the 1,000-ppm rats (143 mL/min) compared with 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, small reductions in the first-order rate constants for metabolism and expiration would also be expected. The model thus estimated that for a 6-hr 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 an increase of 17.6-fold. This increase is somewhat 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 the blood of humans and laboratory animals. Of particular interest is the identification of two groups of humans who apparently differ in their rate of metabolism: those who are apparent “slow metabolizers” in contrast to those who are “rapid metabolizers.” Furthermore, in humans, rats, and dogs, absorption from the lungs at relatively low exposure concentrations (probably at least up to 500–1,000 ppm) seems closely proportional to the exposure concentration. In addition to the work 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 chloride’s distribution to tissue in Fischer 344 rats and/or 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 hr (dogs) or 3 hr (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 earlier unpublished observations from their own laboratory, Kornbrust et al. (1982) noted that after exposing rats to an unspecified inhalation dose of radiolabeled methyl chloride, more than 45% was exhaled as $^{14}CO_2$ and only 6.6% as parent compound, whereas >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 thus 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 hr to 500 ppm of ^{14}C-labeled methyl chloride, uptakes of radioactivity into various tissues immediately postexposure (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), and protein and lipid macromolecules (Kornbrust et al., 1982). Increasing the exposure concentration to 1,500 ppm reportedly 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. Although 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 the latter than in the former in each tissue examined. In the liver, compared with immediately after exposure, the 24-hr 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 in fact 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 hr postexposure 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). It was found that label incorporation into DNA and lipid and protein macromolecules was largely dependent on their uninhibited biosynthesis; 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 largely 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.

Fischer 344 rats were exposed for 24 hr to compare the alkylation of hemoglobin (Hgb) by ^{14}C-labeled methyl chloride, methyl bromide, and methyl iodide (Xu et al., 1990). A "covalent binding index" (pmol bound/mg Hgb per μmol administered/kg bw) for methyl chloride was found to be 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 hr 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 GSH conjugation pathway (Section 3.3).

In a study described only in abstract (Bus, 1980), maternal and fetal tissue from pregnant Fischer 344 rats that had been exposed for 6 hr to 1,500 ppm of methyl chloride on gestation day 19 were examined for NPSH depletion at 0, 2, 4, and 8 hr 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 hr; fetal placental NSPH, also maximally depressed at 0 hr (to 87.5% of the control value), returned to normal within 4 hr. In contrast, fetal liver and carcass NPSH levels were not maximally depressed until 2 hr postexposure (to 66.8% and 71.0% of control values, respectively), and had not returned to normal levels by 8 hr (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. Although the lack of a complete publication is a shortcoming, this experiment suggesting transplacental distribution may be an important contribution to the understanding of how methyl chloride may influence fetal development.

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.

S-methylcysteine was detected in the urine of six chemical workers who were exposed to methyl chloride for 8 hr a day during a 7-day shift, although the levels were very low in two workers who had the highest exposure (van Doorn et al., 1980). Four workers excreted nearly all the "retained" methyl chloride (i.e., that not exhaled unchanged or as CO_2) 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 that, based upon recovery of label from rats exposed by inhalation to radiolabeled methyl chloride for 6 hr, 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). Landry et al. (1983a) exposed male Fischer 344 rats for 6 hr 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 over the range 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 hours to methyl chloride levels of 1,500 ppm and greater.

Dodd et al. (1982) conducted a similar study, exposing male Fischer 344 rats for 6 hr 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 hr of exposure; even at 1,500 ppm, levels had recovered to approximately 90%–95% of control values within 8 hr 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. 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.

When male Fischer F344 rats were exposed for 6 hr 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). Although 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 (although not necessarily all) 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, nonlipid 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 its 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 hr to 4,000 or 10,000 ppm of methyl chloride, formate accumulation was not observed in the blood, and only a slight, 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, whereas that excreted in the urine was approximately doubled. These results suggest, as the study authors conclude, 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. Because exposure to methyl chloride alone does not result in elevated formate levels, but can deplete tissue NPSH and thus presumably inhibit the GSH-requiring enzyme formaldehyde dehydrogenase (FDH) that oxidizes formaldehyde to formate, Kornbrust and Bus (1982) speculated that methyl chloride's toxicity might, in part, result from an accumulation of formaldehyde.

This hypothesis was evaluated in an in vitro experiment in which 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 at least 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 that excreted in the 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 that originally diagramed 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 headspace vials with 1,000 ppm of methyl chloride, and the decline in methyl chloride concentration was 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, whereas 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 of male mice. Hepatic and renal glutathione (GSH + GSSG) levels were monitored in $B6C3F_1$ mice during the course of a 6-hr 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., approximately down to 5%–10% versus 45%–60% of control values, respectively, after 1 hr of exposure; levels had fallen to approximately 1%–5% after 6 hr of exposure in both tissues). In contrast to earlier work reporting the conjugation of methyl chloride to GSH in human erythrocytes, these authors demonstrated that mouse and rat erythrocyte cytoplasm did not metabolize methyl chloride in vitro at 1,000 ppm. Finally, hepatic and renal levels of cytochromes P-450, P-420, and b5 were determined in the four unexposed strains of mice (Hallier et al., 1990). Levels of all three cytochromes in liver 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 more than twofold 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, 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).

The metabolism of methyl chloride by GSH in humans has long been known (Redford-Ellis and Gowenlock, 1971). These investigators determined that human erythrocytes contain a GST isoenzyme that catalyzes this metabolism. GSTs comprise four multigene classes of soluble isoenzymes that catalyze the conjugation of GSH with a wide range of electrophilic, mainly hydrophobic compounds (Kempkes et al., 1996; Warholm et al., 1994). Human erythrocytes have two theta (θ) class isoenzymes, GSTT1-1 and GSTT1-2 (Pemble et al., 1994; Kempkes et al., 1996; Peter et al., 1989a,b; Warholm et al., 1994). In contrast, such erythrocyte GST activity (or, in fact, 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).

GSTT1-1 can transform a number of mono- and dihalogenated methanes (Pemble et al., 1994; Thier et al., 1993). Preliminary data indicated that GSTT1-1 is also present in human liver and kidney (Thier et al., 1997); immunoblotting and immunohistochemical studies have identified GST-α as the predominant form expressed in both normal human renal cortex and in renal tumors (Rodilla et al., 1998). The GST activity towards methyl chloride in erythrocyte preparations from 45 German volunteers (20 male, 25 female) was examined by incubating the preparations in headspace vials and monitoring both the disappearance of methyl chloride and the formation of S-methylglutathione ($GS\text{-}CH_3$) (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 "nonconjugators," displayed no detectable activity. Conjugators can also be thought of as "rapid metabolizers" and nonconjugators as "slow metabolizers," in keeping with terminology used earlier. 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 as nonconjugators or slow metabolizers, whereas 17.5% (7/40) were thus 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 rapid metabolizers, whereas 11.1% (23/208) were slow metabolizers (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 either homozygous (+/+) or heterozygous (+/-), respectively, for the functional GST(θ) allele (i.e., of the GSTT1 gene). Allelic frequencies

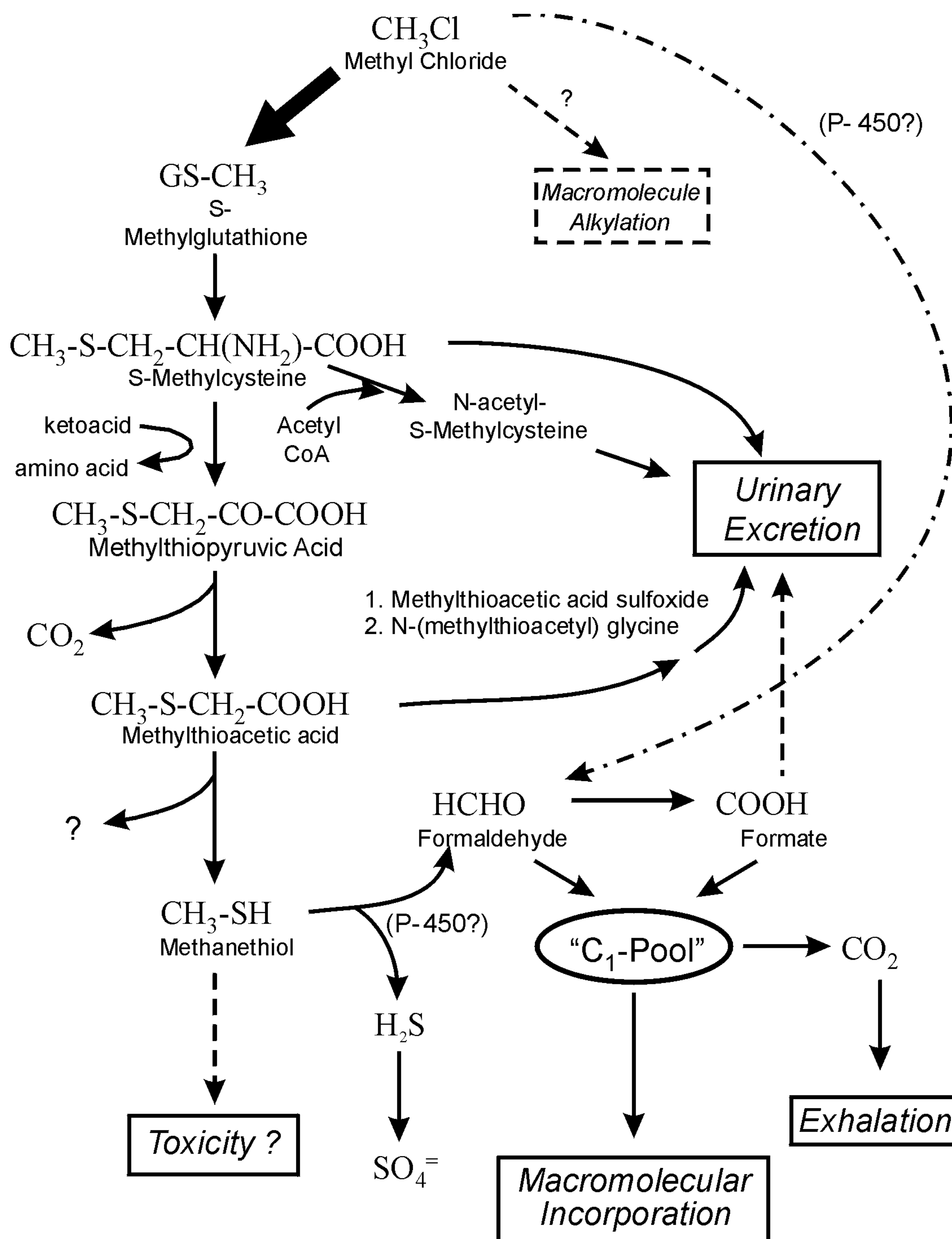

Figure 1. Scheme for metabolism of methyl chloride.
Sources: Kornbrust and Bus, 1983; Hallier et al., 1990, Landry et al., 1983a

of the functional and defective alleles in this population were calculated to be 0.659 and 0.341, respectively. Results were not gender-dependent. These authors cite unpublished results from their laboratory that indicate GST(θ) is probably also expressed in human liver. Shen et al. (1998) determined that the GSTT1 gene is deleted in about 50% of a Chinese population. 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 were controls, Onaran et al. (2000) separated 64 Turkish men and women into two groups on the basis of presence or absence of GSTT1 genes. When collected erythrocytes were exposed to cumene hydroperoxide, levels of the 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 were controls.

More recently, Thier et al. (1998a), using an assay based on ^{35}S-GSH, found species-specific differences in cytosolic activity of GSTT1-1 in hepatic and renal tissues. In rat preparations, GSTT1-1 was present in both liver and kidney at high levels with mouse liver activity at the same order of magnitude; kidney activity was much lower. No significant sex difference with the mouse preparations was seen. In a companion study, Thier et al. (1998b) used methyl chloride to assay GSTT1-1 activity in liver and kidney of rats, mice, hamsters, and humans; erythrocyte activity in humans was also measured. Activity in the mouse liver was sevenfold higher than in kidney, with female mice exhibiting a significantly higher liver activity than males. Similarly in rats liver activity was twofold higher in liver than in kidney. Activity in mouse liver was sevenfold higher than in the rat and twofold higher in kidney than in the rat. No activity was expressed in hamster preparations. In human liver cytosols, all three phenotypes were expressed (i.e., no activity, low activity, and high activity). In kidney the differences were less evident. All three phenotypes were expressed in erythrocytes with activity comparable to that in kidney.

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, Figure 1), were later able to generate the toxic intermediate compound methyl mercaptan (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.

Although it seems clearly established that methyl chloride is principally metabolized via a GSH-conjugation mediated pathway in rodents, the precise contribution of cytochrome P-450 (e.g., CYP2E1) to methyl chloride's in vivo metabolism and toxicity and other members of the P-450 family is less well understood.

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 in 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). CYP2E1 was not detected in human fetal liver and only began a slow rise beginning in the neonatal period up to 1 year (Vieira et al., 1996). 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 (liver) 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 their rates of methyl chloride metabolism (Nolan et al., 1985; Stewart et al., 1980; van Doorn et al., 1980).

In the developing human kidney and lung (and in adult kidney), CYP2E1 RNA content (protein not measured) was expressed at a very low level (Vieira et al., 1998). No CYP2E1 activity was detected in adult human kidney microsomal samples (Amet et al., 1997; De Waziers et al., 1990; Lasker et al., 2000) nor was it detected in freshly isolated proximal tubular cells from human kidney (Cummings et al., 2000). However, human proximal tubular cells were demonstrated to express GSH-dependent enzymes, including GST-θ (Cummings et al., 2000).

The potential of other P-450 isozymes to metabolize methyl chloride in liver or kidney remains to be explored. Using immunoblot analyses of 18 human kidney microsomal nontransformed samples, Amet et al. (1997) identified CYP4A, but not CYP2E1; CYP1A1/1A2 activities were near the detection limit in the eight samples tested. The major P-450 enzymatic activity was found for ω-hydroxylation of fatty acids, mediated by CYP4A, the isozyme previously reported (Imaoaka et al., 1993) to be the major P-450 found in human kidney. Cummings et al. (2000) analyzed freshly harvested human proximal tubular cells and found that CYP4A11 was expressed in all eight samples (from five males and three females), but not CYP2E1; nor was CYP2E1 detected in microsomes from renal cortical slices. The only P-450 enzymes found at significant levels in human renal microsomes are, in addition to CYP4A11, CYP4F2 (Lasker et al., 2000) and CYP3A isoforms (Kharasch et al., 1995; de Waziers et al., 1990). According to Parkinson (1996), no commonly known environmental chemicals appear to be metabolized by the CYP4A family. CYP2C8 and CYP2C9 were not found be expressed in human renal microsomes (Lasker et al., 2000). CYP4F2 and CYP4A11 convert arachidonic acid to 20-hydroxyeicosatetranoic acid (20-HETE), a substance which plays a key role in regulating salt and water balance and arterial blood pressure (Lasker et al., 2000). Guengerich and Shimada

(1991), in their review of the CYP3A family, indicated that a number of isoforms have been found in human liver for which there are many substrates. This assessment was supported by Gonzalez and Gelboin (1994).

Ottenwälder et al. (1989) found that the oxidative P-450 pathway plays a minor role in the metabolism of methyl chloride in vivo. In chamber studies, uptake and metabolism of methyl chloride in $B6C3F_1$ mice was unaffected by pretreatment with specific inhibitors (pyrazole and diethyldithiocarbamate) of P-450, whereas metabolism was greatly reduced for methylene 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 hr 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 P-450 in the metabolism of methyl chloride.

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 evaluated the potential of microsome preparations from female or castrated male mice to form formaldehyde and found they had only 20%–25% the activity of those from naïve males, whereas those from testosterone-treated females had 84%. The rate of formaldehyde formation from methyl chloride observed in kidney microsomes from the female mouse (53 pmol/mg min) was similar to that observed with the castrated male whereas naive males produced 274 pmol/mg/min. 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 antirat 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 all similar, and more than twice those observed in naive 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 threefold range in renal methyl chloride oxidation activities over the four male mouse strains (CD-1, C3H/He, C57BL/6J, and NMRI) examined. The C57BL/6J had the lowest activity, C3H/He intermediate, and the CD-1 and NMRI the highest. On the other hand, either sex of the Sprague-Dawley rat displayed no detectable renal microsome activity for methyl chloride (although CYP2E1 was detected and was induced by ethanol), but an ethanol-inducible, sex-independent activity in liver microsomes was observed that was less than half that observed in CD-1 mice. The study authors 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. Cummings et al. (1999) determined, in the F-344 rat, that the level (and induction of) CYP2E1 and other P-450 isozymes were cell type-specific, with CYP2E1 primarily localized in the proximal and distal tubular cells. Compared to CYP2E1 levels (as measured by chlorozoxazone 6-hydroxylation activity) in kidney microsomes of CD-1 mouse (Speerschneider and Dekant, 1995), the F-344 rat produces only about 25% (Cummings et

al., 1999). Other P-450 isozymes detected in F-344 kidney were CYP2C11, CYP2B1/2, CYP4A2/3.

The correlation of methyl chloride-induced NPSH (GSH) depletion and consequent lipid peroxidation was investigated in liver, kidney, and brain tissues of male $B6C3F_1$ mice and F-344 rats (Kornbrust and Bus, 1984). Over a range of 100–2,500 ppm, single 6-hr 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. For those exposures that depleted mouse liver NPSH levels below 20% of controls, the extent of depletion correlated the capacity of a 9,000g supernatant fraction from liver to undergo lipid peroxidation in vitro (measured as thiobarbituric acid-reactive material, TRM.). Depletion was greatest in liver (at 100 ppm, mouse liver NPSH was approximately 55% that of controls) and least in brain. Most of the depletion occurred during the first 30 min of exposure. After a 6-hr 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 hr postexposure. However, recovery took much longer in brain, reaching only approximately 70% (mice) or 90% (rats) of control levels 18 hr after the end of exposure. Repeated exposure to 2,000 ppm (6 hr/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, in which complete recovery occurred during the 18-hr intervals between each exposure episode. When diethyl maleate (DEM) decreased mouse liver NPSH, a near-lethal injection (2 mL/kg) was less effective at inducing in vitro lipid peroxidation than an exposure to 1,500 ppm methyl chloride. Addition of 0.2–2.0 mM exogenous GSH inhibited lipid peroxidation in a concentration-dependent manner to nearly nondetectable levels after exposure to 1,500 ppm methyl chloride. It was also inhibited by the addition of 2.0 mM EDTA, perhaps because of a sequestration of free iron (a good initiator of lipid peroxidation), and was not observed in animals that were allowed to recover for 4 hr.

The amount of lipid peroxidation varied considerably in the tissues from control animals. No peroxidation was detected in the liver 9,000g supernatants 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 hr 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 dramatically elevated in the liver fraction to near that found in the brain fraction. Using exhalation of ethane as an indicator, in vivo lipid peroxidation during a 6-hr exposure to 2,500 ppm appeared to increase linearly during the last 3 hr of exposure to a level more than 25-fold greater than control levels, then to return to control levels by 1-hr postexposure.

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 are particularly 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. This study did not analyze the subjects' urine for the presence of methyl chloride, but rather for methanol (considered at the time a potentially significant metabolite of methyl chloride); none was detected. Methanol is not a known metabolite of methyl chloride.

In a study of six workers exposed to average methyl chloride concentrations of 30–90 ppm during the course of a 7-day work week, van Doorn et al. (1980) were able to demonstrate significantly elevated, though variable, levels of urinary S-methylcysteine in four of the six. Much lower levels were observed in the other two workers, a finding consistent with the conjugator and nonconjugator subpopulations discussed in Sections 3.1–3.3. Urinary excretion of N-acetyl S-methylcysteine was not detected. As discussed earlier, upon exposure of six volunteers to 10 or 50 ppm of methyl chloride for 6 hr, 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). There was no apparent relationship between urinary S-methylcysteine and exposure to methyl chloride; levels were extremely variable.

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, whereas 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-hr exposure to 4,000 ppm, and only a nonsignificant (at $p < 0.05$) doubling of urinary formate was seen after a 3-hr, 10,000-ppm exposure. Increased urinary formate after methyl chloride exposure was observed only under conditions that inhibited folate-dependent formate metabolism or depleted GSH levels. Bus (1978) reported that when rats were exposed for 6 hr to 1,500 ppm of $^{14}CH_3Cl$,, 63.9%, 32%, and 3.9% of the total excreted radioactivity was recovered in exhaled air, urine, and feces, respectively (U.S. EPA, 1986d,1989).

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 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 hr 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 hours postexposure 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 to be metabolized through a GSH-mediated pathway via formaldehyde and 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 formate appears to be excreted in the urine, although several metabolites resulting from methyl chloride's conjugation with GSH were. Evidence is consistent with the hypothesis that methyl chloride's depletion of GSH in rodents can lead to an accumulation of formaldehyde through inhibition of formaldehyde dehydrogenase that oxidizes formaldehyde to formate and also leads to an increase in lipid peroxidation. Cytochrome P-450 oxidation of methyl chloride also occurs, but to a lesser extent than GSH-mediated reactions. Renal levels of P-450 (and the rate of formaldehyde generated) are substantially higher in male mice than in females and P-450 levels in the mouse are higher than in the rat. CYP2E1, believed to be the principal P-450 isozyme by which methyl chloride is metabolized in liver and in male mouse kidney, has not been detected in human kidney samples in at least three laboratories. The significance of polymorphisms of GST-θ in human subpopulations, which differ in their rates of methyl chloride metabolism, to potential effects of methyl chloride remains to be established.

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 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). Exposure situations typically were accidental overexposure. Typical signs and symptoms of intoxication have been described as appearing within 2–3 hr of exposure and as 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 (body spasms), cold and clammy skin, loss of memory, hallucinations, respiratory depression, unconsciousness, coma, and death (ATSDR, 1998; Ellenhorn and Barceloux, 1988; Farber and Torkelson, 1989; IPCS, 1999; Sittig, 1991). Effects of longer-term, "low-level" exposure are thought to be generally, although 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).

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 more than 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; IPCS, 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 be adversely affected, although the cardiovascular and gastrointestinal effects may largely be secondary to CNS toxicity (ATSDR, 1998; IPCS, 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 levulose tolerance test; and reported renal effects include albuminuria, increased serum creatinine and blood urea nitrogen, proteinuria, anuria, and hematuria (ATSDR, 1998; IPCS, 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 EPA, Dow Chemical Company (1992) presented a study of six cases of methyl chloride intoxication in workers who were chronically exposed to 200–400 ppm for at least 2–3 weeks prior to the onset of symptoms. These cases were also discussed by Scharnweber et al. (1974). Symptoms were similar in each case and included confusion, blurry vision, short-term memory deficits, balance instability, hand tremor nervousness, slurring of speech, and loss of concentration. In most cases, symptoms resolved in 1 to 3 months away from work.

The abovementioned case report is one of numerous case history studies reviewed by Repko (1981). These case reports (e.g., Noetzl, 1952; Rodepierre et al., 1955; Thomas, 1960; Thordarson et al., 1965) provide evidence that methyl chloride exposure (concentrations unknown) resulted in degeneration of areas of the cerebral cortex, spinal cord (sacral and anterior horn area), and frontal and parietal atrophy. Collectively, these case histories demonstrate symptoms of methyl chloride exposure indicative of CNS toxicity in humans: headache, nausea, double vision, mental confusion, vomiting, anuria, nervousness and emotional instability, ataxic gait, low blood pressure, insomnia and anorexia, vertigo, tremor, convulsions, increased pulse and respiration rates, coma, and eventual death. As previously noted, generally these symptoms (other than coma, and death) seem completely reversible, although some memory loss of events during exposure may remain.

In a study by Repko et al. (1976), the neurological and behavioral effects of low-level exposure to methyl chloride in the workplace were evaluated. A group of 122 methyl chloride workers (including 8 females) was compared with another group of 49 workers (including 3 females) that presumably was not exposed to methyl chloride. Both groups were matched as far as practicable for age, sex, and race (age of control group was 4.67 years younger than exposed

group). The mean ambient air concentration of methyl chloride was determined to be 33.6 ppm by conductivity analyses, whereas somewhat higher values were obtained (mean = 45.11 ppm) using charcoal tube sampling. The fact that exposure levels were only measured during the week of test performance in 1975 and no measurements or estimates of prior-year exposures (duration of exposure ranged as high as 25 years) were made represents a serious shortcoming of the study; because correlations between current and past exposure were not established, it would not be possible to associate any effects with quantitative measures of exposure.

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 electroencephalograph (EEG) tracings. Of 73 behavioral measures of task performance, comparison of means between control and exposed groups of cognitive time-sharing tasks and magnitude of finger tremor were of borderline significance, but correlation coefficients from log-transformed test values and ambient air levels for each worker indicated significant positive correlation with increasing exposure concentrations. This latter relationship was judged by the authors to be indicative of an effect of methyl chloride. Various psychological and personality tests showed no difference between exposed workers and controls. The study has been criticized (Farber and Torkelson, 1989) on the grounds that (1) the control group was much younger than the exposed group, (2) the controls were measured later in the study and at a different location, and (3) 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. Anger and Johnson (1985) also expressed concern that the data interpretation was based upon t-tests that were uncorrected for the total number of dependent variables. In summary, the subtle differences observed in finger tremor and time-sharing tasks of the 73 behavorial tasks administered to exposed and control groups for whom there is inadequate exposure characterization are inadequate for drawing any conclusions. (Note: the Repko et al. [1976] study had been used as the basis for a longer-term drinking water health advisory by EPA [1990]).

In an acute exposure study by Stewart et al. (1980), no significant neurological or cognitive abnormalities were observed in any of the 9 male and 9 female individuals exposed on a daily basis to a range of 20-150 ppm methyl chloride for 1, 3, and 7.5 hr, even at 150 ppm for 7.5 hr/day for 2 days for 1 week, or 100 ppm for 7.5 hr/day, for 5 days for 1 week. A battery of cognitive tests, including coordination and arithmetic tasks, was given to each individual. Exposure also had no major effect upon pulmonary mechanics, as evidenced by generally normal pulmonary ventilation rates, and maximum and partial expiratory volumes and flow rates. 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 methyl chloride exposures. Electrically evoked electromyograms (EMGs), designed to evaluate reflexes of the gastrocnemius muscle in methyl chloride-exposed individuals, were not significantly altered from those of controls, nor were subjective responses significantly different between control days and methyl chloride exposure days.

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 hr, 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, $p < 0.053$), whereas 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 plus 10 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. This study indicates a marginal behavioral effect of a 3-hr 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).

Rafnsson and Gudmundsson (1997), investigated mortality and cancer patterns 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 (presumably 4 days prior to discovery and repair) from a refrigerator aboard a fishing trawler, which was located under the quarters of 17 crewmen (deckhands); all but two displayed signs and 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. The first symptoms of intoxication appeared within the next 2 days. One deckhand died within 24 hr 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, whereas the 120 referents, 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). The authors concluded that the long-term sequelae of severe, acute exposure to methyl chloride may include elevated mortality from cardiovascular disease. This excess (5/18) appeared more prominent in the more highly exposed deckhands (RR = 3.9; 95% CI = 1.0-14.4) compared to all study referents (RR=2.3; 95% CI = 0.9-5.5). Because of the wide confidence intervals, which included unity, the data do not appear to be suggestive of an elevated cancer mortality risk, a risk suggested by the investigators.

Holmes et al. (1986) conducted a retrospective cohort study of workers in a synthetic butyl rubber manufacturing plant in order to determine whether there was any indication of increased mortality overall or due to 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 nonwhite) who were employed at the

facility for at least 1 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 deaths 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 reported for nonwhite 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, although 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 study authors 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 further note that the total number of deaths observed in the cohort was significantly less than expected, a finding in line with most occupational cohort studies and attributable to the "healthy worker effect." Inspection of this study's data indicates that 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. 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 would seem not to preclude a possible methyl chloride effect on cardiovascular mortality that is superimposed on a healthy worker phenomenon (resulting from reduced cancer mortality).

The mortality experiences of a cohort of 2,610 Louisiana chemical plant workers were analyzed by Olsen et al. (1989). With respect to noncancer mortality, no excesses were 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 a number of other, mostly older, case reports and human studies that have been previously summarized (ATSDR, 1998; Farber and Torkelson, 1989; IPCS, 1999). They provide descriptions of the CNS, cardiovascular, hepatic, and renal effects that can be caused in humans by exposure to methyl chloride. Most exposures appear to have been acute and of unknown duration; methyl chloride concentrations may have generally been known to be high or low, but rarely were quantified. Although some effects were noticeable within hours or a day or two of exposure, and resolved within days or several months of the cessation of exposure, in some cases, 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, no evidence for an effect of acute, severe exposure to methyl chloride on mortality from all cancers or cardiovascular disease 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 too small to generate reliable indications of carcinogenic potential. The butyl rubber worker cohort study (Holmes et al., 1986), which 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 nonwhite workers was 120 (95% CI = 44–261), whereas that for all malignant neoplasms in white workers first employed during the period from 1951 to 1960 was 107 (CI not reported; observed/expected ratio = 6/5.6). Both these increases were statistically nonsignificant.

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 the 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 nonsignificant. Similarly, mortality due to leukemia and aleukemia was nonsignificantly elevated vs. United States and Louisiana populations (SMRs of 331 and 356, respectively with corresponding 95% CI = 68–963 and 74–1,043), although the increase was statistically significant when compared with the local five-parish area (SMR = 492, CI = 101–1,437). However, the observed cases were only three in number and 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 study authors concluded that the leukemia deaths were most probably not job-related. In any case, the study did not assess the degree of exposure these workers had to methyl chloride or 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, an area specific for methyl chloride exposure. No association between exposure and increased respiratory cancer risk was found. Because of the

limited number of individuals with cancer in the group exposed to methyl chloride and the high prevalence of smoking in the study cases and controls, no conclusions can be drawn between exposure to methyl chloride and the occurrence of respiratory cancer. Earlier retrospective mortality studies had established a nonsignificant 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 ≥20 years of service (18 observed/8.5 expected; SMR = 211.9, CI = 131.9–351.6). The 41 index cases made up 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), whereas the referent group consisted of 4 matched controls for each case. Exposure to the following groups of substances was 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 because of 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 nonsignificant 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 nonsignificant 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 specific zone, although the data were very limited (<10% of the cases had ≥10 yr of service in any specific plant zone). Few cases held common job titles, and only a few of the many job titles reached statistical significance ($p < 0.05$) 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 ≥20 years earlier) generally thought to be etiologically most relevant to respiratory cancer. When the cohort exposed to methyl chloride was examined, it was "found not to be associated with increased cancer risk." Additionally, it was concluded that "the case-control study did not provide clear evidence for an association between workplace exposures at the Dow Corning Midland plant and respiratory cancer 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 very 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 study's authors.

4.2.2. Subchronic and Chronic Inhalation Studies

In a Dow Chemical Company 90-day inhalation study, McKenna et al. (1981b) exposed groups of CD-1 mice (10/sex/concentration), Sprague-Dawley rats (10/sex/concentration), and male beagle dogs (4/concentration) for 6 hr/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). Duration-adjusted exposure levels are: 0, 8.9, 26.8, or 71.4 ppm (0, 18.4, 55.4 mg/m^3). Rodents (5/sex/species/concentration) periodically underwent a simple battery of tests to evaluate sensory and motor function. 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. 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 four mice that died during exposure or were killed moribund were subjected to gross necropsy; two 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. Statistical analyses of the rodent sensory and motor function tests revealed various differences between control and methyl chloride-exposed rodents; however, these statistical differences were judged either unrelated to methyl chloride exposure or of suspect toxicological significance.

A statistically significant decrement observed was in the wire maneuver test with mice, which demonstrated some dependence on methyl chloride concentration and exposure duration. Because this performance decline was not associated with any discernible neuromuscular incoordination or other deficit, it was interpreted as a general muscular weakness 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. The identification of an unequivocal NOAEL/LOAEL for neurotoxicity from this study is questionable.

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 indicative of hepatotoxicity in either group.

Male rats and male dogs (400 ppm) displayed small but statistically significant reductions in mean RBC. Although this reduction in the rat was accompanied by a nonstatistically significant decline in mean Hgb, (1) similar findings were not seen in female rats, (2) there were no indications of dose-response, and (3) 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 postexposure 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 10 150-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 as to whether it represented an adverse or simply a subclinical 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. The gross or microscopic pathological changes observed in rats and mice sacrificed at study termination were considered incidental and typical of spontaneously occurring natural lesions commonly observed in rats of that age and strain. However, subtle reversible changes (e.g., altered tinctorial properties) were noted in the appearance of some hepatocytes from the livers of 5 of 10 male mice in the 400-ppm group. Similar changes were also observed in some control mice and in 1/7 150-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 2/4 400-ppm dogs, 1/4 150-ppm dogs and 2/4 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 by the study authors. 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 a NOAEL of 400 ppm for intermittent subchronic exposure is indicated.

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 hr/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 postexposure 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 and spinal cord) 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.

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. Vacuolar changes in the cytoplasm of hepatocytes were noted in the livers from 9 of 14 rats in the 1,500-ppm group, from 7 of 18 rats in the 750-ppm group, and from 7 of 19 control rats, and were approximately five times more prevalent in females than males. Additionally, hepatic infarctions were observed in one male mouse and one 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.

Subsequently, Battelle conducted a 24-month, chronic inhalation study in F-344 rats and $B6C3F_1$ mice for Chemical Industry Institute of Toxicology (CIIT, 1981). Groups of animals (117-120/sex/species/concentration) were exposed 6 hr/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. The duration-adjusted exposure levels are: 0, 8.9, 40.2, or 178.6 ppm (18.4, 83.0, or 368.8 mg/m^3). Interim sacrifices (typically 10/sex/species/concentration) and toxicological evaluations were scheduled for 6, 12, and 18 months after initiation of the study. There were no data from 1,000-ppm mice at 24 months because of early termination at 21-22 months. A 6-month interim status report of this study was prepared by Mitchell et. al. (1979b). 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 of 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 preselected battery of tissues (including brain) were examined by light microscopy and preselected organs weighed. Porphyrin fluorescence evaluations and neurofunction examinations were also performed.

The results of the chronic study were presented in the unpublished final report by CIIT (1981). The incidence of noteworthy histopathological lesions in mice is presented in Table 2. In the mouse exposures, 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; the effect of this error was considered negligible by the study authors.

Mortality: Mouse mortality was significantly increased in females (beginning at 10 months) at 1,000 ppm compared with controls, but was unaffected at 50 and 225 ppm. The lack of a statistically significant decrease in survival of male mice at 1,000 ppm appeared to be a result of a decreased survival in controls during the 7–18 month period combined with a rapid decline in survival of 1,000-ppm males during the 17–19 month period. In rats, mortality was not significantly affected at any concentration.

Clinical observations: 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 (atrophy of the cerebellum is usually accompanied by forms of ataxia and an unstable gait [Fonnum and Lock, 2000]) described below. 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. No clinical observations pertaining to neurotoxicity were recorded for rats.

Table 2. Principal histopathological effects in female C57BL/6 mice exposed continuously for 11 days

	Exposure Concentration (ppm)						
	0	15	50	100	150	200	400
Number examined	28	12	12	6	12	12	22
Lesion							
Pyknosis/karyorrhexis of granule cells							
Slight	0	0	0	6	0	0	2
Moderate	0	0	0	0	12	0	4
Severe	0	0	0	0	0	12	16
Decreased size of hepatocytes due to decreased glycogen							
Slight	1	0	0	0	1	0	0
Moderate	0	0	0	1	1	1	7
Severe	0	0	0	1	7	11	14
Hepatocyte degeneration or necrosis	0	0	0	0	0	1	18

Source: Landry et al., 1983c, 1985.

Weight changes: Necropsy body weights were significantly decreased in 1,000-ppm female mice at 6 and 12 months, and in 1,000-ppm males at 12 months. Statistically significant organ weight changes observed in male mice typically occurred in the 1,000-ppm group: brain, absolute decrease (6 and 12 mo); kidney, absolute decrease (6 and 12 mo); and liver and lungs, relative increase (12 mo). Those in female mice included heart, absolute increase (12 mo) and relative increase (12 and 18 mo; 225 ppm, 24 mo); brain, absolute decrease (6, 12, and 18 mo); liver, absolute increase (18 mo) and relative increase (6, 12, and 18 mo); and kidneys, relative increase (12 and 18 mo). The relationship between brain weight decreases and the cerebellar lesions (described below) was not known, nor were the reasons for an 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.

In rats, 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. 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. 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. Statistically significant absolute decreases in organ weight occurred in testes (18 and 24 mo in 1,000-ppm males), brain (1,000-ppm males and females at 18 and 24 mo), and liver (1,000-ppm

females only at 12 and 24 mo). It was noted that weight alterations in the brain, kidney, and heart were not reflected in any clinical or pathological findings, although an observation of decreased brain weights could be a reflection of a methyl chloride effect similar to that seen in mice. Heart weight changes (relative increase in 1,000-ppm females at 12 and 24 mo; absolute increase in 1,000-ppm males at 12 mo) 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.

Significant histopathology: In mice, a principal finding was degeneration and atrophy of the cerebellar granular layer. The lesion was found in 1,000-ppm mice that died spontaneously between 0 and 17 months (15/24 males, 9/20 females) and 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. No ophthalmic effects were observed in mice of either sex at any concentration.

Although an increased incidence of minimal to moderate axonal swelling and degeneration of the spinal nerves (thoracic, cervical, and lumbar areas) was observed in all exposed mouse groups (principally females) beginning with the 18-month through the 22-month sacrifice (only 1,000-ppm females examined at 22 months), no dose-response and no functional abnormalities were observed. In addition, at the 24-month end-of-study sacrifice, there was no difference in incidence between exposed and control animals. It was stated in the text that axonal swelling and degeneration of minimal to mild severity was observed in all groups (not clear if this included controls) at 12 months. 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. Significantly elevated ALT levels were observed in male mice (1,000 ppm at 6, 12, and 18 mo, but not at 24 mo; 50 and 225 ppm only at 6 mo), which were consistent with histologically observed hepatocellular degeneration and necrosis at the 1,000-ppm concentration. Elevated ALT levels were not found in female mice at any concentrations (as measured by ANOVA or Kruskal-Wallis tests of variance). AST levels were not elevated. All groups, including controls, were hypoglycemic at 24 months. No other hematological, clinical chemistry, opthamologic, or urinalysis findings in mice could be related to methyl chloride. Porphyrin fluorescence evaluations of fresh liver impression smears made at necropsy were negative.

Renal tubuloepithelial hyperplasia and karyomegaly (severity ranged from minimal to mild) were first apparent in 1,000 ppm male mice at 12 months, subsequently increasing in incidence and severity until the last males in this group were sacrificed at 21 months. Renal tumors were confined to male mice as shown in Table 3. Renal tumors were not detected in either female mice or in both sexes of rats.

Renal tumors were significantly increased in 1,000-ppm males during months 12–21 (due to early termination, no data from this group at 24 months was available): 17 renal neoplasms

Table 3. Renal tumors in male B6C3F1 mice

Exposure (ppm)	0	50	225	1,000
Incidence	0/67	0/61	2/57	22/86
% Incidence	0	0	3.5	26

Source: CIIT, 1981.

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 two 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 seven males and one female from the 1,000-ppm group, as well as in one male and one female from the 225 ppm group at 24 months. Also at 24 months, microcysts were observed in six males from the 50-ppm group, and one 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 possibly methyl chloride-related. However, the low incidence in the 225-ppm group suggests that it may be a spontaneous lesion. Unpublished data (Johnson, 1988) for controls from eight 2-year mouse studies indicate that the incidence values for renal microcysts from the CIIT (1981) study fall within the Dow Chemical Company's historical control incidence for this strain. In addition, examination of nonneoplastic lesions in the $B6C3F_1$ from 122 chronic studies (drinking water, gavage, and inhalation) indicated that in no case was there a dose-response relationship between chemical exposure and cyst formation. In fact, control animals in inhalation studies (e.g., butadiene, acetonitrile, toluene) often evidenced a higher incidence of renal cysts than exposed mice. Secondly, if one considers the incidence of kidney cysts (no microcysts) in the NTP chronic inhalation study (TR 385) for methyl bromide (NTP, 1992), structurally very closely related, there also was no clear dose-response in male $B6C3f_1$ mice.

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 — relatively common in laboratory mice.

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. 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 observed only in 1/10 1,000-ppm males at 6 months; at 12 months, in 4/10 at 1,000 ppm; at 18 months in 10/20 (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 two male rats of the 1,000-ppm group at 6 months, and in one 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. Ophthalmologic examination revealed sporadic corneal cloudiness and opacity across all groups at 6 months, which 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, perhaps as a result of SDA virus outbreaks. In any case, 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.

Conclusions: 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 because tubule degeneration and atrophy at this exposure level occurred upon onset of age-related hyperplasia and compressive adenomas. In mice, 1,000 ppm was identified as an FEL based on high mortality. At this concentration an increased incidence of cerebellar degeneration and atrophy, splenic atrophy, lymphoid depletion and seminiferous tubule degeneration was observed. The NOAEL, based on the absence of the aforementioned effects, is 225 ppm. Axonal swelling and degeneration in areas of the spinal cord in mice are considered not to have neurotoxicological significance because of the absence of functional deficits and lack of a dose-response. Distal axonopathy associated with exposure to neurotoxicants has recently been reviewed by LoPachin et al. (2000), who concluded that axonal swelling and degeneration is a nonspecific phenomenon that is not related to neurophysiological deficits or behavioral toxicity. A shortcoming of this study relates to some incorrect sexing (periodic pregnancies were observed in the mouse population) and misplacement of specific mice. The investigators considered the problem serious but not one that threatened the validity of interpretation of the experimental results.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

No studies in either humans or animals on the reproductive or developmental effects of methyl chloride by the oral route of exposure were located in the available literature.

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 as well. 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).

One study by Huel et al. (1990) attempted to assess the potential adverse reproductive outcomes among former female workers in a New Mexico microelectronics assembly plant. The study appeared to identify an increased risk of spontaneous abortion in 90 former worker-referent pairs after the beginning of employment among the female workers when compared with referents. However, methyl chloride was only one chemical among many (e.g., chlorofluorocarbons, chlorinated hydrocarbons, glycol ethers, isopropanol, acetone, toluene, xylene, "alcohol") to which the women were exposed. 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 so is of limited value.

Exposure of Sprague-Dawley rats for 48–72 hr 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 (Burak 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, multinucleated 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 hr/day on 9 of 11 days to 0, 2,000, 3,500, or 5,000 ppm (4,130 or 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) in F-344 rats and B6C3F$_1$ mice (Mitchell et al., 1979b; CIIT, 1981), degeneration and atrophy of the seminiferous tubules were observed in the testes of rats and mice at 1,000 ppm and possibly an increased incidence of sperm granulomas in rats, especially after 6 months of exposure.

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 hr/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 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 hr was also found to reduce NPSH levels in the testes, epididymis, and liver (relative to 0 hr 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 hr/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 40 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 hr/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. The percentages of females mated by males in the 1,000 ppm and TEM groups were not significantly different from controls at any week postexposure, but were reduced for experiment-1 3,000-ppm males during the first 2 weeks postexposure (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 nonsignificant 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 postexposure 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 postexposure 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 postexposure 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 postexposure, 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 with 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, whereas sperm count in the vas deferens began declining by week 2, 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 postexposure; 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 hr/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 from 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 in large measure be the result of failure of fertilization due to low sperm number and poor sperm quality, i.e., due to 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 hr/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 two untreated females. Twelve hours postmating, 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 postexposure 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 hr, 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.

Studies by Chellman et al. (1986a) had indicated that co-treatment with the anti-inflammatory agent 3-amino-1-[m-(trifluoromethyl) phenyl]-2-pyrazoline (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 postexposure. 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 another report from this laboratory (Chellman et al., 1986b), BW755C (10 mg/kg ip, 1 hr 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) in rats exposed to 7,500 ppm methyl chloride 6 hr/day for 2 days. Rats exposed 6 hr/day for 5 days to 5,000 ppm methyl chloride without BW755C exhibited degenerative lesions in the testis and epididymis (including epididymal sperm granulomas). Additional effects (without BW755C) included hepatocellular cloudy swelling, renal tubular degeneration, adrenal cortex vacuolar degeneration, and cerebellar necrosis of the granule cell layer. With the exception of adrenal gland vacuolation, BW755C co-treatment prevented the detectable occurrence of all these methyl chloride-induced lesions.

To determine whether the observed cytotoxic effects on sperm were the result of methyl chloride's effects on the testes or on the epididymides, Chellman et al. (1987) exposed groups of male F-344 rats (18/group) for 6 hr/day for 5 consecutive days to 0 or 3,000 ppm methyl chloride, with or without concurrent exposure to BW755C, 10 mg/kg by ip injection, 1 hr pre- and postexposure. 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. In the vas deferens, sperm numbers were normal in 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. This study's findings, in conjunction with known epididymal transit times for sperm in F-344 rats, led the authors to conclude that the preimplantation losses observed at weeks 2 and 3 postexposure (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.

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 hr postexposure 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). It was 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 tissues (e.g., brain) where toxicity is not accompanied by an apparent inflammatory response, Chellman et al. (1987) 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.

A two-generation reproduction study with 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 hr/day, 5 days/wk. During one protocol, 10 males were exposed for 10 weeks, after which exposure was 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 2-week breeding period, the 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 gestational day 18 (GD 18) through postnatal day 3 (PND 3); exposure was resumed 6 hr/day, 7 days/wk during PNDs 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; 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. No consistently significant differences in litter size, sex ratio or 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 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 significantly 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 reduction in the number of F_0 males proven fertile and fewer litters with an altered sex ratio produced by the F_1 generation, the two-generation reproductive LOAEL in this study was 475 ppm with a corresponding NOAEL of 150 ppm.

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 hr/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 GD 7 through GD 19, then were sacrificed on GD 20; mice were exposed from GD 6 through GD 17 with sacrifice on GD 18, except for the 1,500-ppm group, in which the animals were sacrificed in extremis during GDs 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 GD 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 GD 9 (the fourth day of exposure), the severity of which required premature sacrifice on GDs 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 nonsignificant 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 (six fetuses), or the bicuspid valve, chordae tendineae, and papillary muscles in the left atrioventricular septum (three 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. In this study, histopathological examination of F_1 brain tissue was not performed.

To further explore the apparent teratogenic effect on the heart in B6C3F_1 fetuses, 74–77 female C57BL/6 mice impregnated by C3H males were exposed 6 hr/day from GD 6 through GD 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 (GD 12), females in the 750-ppm group displayed ataxia, tremors, convulsions, and hypersensitivity to touch or sound; prior to the scheduled sacrifice, six of these animals died and one 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 GD 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 percent 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 and 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 ($p < 0.005$). Histopathological examination of tissues was not performed. The authors thus concluded that in pregnant C57BL/6 female mice and their B6C3F_1 conceptuses, methyl chloride exposure during gestation (GDs

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.

It should be noted that these findings of teratogenicity in the mouse have been questioned (John-Greene et al., 1985). These authors present discussion on 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 hr (GDs 11.5–12.5, the developmental stage they considered the most critical for cardiac structures), or 1,000 ppm for 12 hr (GDs 11.5–12), John-Greene et al. (1985) were unable to demonstrate the lesion's occurrence. In her 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 his staff, and that great care was used in cutting the hearts for examination. She also pointed out the differences in exposure regimen, 6 hr/day for GDs 6–17 versus 12–24 continuous hours on GD 11.5 to GD 12–12.5, discussing in particular why exposure beyond GD 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.

4.4. OTHER STUDIES

4.4.1. Acute and Subacute Inhalation Studies

As described in Sections 3.2 and 3.3, methyl chloride exposure in rats results in time- and concentration-dependent depletions on tissue (e.g., liver, kidney, testes) levels of NPSH. The potentially toxic consequences of NPSH (principally GSH) depletion have not been fully characterized, but may include conversion of methyl chloride-GSH conjugates to toxic intermediates (e.g., methanethiol and formaldehyde), enhancement of the toxicity of other chemicals that are normally detoxified by conjugation with GSH, reduction in the capacity 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. (1986c), using male $B6C3F_1$ mice, examined the role of GSH (measured as NPSH) in mediating the acute toxicity of methyl chloride exposure in brain, liver, and kidney target tissues. They found that when groups of mice (3–5/group) were pretreated (at -1.5 hr) with 4 mmol buthionine-S,R-sulfoximine (BSO), a potent and specific inhibitor of γ-glutamylcysteine synthetase, the rate-limiting enzyme for de novo synthesis of GSH and then exposed for 6 hr to 2,500 ppm methyl chloride, methyl chloride toxicity was prevented. Relative to control (–BSO) values at 0, 3, and 6 hr of methyl chloride treatment, GSH 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 methyl chloride lethality at 18 hours 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 hr/day, 5 days/wk for 2 weeks, ± daily pretreatment with 2 mmol BSO. It was observed that BSO pretreatment 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 hr 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 hr was found 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/L, respectively). With respect to kidney toxicity in animals treated 6 hr/day, 5 days/wk for 2 weeks to 1,500 ppm, 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 threefold in male mice and 8.5-fold in female mice by the 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, thus preventing the formation of methyl chloride-GSH conjugates that would result in the metabolic conversion to toxic intermediates.

In a subacute inhalation study reported by Dow Chemical Company (Burak et al., 1981), Sprague Dawley rats (40/sex/concentration) were exposed for 48 hr 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. 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 hr of exposure, animals exposed to 1,000 or 2,000 ppm appeared progressively less alert, and by 48 hr the 1,000 ppm rats appeared lethargic, while the 2,000-ppm rats were either lethargic, moribund, or dead. After 72 hr of exposure, the 1,000-ppm rats were either sick or moribund, though still alive, while all those in the 2,000-ppm group were dead. The primary cause of death in rats exposed to 1,000 or 2,000 ppm for 48 or 72 hr 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.

After 72 hr 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. 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 hr–1,000 ppm group (although not of males in the 48 hr–1,000 ppm, or in any of the 500- or 200-ppm groups); the testicular weight decreases were considered secondary to concomitant epididymal lesions.

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 hr–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 hr: 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 these rats not eating. Similar but less severe effects were noted in the 72 hr–1,000 ppm group. Decreases in serum AP levels were also observed in all 500- and 200-ppm groups (48 or 72 hr exposures), were statistically significant for 48 and 72 hr–500 ppm males and for 72 hr–200 ppm females, and were considered treatment-related, probably the result of decreased food intake. Altered urinalysis values in the 48 and 72 hr–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 for 48 hr. Liver toxicity was noted in all exposure groups at 72 hours. This ranged from dark livers, necrosis, inflammation, and degenerative changes (increased lipid, variable-sized nuclei, altered tinctorial properties) seen to varying extent in 1,000- and 2,000-ppm rats, to altered tinctorial appearance of hepatocytes and slightly elevated lipid content in 72 hr–200 ppm males and females, respectively; these minimal effects appeared to be reversible. The primary cause of death in rats exposed to 1,000 or 2,000 ppm for 48 or 72 hours was kidney toxicity and subsequent renal failure. 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 hr 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. No brain lesions were seen in any of the treated animals. In summary, this study suggests a subacute LOAEL of 200 ppm methyl chloride, based upon minimal and reversible liver effects seen in rats. Liver and kidney effects predominated at higher doses, with testicular effects occurring at 500 ppm and higher.

In another Dow Chemical Company study (McKenna et al., 1981a), three groups of three male beagle dogs (ages 7–8 mo) and three male cats (ages 8–9 mo) were exposed for approximately 23.5 hr/day for 3 days (i.e., 72-hr treatment regimen) to methyl chloride

concentrations of 0, 200, or 500 ppm (0, 413, or 1,031 mg/m^3). Neurological examinations were performed on all dogs on postexposure day 4 and again on postexposure 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).

Dogs: During the first 24 hr of treatment, no differences in demeanor or condition were observed between control and methyl chloride-exposed dogs. After 48 hr of exposure, 500-ppm dogs appeared more tranquil, with one exhibiting intermittent tremor and slight excess salivation, but all were judged alert and responsive. 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 forelimb 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 hr postexposure. Improvement was noted in all 500-ppm dogs by postexposure day 10, which continued until termination on day 27. The most severely affected dog was able to get up and take several steps by postexposure 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. Dogs in the 500-ppm group exhibited 5%–8% body weight losses that persisted for several days, then slowly recovered to pretreatment values by postexposure days 19–25. 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 postexposure). Immediately after 72 hr of treatment, control and 200-ppm dogs were comparable.

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 postexposure), 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 postexposure, 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 postexposure day 4, and appeared to be fully or nearly completely recovered on postexposure day 26. No significant alterations in urinalysis parameters were observed. Ophthalmoscopic examination revealed no treatment-related eye lesions or abnormalities. Clinical chemistry parameter changes were judged not treatment-related nor toxicologically important. No statistically significant alterations in mean organ weights were observed.

No treatment-related alterations in gross or histopathology were observed in any 200-ppm dogs. 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 brainstem and the lateral and ventral funiculi of the spinal column, and were not observed in the cerebrum, cerebellum, or peripheral nerves. Several characteristics of these lesions (perivascular aggregates of mononuclear cells, location in the cerebrum and cerebellum as well as the midbrain, presence in one of three cats in the control and 200-ppm groups) led the authors to speculate that they were likely the result of either a postvaccinal 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.

Cats: During the first 48 hr of exposure, the 200- and 500-ppm cats evidenced a decline in appetite that then recovered, and after 24 hr 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 2-week recovery period, 200- and 500-ppm cats were comparable to controls. The 500-ppm group experienced a transient loss in body weight that was statistically nonsignificant. No significant alterations in urinalysis parameters or mean organ weights were observed. Ophthalmoscopic examination revealed no treatment-related eye lesions or abnormalities. No statistically significant differences in clinical chemistry values were found. Brain and/or spinal cord lesions were found in control (1/3), 200-ppm (1/3), and 500-ppm (3/3) cats; this likely represents a treatment-related effect at the high dose.

The findings of this study indicate a NOAEL of 200 ppm for a nearly continuous 72 hr 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; about 10 weeks old at time of exposure) "continuously" (22–22.5 hr/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 hr/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. Neurofunctional testing was conducted during the course of the study, which consisted of monitoring mice (previously trained for 2 weeks 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 hr postexposure after 4, 8, and 11 days of exposure. Upon termination, the nonfasted mice were subjected to gross and histopathological examination (to include brain, thymus, liver, and kidneys). Body and organ weights were obtained, as were samples of most major organs and tissues (including spinal cord). Tissue samples from the cerebella of three preselected mice were examined by electron microscopy from each of the 0 and 150 ppm continuously exposed groups after 1, 2, 4, 6, 8, or 10.5 days of exposure.

Continuous exposure: No exposure-related mortality was observed in mice exposed continuously to the lower concentrations of methyl chloride (15 and 50 ppm), whereas exposure to 400 or 200 ppm was lethal after 4 or 5 days, respectively, after leading to loss of appetite and ataxia with frequent falling. Mice exposed to 150 to 400 ppm developed poor motor coordination and deteriorated to a moribund condition with accompanying inanition (i.e., marked

weakness) at a rate that was dose-dependent. Mice in the 200-ppm group were sacrificed on day 5 because one mouse had died prior to scheduled necropsy and most of those remaining were moribund. Mean body weights were significantly diminished in the 200- and 150-ppm groups and somewhat diminished at 100 ppm, but were not affected at 50 ppm. Body weights were not obtained for 400-ppm mice. No significant decrements in rotating rod performance were noted for the control and 15- to 100-ppm groups, while substantially diminished performance was seen in the 150-ppm group after 4 and 8 days, with animals moribund or dead by day 11. Moribund mice in the 200 ppm group scored zero in this test.

No organ weight data were reported for the 200- and 400-ppm groups. Mean relative (but not absolute) kidney weights were increased in the 150-ppm group by 9% relative to controls (no increase at day 8), but not in the 50- and 15-ppm groups. Kidney weight data were not obtained for the 100-ppm group. Statistically significant increases in mean absolute and relative liver weights were reported at the end of exposure, whereas statistically significant decreases in one or both of these values were reported for the 150-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. Statistically significant decreases in mean and relative thymus weights in the 15- and 50-ppm (but not at 100 ppm) groups were judged spurious by the study authors because corroborating histopathology data were not seen; the mean weights were within the range of other control groups in this study. The decrease at 150 ppm was considered exposure-related, with the only histopathological finding being thymic involution (7/12 minimal and 5/12 marked), reflecting decreased body weights and stress.

Gross pathology observations included significant inanition in 400- and 200-ppm mice prior to death or sacrifice, and in some 150-ppm mice after 4 or more days of exposure; no gross pathology observations considered exposure-related were recognizable in 15-, 50- or 100-ppm mice. Exposure to 100 or more ppm resulted in concentration- and duration-dependent degenerative changes to the cerebellum, principally in the granule cells, that were characterized by nuclear pyknosis and karyorrhexis, the latter referring to the rupture of the cell nucleus in which chromatin disintegrates. These effects were observed most frequently in the dorso-medial cerebellar folia. At 150 ppm there was a marked loss of granule cells, a decrease in Purkinje cells, and an increase in macrophages. Lesions were more severe in to 200- and 400-ppm groups. Transient intra- and extracellular vacuolation in the Purkinje and/or molecular cell layer and in the white matter were also noted. Electron microscope observations were consistent with those obtained through light microscopy. Decreased glycogen content in 100- to 200-ppm mice was the principal significant change observed in the liver, although focal periportal hepatocellular degeneration and/or necrosis was noted in the 400-ppm group. No exposure-related histopathological effects were observed in the 15- and 50-ppm groups. The principal histopathological effects for mice continuously exposed for 11 days are in shown in Table 2. Duration-dependency of lesions was examined by serial necropsy of 150-ppm animals on days 1, 2, 4, 6, 8, and 11. Incidences from these necropsies for selected lesions are shown in Table 3.

Intermittent exposure: A transient (i.e., at 0.5 hr, but not 3 hr, postexposure) sedation was observed in 1,600- and 2,400-ppm groups at 4–7 days of exposure, but not after 8 days. Inanition was apparent in the 2,400 group (also slow movement and roughened haircoats), 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, which was microscopically confirmed. The in-life observation of red urine in the 2,400-ppm group was determined to result from hemoglobinuria consistent with intravascular hemolysis (hemoglobinemia), rather than from hematuria. These animals deteriorated (e.g., hind limb extensor rigidity) and were sacrificed moribund on days 8–9. The 1,600-ppm group had decreased ingesta. Mice exposed to 1,600 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-ppm group but were not affected at 1,600 ppm. No significant decrements in rotating rod performance were noted for the control and 150- and 400-ppm groups.

Mean relative (but not absolute) kidney weights were increased 19% in the 2,400-ppm group relative to controls, but not in the 150-ppm group (kidney weight data were not obtained for the 1600-, 800-, or 400-ppm groups). Microscopically, evidence of kidney toxicity was found only in the 2,400-ppm group and consisted of slight multifocal tubular degeneration and regeneration and eosinophilic-staining tubular casts. Statistically significant increases in mean absolute and relative liver weights were reported at the end of exposure for the 1,600-ppm group. No other liver weight changes were statistically significant. Decreased hepatocyte size, without degeneration or necrosis, were variably seen in mice from the 400 through 2,400 groups (Table 4). 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- and

Table 4. Duration dependence of principal histopathological lesions in 150 ppm female C57BL/6 mice exposed continuously for 11 days

	ppm	Day 1	Day 2	Day 4	Day 6	Day 8	Day 11
Number necropsied	0	5	5	5	5	5	12[a]
	150	5	5[b]	5	5	5	12[c]
Cerebellum							
Vacuolation of white matter (slight)	0	0	0	0	0	0	0
	150	0	0	0	1	0	12
Increased pyknosis/karyorrhexis of granule cells (multifocal)	0	0	0	0	0	0	0
	150	0	0	5	2	5	12
Loss of cells in granule layer with focal severe areas containing macrophages	0	0	0	0	0	0	0
	150	0	0	0	0	4	12

[a] These controls were controls for 15- and 50-ppm animals and were actually sacrificed on day 12 with those animals.
[b] Several tissues from one mouse were lost during tissue processing.
[c] This group of 150-ppm animals were sacrificed on day 11, one day prior to the others. Two were removed (one moribund, the other dead) prior to the 11-day sacrifice. Results of these two animals included in the data.
Source: Landry et al., 1983c, 1985.

1,600-ppm groups; the latter group evidenced a decrease in the size of the thymus. No gross exposure-related effects were noted in the 400-ppm or 800-ppm groups. A concentration-related increase in the incidence of pyknosis and karyorrhexis (slight) was observed in the 400-ppm and higher groups (see Table 4).

Conclusions: Based upon cerebellar damage, this study identifies a NOAEL and LOAEL of 50 and 100 ppm, respectively, for continuous exposure. For intermittent exposure, the NOAEL and LOAEL are 150 and 400 ppm, respectively. There was no evidence of damage to spinal or peripheral nerves in either exposure protocol. 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 cerebellar lesions were observed in 150-ppm mice exposed continuously that also demonstrated impaired rotorod performance, suggesting a causal relationship. There was no effect of continuous exposure of 100-ppm mice on rotorod performance, although there was evidence in all animals of cerebellar pyknosis and karyorrhexis; severity was graded as slight.

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 5. Groups of rats (10/sex/concentration) were exposed 6 hr/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 hr/day to 0, 500, 1,000, or 2,000 ppm for 12 consecutive days.

Table 5. Principal histopathological effects in female C57BL/6 mice exposed intermittently

	Exposure concentration (ppm)					
	0	**150**	**400**	**800**	**1,600**	**2,400**
Number examined	28	12	6	6	17	12
Lesion						
Increased pyknosis and karyorrhexis of granule cells						
Slight	0	0	2	4	11	12
Decreased size of hepatocytes due to decreased glycogen						
Slight	8	0	1	0	0	0
Moderate	2	0	2	0	4	2
Severe	0	0	0	3	0	3

Source: Landry et al., 1983c, 1985.

Mice: 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. With the exception of one 1,000-ppm C3H male that died on day 11, 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 C3H females by day 4, in all 1,000-ppm females by day 8, and in 2,000-ppm C3H males on days 4–5.

Focal degeneration of the cerebellar internal granular layer was observed primarily in C57BL/6 females at 1,000 and 2,000 ppm and consisted 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. From Table 5 it appears that the lesion demonstrates a marked strain and sex specificity, occurring principally in C57BL/6 females.

Rat: Following the day 5 exposure, 13 rats were sacrificed in extremis (5,000 ppm: 6 males and 5 females; 3,500 ppm: 2 females) with some having convulsions, hind limb paralysis, and forelimb ataxia. It was noted that the rats seemed to tolerate the methyl chloride exposure much better during the second week (days 8–11). Rats appeared more resistant to methyl chloride's toxicity. There was minimal histopathological evidence of cerebellar degeneration (foci of internal granular layer cells having pyknotic nuclei with distended, hydropic perikarya). 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 males. 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 multinucleate 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.

Conclusion: For intermittent exposure of 9–12 days, the LOAEL in rats based upon renal, hepato- and testicular-related toxicity was 2,000 ppm (lowest concentration tested), whereas the NOAEL and LOAEL for neurotoxicity were 3,500 and 5,000 ppm, respectively. In mice, the LOAEL for hepatotoxicity was 500 ppm, the lowest concentration tested. However, the dose-response was not clearly apparent in all the mouse strains/sexes. Cerebellar degeneration, which was most demonstrable in the female C57BL/6 mouse, supports a NOAEL and LOAEL of 500 and 1,000 ppm, respectively. These levels are in apparent contrast to the intermittently exposed female C57BL/6 mice in the study by Landry et al. (1983c, 1985), in which the NOAEL and LOAEL were 150 and 400 ppm. The reason(s) for this apparent inconsistency are unknown. Species, strain, and sex differences in susceptibility to methyl

chloride neurotoxicity together with histopathological differences between studies highlight the difficulties in extrapolation of animal results to humans.

Jiang et al. (1985) conducted an ultrastructural study of lesions induced in the cerebella of C57BL/6 mice (10 females/concentration) exposed 6 hr/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): (1) a coagulative necrosis (also seen in controls, but in milder form and in substantially fewer cells) involving nuclear and cytoplasmic condensation; and (2) 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.). No incidence data were provided. 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 just two animals), leading the study authors to conclude that the reported 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).

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 minutes 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, whereas 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. All these symptoms were reported to have occurred in four 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.

Table 6. Histopathology of mice and rats exposed to methyl chloride for 9–12 days: severity[a] (incidence)[b]

		C3H Mouse		C57BL/6 Mouse		$B6C3F_1$ Mouse		F-344 Rat	
Lesion	ppm	M	F	M	F	M	F	M	F
Cerebellar	5,000							+ (3/10)	+ (2/9)
degeneration	3,500							– (0/10)	– (0/10)
	2,000	– (0/5)	– (0/5)	– (0/5)	+++ (4/4)	– (0/5)	+ (2/5)	– (0/10)	– (0/10)
	1,000	– (0/4)	– (0/5)	+ (3/5)	++ (5/5)	– (0/5)	– (0/5)		
	500	– (0/5)	– (0/5)	– (0/5)	– (0/5)	– (0/5)	– (0/5)		
Degeneration and	5,000							+++ (10/10)	++ (10/10)
necrosis of renal	3,500							+ (10/10)	+ (5/10)
proximal	2,000	+++	+++	++ (3/5)	++ (5/5)	+ (1/5)	+++ (5/5)	+ (8/10)	– (0/10)
convoluted tubules	1,000	(5/5)	(5/5)	– (0/5)	– (0/5)	– (0/5)	– (0/5)		
	500	+ (1/5)	– (0/5)	– (0/5)	– (0/5)	– (0/5)	– 0/5)		
		– 0/5)	– 0/5)						
Basophilic renal	5,000							– (0/10)	– (0/10)
tubules	3,500							– (0/10)	– (0/10)
	2,000	– (0/5)	– (0/5)	– (0/5)	– (0/5)	– (0/5)	– (0/5)	– (0/10)	– (0/10)
	1,000	+ (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	5,000							+ (10/10)	+ (9/10)
degeneration	3,500							+ (9/10)	+ (9/10)
	2,000	+ (4/5)	– (0/5)	+++ (5/5)	– (0/5)	++ (5/5)	+ (4/5)	– (0/10)	+ (8/10)
	1,000	– (0/4)	– (0/5)	+ (3/5)	+ (3/5)	– (0/5)	– (0/5)		
	500	+ (2/5)	– (0/5)	+ (3/5)	+ (2/5)	– (0/5)	– (0/5)		
Testicular	5,000							+++ (10/10)	
degeneration	3,500							+ (10/10)	
	2,000							+ (10/10)	
Adrenal fatty	5,000							+++ (10/10)	+++ (10/10)
degeneration	3,500							+ (4/10)	++ (10/10)
	2,000							– (0/10)	– (0/10)

[a] Severity grades (overall for the group): (–) = not observed, (+) = minimal, (++) = moderate, (+++) = severe

[b] Incidence = (number affected/number examined).

Source: Modified from Morgan et al. (1982).

4.4.2. Genotoxicity Studies

A number of studies have explored methyl chloride's potential for genotoxicity. In the *Salmonella typhimurium*/microsome reverse mutation assay (the Ames test), methyl chloride was tested at concentrations of 1% (10,000 ppm), 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); methyl chloride (concentration not specified) also was positive in transformation of baby hamster kidney fibroblast (BHK21) cells. Similarly, 2.5% to 15% methyl chloride (with or without S9 at 37C for 8 hours) was found to be mutagenic in TA100, with the number of revertants increasing with concentration (Simmon et al., 1977); 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, Fostel et al. (1985) also demonstrated that a 3-hr 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 SCE; approximate increases were 14- and 3-fold, respectively. 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). Of interest with respect to SCE are the findings of Hallier et al. (1993) that methyl bromide, ethylene oxide, and dichloromethane induced SCE in lymphocytes from a human subgroup characterized as "slow metabolizers" in terms of GST polymorphism, but not in "fast metabolizers." It remains to be established whether methyl chloride behaves similarly.

Alkaline elution was also used to analyze kidney cell DNA from male B6C3F$_1$ mice that had been exposed 6 hr/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-hr 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 hr postexposure 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, although 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 hr to 1,000 ppm of methyl chloride and sacrificed at 0, 5, or 48 hr postexposure, or exposed 6 hr/day for 4 days and sacrificed 0 or 5 hr postexposure (Ristau et al., 1990). In the single-exposure groups, evidence for DPC, but not SSB, was again observed immediately after exposure; at 5 hr postexposure, DPC were no longer apparent, but there was evidence of SSB; by 48 hr, no significant indications of either DPC or SSB were found. In the 4-day exposure groups, only a slight indication of DPC at 0 hr postexposure and low levels of SSB 5 hr 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 through 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-labeled 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 unmodified purine bases, suggesting anabolic incorporation. 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 hr/day exposure to 3,500 ppm, but weakly positive after a single 3-hr 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 hr/day to 3,500 ppm for 1, 3, 5, or 9 days, or for a single 3-hr 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 postmitotic germ cell stages (University of Wisconsin, 1982; Bioassay Systems, 1984); the enhancement of viral transformation in cultured Syrian hamster embryo (SHE) cells by a 20-hr 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 that (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 postexposure, and a significant increase in preimplantation loss throughout 8 weeks of postexposure 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, although methyl chloride appears to possess direct genotoxic activity at relatively high concentrations (5,000 ppm and greater) 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. Although these data suggest that a genotoxic response in humans may be marginal in magnitude, the observation that (1) single-strand breaks do occur, (2) SCE are induced by methyl chloride, and (3) SCE are induced by a structurally related chemical, methyl bromide, in human subgroups on the basis of their GST polymorphisms are cause for concern and warrant characterizing methyl chloride as a possible human mutagen.

4.4.3. Mode of Action in Relation to Toxicity

Although 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, 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 weak (i.e., at high concentrations) direct-acting mutagenicity has been demonstrated 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. However, it has been shown to cause SCE.

Second, in some cases inflammatory responses have been demonstrated to be responsible for methyl chloride's dominant lethal effects, both 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 endproducts (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. Specific inhibition of GSH formation has been shown to prevent lethality and target organ toxicity, including cerebellar lesions, in acute exposures of laboratory animals to methyl chloride (Chellman et al., 1986b). 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. There are no data at present, however, to support the proposed differences in toxicological outcomes other than the finding that methyl bromide induces an increase in SCEs in those characterized as "slow metabolizers" compared with "rapid metabolizers."

Finally, data from several studies (Jäger et al., 1988; Hallier et al., 1990; Dekant et al., 1995; Hu et al., 1990; Cummings et al., 1999, 2000) 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, was negligible in preparations from either sex of rat (Dekant et al., 1995) and CYP2E1 expression in female mice was elevated by testosterone (Hu et al. 1990). 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. Because CYP2E1 has not been found in the kidneys of humans of either sex (Amet et al., 1997; Cummings et al., 2000; Lasker et al., 2000), extrapolation of the findings of renal tumors in the chronic mouse

study (CIIT, 1981) to humans may not be warranted. It must be recognized, however, that other P-450 families (i.e., CYP3A and CYP4F) are found in human kidney that may metabolize methyl chloride to toxic intermediates in the kidney, although confirmation is needed.

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 human exposure via the oral route. However, both short- and long-term drinking water health advisories have been established (U.S. EPA, 1990) based on inhalation studies (Putz-Anderson et al., 1981 and Repko et al., 1976, respectively) that were extrapolated to drinking water situations. Discussion and limitations of these studies are described in Section 4.1.1. Similarly, insufficient data exist for evaluating the effects in animals of oral exposure to methyl chloride. 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 some dermal exposure could be possible).

Following acute inhalation exposure, the most prominent symptoms 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 (that also include observed degenerative changes in the brain and spinal cord) 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 generally considered relatively mild. In the only occupational study, stated findings of decrements in two administered tests associated with low-level exposures at the time of testing are equivocal because prior exposures were not characterized.

There were two clinical studies in which previously unexposed individuals were exposed to measured levels of methyl chloride and then administered performance tasks (Stewart et al., 1980; Putz-Anderson et al., 1981). Exposure of volunteers to 20–150 ppm for up to 7.5 hr/day over 2–5 consecutive days revealed no detrimental effects on physiological and clinical parameters, nor were any neurological abnormalities or biologically significant decrements seen in cognitive functioning, including arithmetic task performance. In another clinical study, exposure of volunteers for 3 hours to 200 ppm did produce marginally significant decrements in

two performance tasks; visual vigilance and time discrimination were the most sensitive indicators for methyl chloride impairment.

Studies in laboratory animals, both short- and long-term, provide confirmation that CNS effects are the most relevant for human exposure scenarios. The principal adverse effects (relevant to known clinical manifestations of methyl chloride toxicity in humans) observed in acute (mouse, rat, guinea pig, dog, and cat) and chronic studies (mouse) are degenerative brain (cerebellum) cell changes. High level exposure (e.g., 1,000 ppm, 6 hr/day, 5 days/week) have also caused degenerative changes in rat testes. Five subacute studies (continuous or intermittent exposures over 2–12 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 (Burak et al., 1981; Jiang et al., 1985; Landry et al., 1983c, 1985; Morgan et al., 1982; McKenna et al., 1981a).

The study by CIIT (1981) in the $B6C3F_1$ mouse provided clear evidence that a 2-year exposure, 6 hr/day, 5 days/wk resulted in cerebellar degenerative lesions, testicular lesions, renal toxicity, splenic atrophy, and hepatocellular lesions, all at 1,000 ppm, a concentration that also produced mortality, particularly in females. Testicular toxicity was the predominant lesion seen in the rat at this concentration; no cerebellar lesions were observed, nor was there treatment-related mortality. Intermittent exposure (over 9–12 days) of several mouse strains and the F344 rat to concentrations of 500–5,000 ppm (Morgan et al., 1982) provided confirmation of effects seen in the sole chronic study. The female C57BL/6 mouse appeared to be more susceptible to cerebellar lesions than either the male C57BL/6 or either sex of the $B6C3F_1$ mouse because moderate to severe lesions were observed in the female C57BL/6 from 1,000 to 2,000 ppm, respectively, whereas only 3/5 C57BL/6 males (at 1,000 ppm) and 2/5 $B6C3F_1$ females (at 2,000 ppm) evidenced lesions of minimal severity; no cerebellar lesions were observed in the C3H mouse (both sexes) and only lesions of minimal severity were seen in some F344 rats (both sexes) at 5,000 ppm. No cerebellar lesions were seen in any species at 500 ppm.

In contrast to the occurrence of cerebellar lesions (and mortality) at 1,000 ppm (but not at 50 and 225 ppm) in $B6C3F_1$ mice exposed intermittently for 2 years, a continuous exposure (22–22.5 hr/day) of female C57BL/6 mice for 11 days revealed that cerebellar degeneration (as well as liver glycogen depletion and necrosis) occurred at levels from 100 to 400 ppm (Landry et al., 1983c, 1985) with an absence of effects at 15 and 50 ppm. Incidence of cerebellar degeneration exhibited both concentration- and duration-dependence along with increasing severity. Marked weakness was observed in mice at 150 ppm whereas 200 ppm resulted in mortality (compared to 1,000 ppm in the chronic study) prior to exposure termination. When exposure (150 to 2,400 ppm) of another group of female C57CL/6 mice was intermittent (6 hr/day) for 11 days (Landry et al., 1983c, 1985), a concentration-related increase in the incidence of cerebellar lesions of severity described as "slight" occurred at levels of 400 to 2,400 ppm. In contrast to the continuous exposure, there was no mortality at any concentration (however, 2,400-ppm mice were moribund by day 8–9 and were sacrificed). Neurofunctional testing of the 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.

Evidence that could explain any particular susceptibility of the female B57BL/6 mouse to cerebellar lesions or the mode of action by which they occur 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). However, both in vitro and in vivo data indicate that methyl chloride's primary metabolic pathway involves conjugation with GSH and may result in the formation of methanethiol, which has neurotoxic effects similar to those described for methyl chloride. 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. Both formaldehyde and formate could contribute to noncerebellar toxicity. Methylation of cellular macromolecules does not appear to be a significant feature of methyl chloride's metabolic profile. Although human subpopulations have shown polymorphisms in erythrocyte GST-θ in conjunction with differences in metabolism of methyl chloride, relationships to potential exposure toxicity outcomes remain to be established. However, it is noted that methyl bromide induces SCE in a human subpopulation deficient in GST and that methyl chloride also induced SCE, but in human volunteers uncharacterized for their GST status.

Target tissue depletion of GSH by methyl chloride has also been associated with lipid peroxidation, both of which were found more extensively in mouse liver than in rat liver, paralleling the species' sensitivities to methyl chloride-induced hepatotoxicity. When mice were subjected to pretreatment with a specific inhibitor of γ-glutamylcysteinyl synthetase (also known as γ-glutamate-cysteine ligase), the rate-limiting enzyme for de novo synthesis of GSH, NPSH levels in the brain, liver, and kidney were depleted, and lethality and target organ toxicity (including cerebellar lesions) from subsequent methyl chloride exposure were eliminated; these findings implicate a pivotal role for methyl chloride-GSH conjugation in methyl chloride's mode(s) of action. In addition, pre- and posttreatment of methyl chloride-exposed rats with an anti-inflammatory investigational compound (BW755C) prevented cerebellar necrosis, renal tubular degeneration and hepatocellular swelling. This raises the possibility that cerebellar lesions may result, in part, from permeability changes or edema induced by methyl chloride or one of its metabolites on perturbations of normal leukotriene and/or prostaglandin processes.

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 conclusions. At high exposure concentrations (1,000 to 5,000 ppm), studies in rats have demonstrated that methyl chloride results in 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. The collective data, including studies with the anti-inflammatory agent 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.

In male rats exposed to 475 ppm and mated with exposed and unexposed females, there was a small reduction in F_0 male rat fertility that resolved during postexposure. A statistically significant decreased percentage of males and temporary pup weight reductions in F_1 litters were also observed at 475 ppm. At 1,500 ppm, males were infertile. No effects on fertility were seen at the only other concentration tested, 150 ppm.

There was no evidence of teratogenicity or embryo- or fetotoxicity in rats exposed 6 hr/day during gestation to up to 1,500 ppm. After C57BL/6 female mice impregnated by C3H males were exposed during gestation to 100 to 1,500 ppm methyl chloride, concentration-related heart malformations were observed in the $B6C3F_1$ fetuses at 500 ppm and above, with more malformation occurring in females than males. However, no studies have examined histopathologically the cerebellum of either postnatal rats or mice of F_1 generations of exposed dams. The absence of such histopathological data postnatally should be regarded as an area for further research. It is not currently known if and to what extent exposure in utero to methyl chloride continuing with exposure postnatally results in abnormal development of the cerebellum or leads to degeneration of granule or Purkinje cells. The Purkinje and granule cells have been described as important targets for toxic substances (Fonnum and Lock, 2000). The Purkinje cells appear in the rat brain on prenatal day 14-16, whereas the granule cells appear postnatally.

4.6. WEIGHT-OF-EVIDENCE EVALUATION 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 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, in which a statistically significant increased incidence of renal benign and malignant tumors occurred only in male $B6C3F_1$ mice at the high concentration (1,000 ppm). Two renal adenomas occurred in 225-ppm males and should be considered related to exposure. Renal cortical tubuloepithelial hyperplasia and karyomegaly were also confined to 1,000-ppm male mice. Neoplasia was not found at lower concentrations or at any other site in the male $B6C3F_1$ mouse, nor at any site or concentration in female mice or F-344 rats of either sex. Why only the male mice develop renal tumors may have its basis in the expression of CYP2E1 protein. Of the two rodent species examined thus far (mouse and rat), the mouse is the only species that produces high levels of CYP2E1 and significant amounts of formaldehyde. 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 fivefold higher in male mouse kidney than in female kidney. F-344 male rats (Cummings et al., 1999) have been shown to produce about 25% of the amount of CYP2E1 than male CD-1 mice (Speerschneider

and Dekant, 1995). Kidney microsomes from Sprague-Dawley rats did not catalyze the formation of formaldehyde from methyl chloride, and both sexes exhibited only low rates of chlorozoxazone oxidation (Dekant et al., 1995).

Thus, 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 (see below), 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.

This mode of action may not be relevant for humans. Human kidney CYP2E1 protein has not been detected in proximal tubular cells (Amet et al., 1997; Cummings et al., 2000); very low levels of CYP2E1 RNA (protein was not evaluated) have been detected in fetal, developing kidney, and adult human kidney (Vieira et al., 1998) whereas 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).

However, other modes of action that may be relevant to humans cannot be discounted. For example, P-450 isozymes other than CYP2E1 (e.g., CYP3 and CYP4 isozymes) may have the potential to metabolize methyl chloride. Additionally, potentially genotoxic metabolites originating from metabolism via GSH, the predominant pathway for methyl chloride, and potential inhibition of the GSH-requiring formaldehyde dehydrogenase, which converts formaldehyde to formate, provide a plausible mode of action that may be of importance.

Exposure of B6C3F$_1$ mice to 1,000 ppm for 8 hr, or 6 hr/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, which in 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).

In several reverse or forward mutation strains of *Salmonella*, methyl chloride demonstrated weak-to-moderate direct mutagenic activity at high concentrations (0.5%–30%) (Andrews et al., 1976; du Pont, 1977; Fostel et al., 1985; Longstaff et al., 1984; Simmon et al., 1977). At concentrations of 0.3%–5%, it was reported to induce mutations and SCE, but not alkaline-labile DNA damage in an established human lymphoblast cell line (Fostel 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%, whereas 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, 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.

Based upon inadequate evidence of carcinogenicity to both humans and animals, IARC (1999) has classified methyl chloride as a Group 3 chemical — “not classifiable as to its carcinogenicity to humans.” It must be noted that IARC last reviewed these data in 1986, at which time only an abstract of the 1981 CIIT study was available. Applying the criteria for evaluating the overall weight of evidence for carcinogenicity to humans outlined in EPA’s Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1986a), methyl chloride is most appropriately designated a “Group D — not classifiable as to its human carcinogenicity.” 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 best be described as *cannot be determined.* Limited human epidemiology studies show no suggestive evidence that methyl chloride exposure was associated with a carcinogenic response. However, weak-to-moderate mutagenicity has been demonstrated in *S. typhimurium* (albeit at high concentrations), and an increased incidence of tumor formation (benign and malignant) in male mouse kidneys does provide some *suggestive* information of carcinogenic risk, although no renal tumors were found in female mice or in either sex of rats tested in the same study. In addition, induction of SCE by methyl chloride has been observed in human lymphoblasts, and by a congener, methyl bromide, in lymphocytes from a human subgroup categorized as “slow metabolizers,” a group known to be genetically predisposed (have polymorphisms in glutathione transferase) to have a lower rate of metabolism compared to the majority of human populations studied.

The lack of detectable CYP2E1 protein in human kidney (in contrast to mice, which have high levels) suggest that the metabolism of methyl chloride by P-450 (presumably leading to elevated formaldehyde concentrations), that could be responsible for the induction of male mouse kidney tumors, may not be relevant to humans. However, the role of hepatic (and/or kidney) metabolism (leading to potential genotoxic metabolites) via the predominant GSH pathway (or even by P-450 isozymes other than CYP2E1) in this regard cannot be discounted; in vivo metabolism of methyl chloride to formate in liver is GSH-dependent, via the GSH-requiring

formaldehyde dehydrogenase that oxidizes formaldehyde to formate. Inasmuch as methyl chloride exposure can lower tissue nonprotein sulfhydryl concentrations, it thus has the potential to inhibit formaldehyde dehydrogenase and increase formaldehyde levels. The extent to which this may or may not take place in the human kidney is an area for further research.

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 or slow metabolizers," 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). Inasmuch as GST(θ) also has been identified in human kidney (Cummings et al., 2000), differences between these subpopulations in metabolizing capability may be significant for kidney toxicity as well. However, because of the complexities and uncertainties of methyl chloride's metabolism, especially with respect to mechanisms of toxicity, it is not clear whether these subpopulations would have significantly altered susceptibility (or resistance) to effects of methyl chloride, and if so, whether it would be lesser or greater (e.g., Nolan et al., 1985; Warholm et al., 1994). Those characterized as "slow metabolizers" have been shown to be susceptible to SCE induced by the congener methyl bromide when their erythrocyte cytoplasm was incubated with peripheral lymphocytes (Hallier et al., 1993). On the other hand, demonstration that when NPSH levels are lowered in laboratory animals prior to methyl chloride exposure, lethality and target organ toxicity can be largely prevented may suggest that "slow metabolizers" are less susceptible. It also bears mentioning that 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 influence their susceptibility to methyl chloride, no specific information was identified on methyl chloride exposure in infants or children. Limited animal data suggest that methyl chloride can cross the placental barrier and thus may cause fetal effects. There are no studies in laboratory animals that have examined the potential of methyl chloride on the neurodevelopment of fetuses in F_1 or F_2 generations. However, exposure of F_0 generation F344

rats to a high concentration of methyl chloride (475 ppm) led to a decreased percentage of males in F_1 generation litters.

4.7.2. Possible Gender Differences

In the various studies referenced above concerning the "metabolic" subpopulations, no gender differences were noted, nor were any discernable in other clinical, case report, or epidemiological studies of methyl chloride. 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. For example, female C57BL/6 mice appeared to be 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 were 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 GSH 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)

Not applicable. No oral dose-response assessment was performed for methyl chloride and there have been no oral studies in laboratory animals.

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 hr/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 for chronic inhalation exposure to methyl chloride were 1,000 ppm and 225 ppm, respectively, based on a variety of lesions in liver, brain, kidney, and spleen of mice at 1,000 ppm. These effects were not apparent in exposed rats.

As indicated in Section 4.2.2, this study, which suffered some procedural errors (e.g.,

some misidentification of mice, pregnancy of some mice, and an exposure error early in the study), would typically have been chosen for identification of the critical effect (e.g., cerebellar lesions) because it satisfies the criteria set forth in U.S. EPA (1994b). However, the continuous (22–22.5 hr/day) 11-day exposure of the female C57BL/6 mouse (Landry et al., 1983c, 1985) is considered more appropriate in the context of protecting public health for the following reasons: (1) cerebellar lesions (considered the most critical effect in the context of known CNS deficits from human case reports) occurred at levels (100 ppm) far below those in the $B6C3F_1$ strain exposed chronically (1,000 ppm); even C57BL/6 females intermittently exposed (400 ppm) over 11 days evidenced cerebellar lesions, and (2) continuous exposure of C57BL/6 mice resulted in mortality at 200 ppm whereas intermittent 2-year exposure of the B6C3F1 mouse did not cause mortality below 1,000 ppm. Additionally, intermittently exposed female mice (Morgan et al., 1982), particularly the C57BL/6 strain, had a greater and more severe incidence of cerebellar lesions than C57BL/6 males or both sexes of the B6C3F1 and C3H strain (no cerebellar lesions in C3H mice at exposure levels up to 2,000 ppm). Thus, the Landry et al. (1983c, 1985) study was chosen as the principal study for RfC derivation, with cerebellar lesions as the critical effect.

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

Derivation of the RfC was not based on the Benchmark Dose approach because the critical effect (cerebellar lesions) did not show a concentration-response incidence trend; cerebellar lesions occurred in all mice at 100 ppm (designated the LOAEL) and higher. Therefore, the NOAEL/LOAEL approach was used in all further analysis, with 50 ppm designated as the NOAEL.

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

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. For methyl chloride, a NOAEL has been identified from the Landry et al. (1983c, 1985) 11-day continuous exposure study. This NOAEL of 50 ppm (103.2 mg/m^3) was adjusted to a continuous exposure by the following calculation: NOAEL(ADJ) = 103.2 $mg/m^3 \times$ (22 h/24 h) $\times$ (7/7 days) = 94.6 mg/m^3.

The physicochemical 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). Accordingly, the human equivalent concentration (HEC) for methyl chloride was derived by multiplying the NOAEL(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 determined to be approximately 1 based on the blood:gas partition coefficients for humans (Nolan et al., 1985) and for the rat (Gargas et al., 1989). The assumption is that the partition coefficient for the mouse would be similar to that for the rat based on the tabulation of Gargas et al. (1989), who reported

that blood:gas partition coefficients for 6/7 chemicals are similar for both the rat and mouse. In addition, it is a defensible assumption that is within the range of current modeling practice. Thus, the NOAEL, multiplied by the RGDR to yield the NOAEL (HEC) is: $NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR = 94.6\ mg/m^3 \times 1.0 = 94.6\ mg/m^3$.

Uncertainty factors (UF) were applied as follows:

(1) Because of the dosimetric adjustment in conversion to the HEC, a UF of 3, instead of the default 10, was applied for interspecies extrapolation. Only the C57BL/6 female mouse was examined under continuous exposure conditions. We do not know how the male or $B6C3F_1$ mice would react upon similar exposure conditions. The only strain comparisons that were made were those of Morgan et al. (1982), and they were under intermittent exposure conditions at relatively high concentrations.

(2) A factor of 10 is used to extrapolate from an 11-day continuous study to a lifetime inhalation study. In the typical situation in which only a subchronic intermittent rodent inhalation exposure study is available, a full factor of 10 is generally applied to account for the lack of chronic intermittent exposure results. Although the 11-day study is not fully equivalent in duration to a subchronic study, it is a valuable continuous inhalation study supported by the conclusions of a chronic study. A factor of 10 is thus considered protective to account for using a less than chronic study for the derivation of the RfC.

(3) A factor of 10 was applied to account for the fact that there are two identifiable human subpopulations who are known to differ in their rates of metabolism of methyl chloride because of genetic polymorphisms. These populations may differ in the type or severity of methyl-chloride-induced lesions.

(4) A factor of $10^{1/2}$ or 3 is applied to account for the lack of histopathology of the cerebellum in postnatal F_1 generation $B6C3F_1$ mice. Although cerebellar lesions have been demonstrated in adult $B6C3F_1$ mice (both sexes) and in C57BL/6 adult female mice, the effect of exposure on in utero development of the brain in mice has not been examined and remains an important data gap.

Because two factors of 3 ($10^{1/2}$) coalesce to a 10, the total uncertainty factor applied to the $NOAEL_{HEC}$ of 94.6 mg/m^3 is 1,000, yielding an RfC of 9E-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 bioassay in $B6C3F_1$ mice and F-344 rats that found a statistically significant increased incidence of renal tumors only in male 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 was 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 occurrence of renal tumors only in male mice is a pattern seen with structurally related chemicals. Renal tubular adenomas and carcinomas have also been seen in male BDF_1 mice exposed by inhalation to chloroform (Nagano et al., 1998) and in male Swiss-Webster mice (Maltoni et al., 1984) exposed by inhalation to 1,1-dichloroethene (vinylidene chloride). These studies were reviewed by IARC (1999). Metabolic data suggest that mouse renal neoplasms are the result of conversion of methyl chloride to metabolites with carcinogenic potential as a result of the mouse kidney possessing high levels of CYP2E1. Several studies that examined CYP2E1 content and/or activity of human kidney microsomes have found that CYP2E1 was undetectable (Amet et al., 1997; De Waziers et al., 1990; Lasker et al., 2000; Cummings et al., 2000).

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 cannot be determined. Although human epidemiological studies that have been performed are negative, positive mutagenicity results (albeit at high concentrations) and renal tumors (benign and malignant) observed in male mouse kidney provide some *suggestive* information of carcinogenic risk to humans. Mechanistic considerations (e.g., expression of CYP2E1 protein in the mouse kidney and none detected in human kidney) and the occurrence of tumors in only one organ of one sex of a single rodent species (the $B6C3F_1$ mouse) suggest that the induction of kidney tumors in male mice may not be relevant to humans. However, in vitro induction of SCE by methyl chloride, evidence with methyl bromide that those deficient in GST are at increased risk of SCE, and evidence that methyl chloride has mutagenic potential in the Ames assay indicate that further clarification of this potential with respect to human carcinogenicity is needed.

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

6.1. HUMAN HAZARD POTENTIAL

Methyl chloride is found ubiquitously in nature; the vast majority comes from such natural sources as the ocean, microbial fermentation, and biomass fires. These 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 to the environment (ATSDR, 1998; Farber and Torkelson, 1989; U.S. EPA, 1986d). Industrially produced 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. Methyl chloride exists as a gas that is slightly soluble in water; thus, the majority of exposures are through inhalation. Although drinking water advisories based on inhalation studies had previously been established (U.S. EPA, 1990), these studies (Putz-Anderson et al., 1981; Repko et al., 1976) have limitations that preclude their usefulness for the purpose intended.

Methyl chloride is readily absorbed in the respiratory tract and distributes throughout the

body. Its toxicity appears to be a result of its metabolism. The effects noted in the cerebellum of exposed rodents, coupled with the known CNS effects in humans at high levels of exposure, suggest that inhalation of methyl chloride may pose risks to CNS functioning at levels above the RfC.

The carcinogenic potential of methyl chloride for humans exposed in ambient environments is difficult to assess. Renal adenocarcinomas have been shown to occur only in male mice (and not in rats of either sex) at levels of exposure (i.e., 1,000 ppm) unlikely to be approached in the ambient environment outside of a catastrophic exposure scenario. Additionally, species-specific metabolic differences in how the kidney processes methyl chloride strongly suggest that renal mouse neoplasms via P-450 oxidation may not be biologically relevant to humans given that humans lack the key enzyme (CYP2E1) known to convert methyl chloride to toxic intermediates (e.g., formaldehyde) having carcinogenic potential. However, it is not known if there are species-specific metabolic differences leading to potential mutagens via the predominant GSH pathway or via P-450 isozymes other than CYP2E1. In addition, methyl chloride does induce SCE and is weakly to moderately mutagenic at high concentratiuon in the Ames assay, although it does not methylate DNA in rodent species. Thus, the carcinogenic potential of methyl chloride for humans cannot be determined based on data available.

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 adequate characterization of exposures have been reported. The RfC for methyl chloride was derived from an 11-day continuous exposure of female mice that exhibited concentration- and duration-related increase in atrophy and degeneration of cerebellar granule cells, effects also seen when female mice were exposed intermittently for 11 days, but at much higher concentrations. It is not known if longer duration continuous exposures would yield cerebellar lesions at lower concentrations than those observed in the 11-day continuous exposure period.

The overall confidence in the RfC assessment is medium. The confidence in the principal study (Landry et al., 1983c, 1985) is high. The overall confidence in the database is medium. Although reproductive parameters were evaluated in two generations of rats, the lack of data on cerebellar effects in the developing mouse or rat represents a data gap that is important when risks to children’s health are considered. Confidence in the RfC is judged to be medium.

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APPENDIX A: EXTERNAL PEER REVIEW—SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for methyl chloride have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The three external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. The reviewers made a number of editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further. Public comments were received and considered. A summary of significant comments made by the external reviewers and EPA's response to these comments follows:

(1) ***General Comments***

A. Data Presentation

One reviewer felt that discussion in the Toxicological Review was too disorganized in places, with key studies cross-referenced across several sections, making understanding difficult. In addition, too many study details were presented, there was inadequate presentation of data in tabular form, and statistical significance of findings was rarely presented. This reviewer also indicated that the IRIS Summary was too brief and lacked broad coverage of the spectrum of effects in the Additional Studies/Supporting Documentation section. In contrast, a second reviewer felt the narrative and conclusions in both draft documents were clear and well-founded, although more tabular presentation would have been beneficial to the reader. The third reviewer did not comment on this aspect.

Response to comments: The draft sent to the reviewers did suffer from lack of tabular presentation of results, excessive presentation of study details, and cross-referencing of some studies across several sections of the Toxicological Review. These suggestions and those pertaining to the summary document provided the impetus to address and correct these concerns in the following manner: (1) details of study conduct and discussion of interim results were pared back throughout the Toxicological Review, (2) discussions of key studies were reorganized to eliminate duplicate or piecemeal discussion across several sections, (3) discussion of findings in key studies was compartmentalized within the body of the text to make the findings more accessible to the reader, (4) findings of key studies were presented in tabular form by creating additional tables, and (5) the IRIS summary document was expanded to include information from studies that examined the spectrum of biologically relevant adverse effects.

B. Are there additional data/studies that should be included?

One reviewer felt that although the information in the Toxicological Review appeared to encompass the relevant studies, the summary document did not reflect the diversity of

information on neurotoxicity that was present in the Review. The other two reviewers recommended the citation of two additional ancillary studies.

Response to comments: The IRIS summary was expanded to include additional discussion of supporting studies that delineated the types of effects associated with adverse reactions in laboratory animals. Those specific references cited by the reviewers were added to the text where deemed appropriate.

C. For the RfC, has the most appropriate critical effect been chosen, and for the cancer assessment, are the tumors observed biologically significant and relevant to humans?

One reviewer felt that the critical effect (axonal swelling in the spinal cord of the mouse) was not the appropriate effect because there was no statistically or toxicologically significant association between axonal degeneration and exposure. If the CIIT (1981) study were chosen, this reviewer felt that degeneration and atrophy of seminiferous tubules of the rat testes should be the critical effect. A second reviewer was of the opinion that axonal swelling in the mouse spinal cord was a “less-than-perfect” endpoint and that cerebellar degeneration (in the CIIT [1981] study) was a better choice. The third reviewer felt that axonal swelling and degeneration appeared to be the lowest response on the dose-response continuum. The cancer subquestion was answered by two reviewers who were of the opinion that the evidence that methyl chloride is carcinogenic in humans and laboratory animals is inadequate.

Response to comments: While these documents were being externally reviewed, it was independently decided by EPA that the strength of the evidence for axonal swelling and degeneration in the mouse spinal cord (CIIT, 1981) was insufficient to warrant selecting this endpoint as the critical effect because of the lack of a dose-response and information that this endpoint has no functional correlate. The most appropriate endpoint, given the knowledge that methyl chloride is a potent neurotoxicant in humans and that histopathological evidence of cerebellar damage has been observed in the rat and mouse, would be the occurrence of cerebellar lesions in the mouse. Accordingly, all aspects of the external review documents that discussed axonal effects as the critical endpoint were revised to focus on the information for cerebellar degeneration and the revised weight of evidence led to an RfC based on this endpoint.

After taking into account the reviewers’ comments and additional information relating to the potential of methyl chloride and a congener, methyl bromide, to induce SCE, EPA has revised its original position and now recommends that the carcinogenic evidence for methyl chloride be regarded, as per U.S. EPA (1996), as “cannot be determined.” The evidence from the sole bioassay (CIIT, 1981) in the male mouse and in vitro studies with rat, mouse, and human kidney microsomes together provide suggestive evidence that renal tumors in the male mouse (CIIT, 1981) are due to species- and sex-specific metabolism. Adult human kidney (proximal tubular cells and kidney homogenates) has been shown to lack CYP2E1 (Cummings et al., 2000; Amet et al., 1997), the key P-450 isozyme believed to be responsible for conversion of methyl chloride to formaldehyde, and CYP2E1 RNA is only present at very low levels in kidney of a fetus, a newborn, and an adult (Vieira et al., 1998). These studies had not been cited in the external review given to the peer reviewers, but were brought to EPA’s attention by one of the reviewers during the external review process. In addition, sex specificity of renal CYP2E1 in the

male mouse was strongly influenced by testosterone (Hu et al., 1990). Rat kidney microsomes do not catalyze the formation of formaldehyde, unlike microsomes from the male mouse, whereas the female mouse can be induced to metabolize methyl chloride with exogenous testosterone (Dekant et al., 1995). Information from studies with 1,1-dichloroethene (Speerschneider and Dekant, 1995) supports the species- and sex-specific renal metabolism of methyl chloride. This chemical was associated with renal adenomas only in male mice, and metabolism due to CYP2E1 was lacking in both sexes of the rat and in six samples of human male kidney. On the other hand, methyl chloride induced SCE in human lymphocytes, and methyl bromide also was shown to induce SCE in human lymphocytes, particularly in those individuals with impaired metabolic capability for methyl chloride as measured by erythrocyte glutathione transferase (theta) activity. Thus, this potential for modulation (including demonstrated mutagenicity) of genetic determinants, counterbalanced by the fact that methyl chloride does not directly methylate DNA and some uncertainty as to whether P-450 isozymes (e.g., CYP3a and 4F families) other than CYP2E1 (not found in human kidney) have capability to metabolize methyl chloride in human kidney has led to a revised cancer classification.

Thus, the most appropriate weight-of-evidence descriptor under the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a) is "cannot be determined." A full characterization of this determination may be found in the text.

D. Have the noncancer and cancer assessments been based on the most appropriate studies?

One reviewer felt that because the CIIT (1981) study was the only long-term bioassay, it should be considered. However, this reviewer also stated that the study was poorly designed and implemented and because of these and other shortcomings its credibility was in question. This reviewer suggested the Repko (1976) human occupational study as a possible basis for deriving an RfC in spite of published criticisms of the study's findings. On the other hand, a second reviewer felt that it was "a fairly good study." The third reviewer considered this study appropriate.

Response to comments: As indicated in the previous response to (C), a reevaluation of the critical effect during the external review period resulted in significant changes in selection of the appropriate critical study and critical effect. The CIIT (1981) study had several shortcomings: (1) misidentification of mice, (2) pregnancy of some mice, (3) a 3-day exposure mistake involving low- and high-dose mice, and (4) lack of brain histopathology at time points prior to the 12-month interim sacrifice. In spite of these shortcomings, the results do not appear to have been compromised because the effects and the exposure-response relationships were consistent with those demonstrated in other, shorter term studies.

Because cerebellar effects were selected as the critical effect, it seemed appropriate, from a conservative public health perspective, to rely on the 11-day continuous exposure study of Landry et al. (1983c, 1985) as the study from which an RfC should be calculated rather than the intermittent exposure study of CIIT (1981). Although this approach represents a departure from the criteria set forth in U.S. EPA (1996), the availability of shorter term continuous studies was not addressed when these criteria were formulated. With few exceptions, RfCs are typically

derived from subchronic or chronic intermittent-exposure studies in which duration adjustments are made to lifetime, 24-hour/day exposures. It is rare indeed to have toxicological data from continuous exposures of a sensitive laboratory animal species and even rarer, such as with the Landry et al. (1983c, 1985) study, to have data from both continuous and intermittent exposures. Notwithstanding the issue of whether the C57BL/6 female is unusually sensitive to the cerebellar effects of methyl chloride, these studies did convincingly demonstrate that continuous exposure did result in effects at much lower concentrations than intermittent exposures. This alone should preclude consideration of using the intermittent exposure mouse data from the CIIT (1981) and other studies. It can be foreseen that if continuous exposure of the C57BL/6 female were to be carried beyond 11 days cerebellar effects would occur at even lower levels. Inasmuch as identification of a NOAEL and/or LOAEL associated with effects in humans exposed for 24 hours/day for a lifetime is the principal objective in an RfC derivation, the C57BL/6 continuous data provide a more scientifically meaningful base from which to extrapolate to human exposure scenarios than if intermittent exposure data were to be used.

The selection of the Landry et al. (1983c, 1985) study is a departure from previous EPA assessments. In 1990, EPA published a drinking water health advisory for 15 volatile chemicals, of which methyl chloride was one (U.S. EPA, 1990). At that time, the Repko et al. (1976) occupational study was selected as the basis for derivation of an RfD, with humans being considered as more sensitive than the mouse. As cited elsewhere in this external peer review document, the findings of the Repko et al. (1976) study have been criticized on a number of points. In this assessment, the Repko et al. (1976) study is deemed inadequate for derivation of the RfC for reasons not specifically addressed in the health advisory. The principal limitations are that prior exposures to methyl chloride in the study population were not taken into account and the slight decrements in only two of many tasks administered are insufficient to draw cause-effect conclusions.

E. Should other studies be included under the Supporting/Additional category to lend scientific justification for the designation of critical effect?

Two of three reviewers felt that a more comprehensive perspective of the noncancer effects (in humans and laboratory animals) and mode-of-action information should be presented. The third reviewer felt that all relevant studies were included. One reviewer addressed the cancer issue specifically. Unpublished and published NIOSH reports in the 1980s considered methyl chloride to be carcinogenic (based on the CIIT [1981] study) and a mutagen. The reviewer suggested discussing these previous evaluations in light of the current position taken by EPA in the external review draft.

Response to comments: EPA agrees that a broader perspective in the Supporting/Additional Studies section be included. Accordingly, this section now provides that perspective by including of information from relevant human and laboratory animal studies that address the neurotoxic, hepatic, renal, teratogenic/reproductive, and genotoxic effects of methyl chloride.Additionally, this section addresses the current weight of evidence in contrast to previous positions on this chemical by EPA and NIOSH.

F. For the noncancer assessments, are there other data that should be considered in developing uncertainty factors or the modifying factor? Do the data support the use of different values from those proposed?

One reviewer suggested that perhaps even lower values could be used because of the consistency from one species to another. A second reviewer felt that the use of either 300 or 1,000 was not a critical element in the uncertainty factor approach. The third reviewer felt that reasons for using the default uncertainty factors should be stated, such as mention of the GST polymorphisms in humans, and also that a modifying factor of 10 should be used if the CIIT study is deemed the critical study for the RfC.

Response to comments: Because the critical study and effect was changed to that of Landry et al. (1983c, 1985), the uncertainty factors were reevaluated and justification provided as suggested.

G. Do the confidence and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects (cancer and noncancer) to humans, and the comprehensiveness of the database? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments?

One reviewer indicated that the reasoning and statements were very clear, provided concerns about axonal swelling/degeneration were more fully addressed. Another reviewer suggested that the assumptions should be better described and was concerned that the proposed cancer classification ("not likely") was not supported by the data and what was considered to be a "speculative" mode of action. In addition, IARC had classified methyl chloride as "not classifiable to its carcinogenicity to humans." The third reviewer suggested that comprehensiveness be expanded to reconcile the proposed assessment with previous evaluations where this substance was regarded as a mutagen, teratogen, and carcinogen.

Response to comments: Both the confidence and weight-of-evidence statements were extensively revised and expanded to account for the changes in the critical effect and critical study and to account for the expanded discussion of the mode of action. Discussion has also been included to place in perspective the noncancer and cancer information with those previous evaluations done by NIOSH, EPA, and IARC. It should be noted that the IARC classification in 1986 (and in the 1999 IARC reference) was based on the fact that it only had an abstract of the CIIT study findings and thus could not comment further. The NIOSH (1984a,b) cancer assessment concluded that methyl chloride should be regarded as a potential occupational carcinogen based on (1) its mutagenic ability and (2) the kidney tumors found in the CIIT (1981) study. In the EPA drinking water advisory (U.S. EPA, 1990), methyl chloride was judged to be a possible human carcinogen based on the kidney tumors observed in the CIIT (1981) study. The data do indicate that methyl chloride is a mutagen; however, it has been shown in Ames assays to be mutagenic only at exceedingly high concentrations (e.g., >2,500 ppm) and has not been demonstrated to be a direct-acting mutagen. When viewed in the context of ambient air exposure concentrations, which appear to be in the high parts-per-trillion range, one may question whether the mutagenic data really represent a real-world concern. The kidney tumors also have limited

relevance to humans for reasons discussed in this document, particularly what is known about the mode of action. Significant amounts of new metabolic information and cancer information from structurally related compounds (e.g., vinylidene chloride), previously unavailable to NIOSH, EPA and IARC, support the view that certain kidney tumors in the mouse are species- and sex-specific and may not be relevant to extrapolation to human ambient exposure scenarios. The wealth of metabolic information now available is sufficient to discount the mode of action as being "speculative." Although human kidney does not possess CYP2E1, the principal isozyme believed to be responsible for mouse kidney tumor formation, the human kidney does have measurable amounts of other P-450 enzymes, but it is not known if they metabolize methyl chloride. Until this avenue of research is explored, a definitive characterization that methyl chloride is not likely to be a human carcinogen must be tempered.

(2) ***Chemical-Specific Comments***

A. Do the results of Landry et al. (1983c, 1985) and particularly those of Morgan et al. (1982) support the statement in Section I.B.5 of the summary that the female C57BL/6 mouse may be particularly sensitive to the neurotoxic effects of methyl chloride?

One reviewer felt that additional dose-response data were needed, particularly at lower concentrations, to strengthen this argument. Another reviewer considered the available data inadequate to conclude particular sensitivity for this strain. The third reviewer felt that the $B6C3F_1$ female was particularly sensitive.

Response to comments: Given the spectrum of responses, there is no unanimity. The only data that specifically address this issue are the findings of Morgan et al. (1982) in which cerebellar lesions occurred in the female C57BL/6 mouse at concentrations that either did not cause lesions in the male or other strains or did so at significantly lower incidence and severity levels. Additional data drawn from studies in several strains using similar exposure protocols (preferably longer term) would be needed to resolve this issue.

B. Histopathology of the spinal nerves apparently was not part of the CIIT (1981) protocol at the 6- and 12-month time points. Are this lack of data, the lack of a dose-response, and the exposure error (low-dose group exposed to 1,000 ppm for 3 days) sufficient reasons for giving a low confidence rating to this study?

All three reviewers agreed that low confidence should be assigned to this study; one considered the study well executed and reported for its time whereas the other felt that if this study were to be used, a modifying factor of 10 should be applied because the study was compromised on a number of points. One reviewer did not consider spinal cord histopathology as a treatment-related effect; the other had reservations.

Response to comments: Given the responses of the three reviewers and the independent reexamination of this issue by EPA, it was decided not to use this study or the spinal cord effects in the RfC derivation, in part, because of the lack of a dose-response, the absence of corroboration in the Landry et al. (1983c; 1985) study, and the review by LoPachin et al. (2000), which discounted such spinal cord lesions as related to functional abnormalities.

C. Does the absence of axonal degeneration in the C57BL/6 mouse in the Landry et al. continuous study cause one to doubt the significance of the evidence of axonal degeneration in the CIIT intermittent exposure study with the $B6C3F_1$?

Two reviewers thought the lack of confirmation of effect in the Landry et al. study was reason to doubt the significance; the third reviewer suggested the slides be reviewed by an independent pathologist, although it was felt that the CIIT results were not negated by the lack of confirmation by Landry et al.

Response to comments: The critical study and effect was changed, with text discussion modified to reflect lack of significance of the axonal lesions.

D. Is the lack of a developmental neurotoxicity study in the mouse sufficient justification for the inclusion of an uncertainty factor of 3?

All reviewers felt that an additional uncertainty factor of 3 was necessary, although one felt the factor should reflect the lack of a specialized neurotoxicity study in adults, using current test guidelines, and the lack of a *good* (sic) chronic bioassay while considering the lack of a developmental neurotoxicity study to be premature.

Response to comments: It was decided that a factor of 3 was appropriate and based on the lack of brain histopathology in F_1 generation mice. Although some developmental aspects of F_1 generation mice were examined, no studies performed brain histopathology on such mice. Given the suggestive evidence that methyl chloride crosses the placenta, coupled with methyl chloride's known propensity to cause cerebellar lesions, the lack of such data warrants an uncertainty factor.

E. Because the mode of action may operate through modulation of prostaglandins and leukotrienes, should an additional uncertainty factor be included for lack of immunological studies?

None of the reviewers were in favor of an additional uncertainty factor in this instance. One considered the mode of action unknown and that it would be premature to consider prostanoid metabolism at this point. Another felt there were no data to suggest immunological involvement.

Response to comments: No additional uncertainty factor was included.

F. Are you aware of any links between leukotriene and prostaglandin synthesis and GST-theta metabolism?

One reviewer was aware of links of other GSTs with prostanoid metabolism, but not theta. The other reviewers were not aware of any data.

Response to comments: The text, where appropriate, was revised to include these sentiments.

G. Given the amount of text data reported for the CIIT (1981) study (pages 42-48), which data could be presented in tabular form with minimal text discussion?

All three reviewers felt that more tabular information was in order, although two considered it more a matter of style than substance. One reviewer was adamant about rewriting the discussion of the CIIT (1981) study to subdivide the headings, as well as including tables. This reviewer also suggested rewriting the studies in Sections 4.1- 4.4 to make the text more focused with less detail.

Response to comments: The suggestion to revise Sections 4.1 through 4.4 was adopted. Accordingly, text discussion throughout was (1) reduced to eliminate what was considered excessive detail; (2) incorporated data from text to tabular form; (3) reorganized to have text data, particularly with the CIIT study, placed under subheadings; and (4) eliminated Section 4.4.1 (neurological studies) altogether because findings from a particular study were discussed in more than one location; information from 4.4.1 was placed either in 4.2.2 or 4.4.2 where appropriate. The subheadings (mortality, clinical observations, weight changes, significant histopathology, and conclusions) for the CIIT study were selected so as to focus the readers' attention to main topics rather than to subdivide further along organ systems. No tables for this information were considered to be necessary inasmuch as all adverse effects occurred at 1,000 ppm. On the other hand, the discussion of the Landry et al. (1983c, 1985) data was revised to include three tables along with some subdivision of text (i.e., continuous exposure and intermittent exposure) to present the results more effectively.