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# **Methylene Chloride**

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TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

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## Acronyms and Abbreviations

Acronyms and Abbreviations	Definition
ACGIH	American Conference of Governmental Industrial Hygienists
ADAF	age-dependent default adjustment factors
AEGL	Acute Exposure Guideline Level
AMCV	Air monitoring comparison values
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMR	benchmark response
C	concentration
CalDHS	California Department of Health Services
CalEPA	California Environmental Protection Agency
CHO	Chinese hamster ovary
CNS	central nervous system
CO	Carbon monoxide
COHb	carboxyhemoglobin
CSAF	chemical-specific adjustment factor
CSF	Cancer slope factor
DNA	deoxyribonucleic acid
DSD	development support document
ESL	Effects Screening Level
<sup>acute</sup> ESL	acute health-based Effects Screening Level for chemicals meeting minimum database requirements
<sup>acute</sup> ESL <sub>generic</sub>	acute health-based Effects Screening Level for chemicals not meeting minimum database requirements

<b>Acronyms and Abbreviations</b>	<b>Definition</b>
$^{acute}ESL_{odor}$	acute odor-based Effects Screening Level
$^{acute}ESL_{veg}$	acute vegetation-based Effects Screening Level
$^{chronic}ESL_{linear(c)}$	chronic health-based Effects Screening Level for linear dose response cancer effect
$^{chronic}ESL_{linear(nc)}$	chronic health-based Effects Screening Level for linear dose response noncancer effects
$^{chronic}ESL_{nonlinear(nc)}$	chronic health-based Effects Screening Level for nonlinear dose response noncancer effects
$^{chronic}ESL_{veg}$	chronic vegetation-based Effects Screening Level
F	exposure frequency, days per week
FDA	Food and Drug Administration
GSH	glutathione
GST	glutathione transferase
h	hour
$H_{b/g}$	blood:gas partition coefficient
$(H_{b/g})_A$	blood:gas partition coefficient, animal
$(H_{b/g})_H$	blood:gas partition coefficient, human
HEC	human equivalent concentration
HPRT	hypoxanthine phosphoribosyl-transferase
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
LED	lowest effective dose
LMS	linearized multistage
LOAEL	lowest-observed-adverse-effect-level
MC	Methylene chloride
MCMC	Markov chain Monte Carlo
MW	molecular weight

<b>Acronyms and Abbreviations</b>	<b>Definition</b>
µg	microgram
MFO	Mixed function oxidase
min	minute
MOA	mode of action
MRL	Minimal Risk Level
NA	Not applicable
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect-level
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PBPK	physiologically-based pharmacokinetic model
PCBs	polychlorinated biphenyls
PK	pharmacokinetic
POD	point of departure
POD <sub>ADJ</sub>	point of departure adjusted for exposure duration
POD <sub>HEC</sub>	point of departure adjusted for human equivalent concentration
ppb <sub>v</sub>	parts per billion by volume
ppm <sub>v</sub>	parts per million by volume
REL	Reference Exposure Level
ReV	Reference Value
RfC	Reference Concentration
RGDR	regional gas dose ratio
SMR	Standard mortality rate
SSB	Single strand break
T	time or exposure duration
TCEQ	Texas Commission on Environmental Quality
TD	Toxicology Division

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<b>Acronyms and Abbreviations</b>	<b>Definition</b>
TD <sub>05</sub>	Tumor dose (5% excess tumor rate)
TWA	Time-Weighted Average
UF	uncertainty factor
UF <sub>H</sub>	interindividual or intraspecies human uncertainty factor
UF <sub>A</sub>	animal to human uncertainty factor
UF <sub>L</sub>	LOAEL to NOAEL uncertainty factor
UF <sub>D</sub>	incomplete database uncertainty factor
URF	Unit Risk Factor
US	United States
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WOE	weight of evidence

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## Chapter 1 Summary Tables

Table 1 for air monitoring and Table 2 for air permitting provide a summary of health- and welfare-based values from the acute and chronic evaluations of methylene chloride (MC). Please refer to the Air Monitoring Comparison Value document (AMCV Document) available at [AMCVs at TCEQ](#) for an explanation of the values used for review of ambient air monitoring data and air permitting. Table 3 provides summary information on MC's physical/chemical data.

**Table 1. Air Monitoring Comparison Values (AMCVs) for Ambient Air**

Short-Term Values	Concentration	Notes
Acute ReV	12,000 $\mu\text{g}/\text{m}^3$ (3,500 ppb) <b>Short-Term Health</b>	<b>Critical Effect:</b> CNS depression as indicated by decreased visual-peripheral performance in humans
$^{\text{acute}}\text{ESL}_{\text{odor}}$	560,000 $\mu\text{g}/\text{m}^3$ (160,000 ppb) <b>Odor</b>	50% odor detection threshold
$^{\text{acute}}\text{ESL}_{\text{veg}}$	---	No relevant data found
Long-Term Values	Concentration	Notes
Chronic ReV	1,300 $\mu\text{g}/\text{m}^3$ (360 ppb)	<b>Critical Effect:</b> significant increases in the incidence of hepatocellular cytoplasmic vacuolization and multi-nucleated hepatocytes (i.e., liver histopathology) in female rats
$^{\text{chronic}}\text{ESL}_{\text{linear}(c)}$	350 $\mu\text{g}/\text{m}^3$ (100 ppb) <sup>a</sup> <b>Long-Term Health</b>	<b>Critical Effect:</b> liver and lung tumors in mice
$^{\text{chronic}}\text{ESL}_{\text{veg}}$	---	No relevant data found

<sup>a</sup> Based on an inhalation unit risk factor (URF) of 2.8E-08 per  $\mu\text{g}/\text{m}^3$  (9.8E-08 per ppb) and a no significant risk level of 1 in 100,000.

Abbreviations used in Tables 1 and 2: **AMCV**, Air Monitoring Comparison Values; **ppb**, parts per billion;  **$\mu\text{g}/\text{m}^3$** , micrograms per cubic meter; **h**, hour; **HQ**, hazard quotient; **ESL**, Effects Screening Level; **ReV**, Reference Value;  $^{\text{acute}}\text{ESL}$ , acute health-based ESL;  $^{\text{acute}}\text{ESL}_{\text{odor}}$ , acute odor-based ESL;  $^{\text{acute}}\text{ESL}_{\text{veg}}$ , acute vegetation-based ESL;  $^{\text{chronic}}\text{ESL}_{\text{linear}(c)}$ , chronic health-based ESL for linear dose-response cancer effects;  $^{\text{chronic}}\text{ESL}_{\text{nonlinear}(nc)}$ , chronic health-based ESL for nonlinear dose-response noncancer effects; and  $^{\text{chronic}}\text{ESL}_{\text{veg}}$ , chronic vegetation-based ESL

**Table 2. Air Permitting Effects Screening Levels (ESLs)**

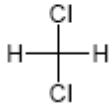
<b>Short-Term Values</b>	<b>Concentration</b>	<b>Notes</b>
<sup>acute</sup> ESL [1 h] (HQ = 0.3)	3,600 $\mu\text{g}/\text{m}^3$ (1,100 ppb) <sup>a</sup> <b>Short-Term ESL for Air Permit Reviews</b>	<b>Critical Effect:</b> CNS depression as indicated by decreased visual-peripheral performance in humans
<sup>acute</sup> ESL <sub>odor</sub>	560,000 $\mu\text{g}/\text{m}^3$ (160,000 ppb)	50% odor detection threshold
<sup>acute</sup> ESL <sub>veg</sub>	---	No relevant data found
<b>Long-Term Values</b>	<b>Concentration</b>	<b>Notes</b>
<sup>chronic</sup> ESL <sub>nonlinear(nc)</sub> (HQ = 0.3)	390 $\mu\text{g}/\text{m}^3$ (110 ppb) <sup>b</sup>	<b>Critical Effect:</b> significant increases in the incidence of hepatocellular cytoplasmic vacuolization and multinucleated hepatocytes (i.e., liver histopathology) in female rats
<sup>chronic</sup> ESL <sub>linear(c)</sub>	350 $\mu\text{g}/\text{m}^3$ (100 ppb) <sup>c</sup> <b>Long-Term ESL for Air Permit Reviews</b>	<b>Critical Effect:</b> liver and lung tumors in mice
<sup>chronic</sup> ESL <sub>veg</sub>	---	No relevant data found

<sup>a</sup> Based on the acute ReV of 12,000  $\mu\text{g}/\text{m}^3$  (3,500 ppb) multiplied by 0.3 to account for cumulative and aggregate risk during the air permit review.

<sup>b</sup> Based on the chronic ReV of 1,300  $\mu\text{g}/\text{m}^3$  (360 ppb) multiplied by 0.3 to account for cumulative and aggregate risk during the air permit review.

<sup>c</sup> Based on an inhalation unit risk factor (URF) of 2.8E-08 per  $\mu\text{g}/\text{m}^3$  (9.8E-08 per ppb) and a no significant risk level of 1 in 100,000.

**Table 3. Chemical and Physical Data**

Parameter	Value	Reference
Molecular Formula	$\text{CH}_2\text{Cl}_2$ 	ATSDR (2000)
Molecular Weight	84.93 (g/mole)	TRRP (2006)
Physical State	liquid	ATSDR (2000)
Color	colorless	ATSDR (2000)
Odor	sweet, pleasant	ATSDR (2000)
CAS Registry Number	75-09-2	TRRP (2006)
Synonyms and Trade Names	Synonyms: dichloromethane Trade names: Narkotil, Solaesthin, Solmethine	ATSDR (2000)
Solubility in water	15,400 mg/L	TRRP (2006)
Log $K_{ow}$	1.34	TRRP (2006)
Vapor Pressure	455 mm Hg at 25°C	TRRP (2006)
Vapor Density (air = 1)	2.93 g/L	ATSDR (2000)
Density (water = 1)	1.3182 g/ml at 25°C	ATSDR (2000)
Melting Point	-95.1°C	ATSDR (2000)
Boiling Point	40°C	ATSDR (2000)
Conversion Factors	$1 \text{ :g/m}^3 = 0.28 \text{ ppb}$ $1 \text{ ppb} = 3.53 \text{ }\mu\text{g/m}^3$	ATSDR (2000)

## Chapter 2 Sources, Air Concentrations, and Uses

### 2.1 Sources and Air Concentrations

General information on MC sources, taken from the Agency for Toxic Substances and Disease Registry (ATSDR 2000), is given below. See ATSDR (2000) for the cited references.

According to the Toxics Release Inventory, in 1998, the estimated releases of methylene chloride of 40 million pounds (18.3 million kg) to air from 714 large processing facilities accounted for about 97.4% of total environmental releases (TRI98 2000). Table 5-1 lists amounts released from these facilities. The TRI data should be used with caution because only certain types of facilities are required to report. This is not an exhaustive list.

Because methylene chloride is a highly volatile substance, most environmental releases are into the atmosphere. Methylene chloride is released to the atmosphere during its production, storage, and transport, but most (more than 99%) of the atmospheric releases

result from industrial and consumer uses (EPA 1983c, 1985e). In 1992, 32.5% of the total global emission of methylene chloride was attributed to North America (McCulloch and Midgley 1996). It has been estimated that 85% of the total amount of methylene chloride produced in the United States is lost to the environment (EPA 1985e), about 86% of which is released to the atmosphere (EPA 1982a).

Consumer products containing methylene chloride in wide use include paint strippers, aerosols, adhesives and glues, and cleaning fluids and degreasers (CPSC 1990). Virtually all the methylene chloride in these products is released to the atmosphere during use.

Methylene chloride is formed during water chlorination (NAS 1977) and is emitted to the air from waste waters in treatment plants (Corsi et al. 1987; Dunovant et al. 1986; Namkung and Rittmann 1987). Declining production amounts of methylene chloride will result in a decrease in the volume emitted to the atmosphere.

Methylene chloride is produced by the chlorination of methane with chlorine or by the chlorination of methanol with hydrogen chloride followed by chlorination of methyl chloride (Mannsville Chemical Products Corporation 1988). Production of methylene chloride grew steadily through the 1970s and early 1980s at about 3% each year, with a peak production of about 620 million pounds in 1984. By 1988, methylene chloride production volume had dropped due to declining demand to about 500 million pounds (Mannsville Chemical Products Corporation 1988; USITC 1989). This decline in the demand for methylene chloride was expected to continue at a rate of about 1–2% per year through 1993, as more manufacturers move toward water-based aerosol systems in anticipation of further regulation of methylene chloride (HSDB 1999; NTP 1989). In 1994, the latest year for which data are available, 403 million pounds of methylene chloride were produced (C&EN 1996).

The Toxics Release Inventory (TRI98 2000) reports that methylene chloride is produced at 23 facilities in 14 states. Table 4-1 summarizes information on the U.S. companies that reported the manufacture and use of methylene chloride in 1998 (TRI98 2000).

According to Table 5-1 of ATSDR (2000), air emissions of MC from Texas facilities accounted for approximately 2% of the total air emissions reported for the 1998 TRI. Ambient air data collected by the Texas Commission on Environmental Quality (TCEQ) from 1999-2007 indicate that annual average air concentrations of MC at monitoring sites around Texas range from nondetectable levels to an approximate maximum annual average of 1.6 ppb, with a statewide mean annual average (including ½ the detection limit as proxy values for nondetects) for 1999-2007 of about 0.06 ppb and a statewide median of about 0.04 ppb. The maximum site average in Texas for 1999-2007 (1.6 ppb) is near the upper end of the range reported for ambient air monitoring sites in Canada based on surveys conducted between 1988 and 1990 (1.0 to 6.2  $\mu\text{g}/\text{m}^3$  or 0.28 to 1.8 ppb) (Long et al. 1994), and the Texas statewide average (0.06 ppb) is below the lower end of the range.

MC levels in indoor air may be significantly higher than in ambient air, most likely due to its presence in many consumer products (e.g., paint removers, spray paints, stains/varnishes) (Health Canada 1993). For example, the mean concentration reported for a Canadian indoor air survey of 757 homes ( $16.3 \mu\text{g}/\text{m}^3$ ) is over six times higher than the mean from a Canadian urban air survey ( $2.6 \mu\text{g}/\text{m}^3$ ) (Long et al. 1994), and the indoor air means in metro Toronto ( $9.1$  to  $26.9 \mu\text{g}/\text{m}^3$ ) were well above the corresponding ambient air means for MC ( $1.6$  to  $2.0 \mu\text{g}/\text{m}^3$ ) (Health Canada 1993). In regard to indoor air concentrations, ATSDR (2000) indicates:

Indoor air concentrations resulting from the use of methylene chloride-containing consumer products have been estimated to range from 0.06 to 5,472 ppb (Callahan 1981; NAS 1978; Otson et al. 1983). Previous estimates indicated that a concentration of 50 ppm ( $174 \text{mg}/\text{m}^3$ ) of methylene chloride would have been expected in the breathing zone of consumers following hair spray use (FDA 1985), resulting in a time-weighted average exposure of 0.174 ppm. Hair care specialists would have been exposed to 10 times this level (FDA 1985). However, this source of exposure has been virtually eliminated since the FDA (1989) banned the use of methylene chloride in hairsprays.

See ATSDR (2000) for additional information on sources and monitored environmental levels.

## **2.2 Uses**

Information on MC uses, taken from ATSDR (2000), is given below. See ATSDR (2000) for the cited references.

Methylene chloride is used as a solvent in paint strippers and removers (25%), as a propellant in aerosols (25%), as a process solvent in the manufacture of drugs, pharmaceuticals, and film coatings (20%), as a metal cleaning and finishing solvent (10%), in electronics manufacturing (10%), and as an agent in urethane foam blowing (10%) (NTP 1989). Aerosol products in which methylene chloride may be found include paints, automotive products, and insect sprays. However, because of labeling regulations and concerns over health and environmental issues, the use of methylene chloride in consumer aerosol products has declined (Mannsville Chemical Products Corporation 1988). Methylene chloride was once used in hair sprays but this use was banned in 1989 (FDA 1989).

Methylene chloride is also used as an extraction solvent for spice oleoresins, hops, and for the removal of caffeine from coffee. These uses of methylene chloride are approved by the Food and Drug Administration (see Chapter 7). However, it has been reported that because of concern over residual solvent, most decaffeinator no longer use methylene chloride (Mannsville Chemical Products Corporation 1988). Methylene chloride is also approved for use as a post-harvest fumigant for grains and strawberries and as a degreening agent for citrus fruit (Hearne et al. 1990; Mannsville Chemical Products Corporation 1988; Meister 1989; NTP 1989).

Individuals using consumer products containing substantial amounts of methylene chloride have the potential for high exposure to this compound (CPSC 1987). Paint strippers, adhesive removers, spray shoe polishes, adhesives, glues, paint thinners, and many other household products contain enough methylene chloride to expose consumers to significant amounts of methylene chloride vapor when the products are used, especially indoors (CPSC 1987, 1990).

See ATSDR (2000) and CalEPA (2000) for additional information on the uses of MC.

## **Chapter 3 Acute Evaluation**

### ***3.1 Health-Based Acute ReV and ESL***

MC is a small lipophilic molecule that is rapidly absorbed from the alveoli of the lung into the systemic circulation where it is distributed into all body tissues and cellular lipids, particularly adipose tissue, and rapidly crosses the blood-brain barrier (Health Canada 1993). While MC is capable of producing point-of-entry effects (e.g., respiratory and eye irritation) at relatively high concentrations, the most sensitive effects of lower concentration MC exposure are systemic in nature (e.g., neurobehavioral, increased blood carboxyhemoglobin).

#### **3.1.1 Physical/Chemical Properties and Key Studies**

##### ***3.1.1.1 Physical/Chemical Properties***

The main chemical and physical properties of MC are summarized in Table 3. MC is a colorless liquid, halogenated hydrocarbon (commonly known as dichloromethane) with a sweet, pleasant odor (ATSDR 2000).

##### ***3.1.1.2 Essential Data and Key Studies***

Available data indicate that the central nervous system (CNS) is the most sensitive target of acute inhalation exposure to MC in humans, rats, guinea pigs, rabbits, dogs, and monkeys, while the liver is the primary target of chronic inhalation exposure (ATSDR 2000, Haber et al. 2002). Signs and symptoms of MC-induced CNS depression include dizziness, incoordination, and decreased performance in tests of sensory and motor functions. These effects are likely caused by a combination of the anaesthetic properties of MC and accumulation of carboxyhemoglobin (COHb) which forms as a result of MC metabolism (ATSDR 2000) (see Section 3.1.2). In addition to the likely contribution of MC-induced increases in COHb to CNS effects, cardiac effects resulting from COHb formation following MC exposure, such as those observed in sensitive human populations following carbon monoxide (CO) exposure (Kleinman et al. 1989, Allred et al. 1989), are considered a possible endpoint of concern for MC toxicity (CalEPA 1999a).

##### **3.1.1.2.1 Human Studies**

The CNS is the primary target for short-term inhalation exposure in humans based on human experimental and occupational studies which reported alterations in behavioral performance and various psychomotor tasks following exposure to MC. For example, all 33 cases of acute inhalation exposure to MC reported to occupational health authorities in the United Kingdom between 1961 and 1980 involved depression of the CNS (ATSDR 2000). A summary of human (and animal) studies may be found in ATSDR (2000). Human data on the short-term (i.e., acute) CNS effects of MC are available and preferred over animal study data for the calculation of an acute Reference Value (acute ReV) and acute Effects Screening Level (<sup>acute</sup>ESL).

#### ***3.1.1.2.1.1 Key Study - Putz et al. (1979)***

In Putz et al. (1979), twelve volunteer nonsmokers (6 male, 6 female) were exposed to 195 ppm of MC (analytical concentration) for 4 hours (h). This exposure was intended to produce a COHb level of approximately 5% (maximum acceptable limit recommended by NIOSH at the time) during the fourth hour of exposure. The American Conference of Governmental Industrial Hygienists (ACGIH 1999) currently has an occupational biological exposure index (BEI) of 3.5% COHb to prevent neurobehavioral changes and maintain cardiovascular exercise capacity in workers, and increase protection for pregnant workers, the fetus, and workers with chronic heart and respiratory disease. Neurobehavioral/CNS effects were assessed by performance on a visual-manual task, dual-task, and an auditory vigilance task. Various tests were performed at specific time intervals throughout the 4-h exposure, including breath sample analysis for CO, blood sample analysis for COHb, and several behavioral performance tests (e.g., dual-task eye-hand coordination tracking in conjunction with monitoring peripheral visual stimuli). In addition to a dual-task test to assess behavioral effects, an auditory vigilance task test was performed (modeled after the Winneke 1974 supporting study). Each subject served as their own control.

The 4-h exposure was divided into three 80-minute blocks, and each block had four main activity time periods: dual task, auditory vigilance, dual-task, and rest. The dual-task period lasted from time 0 to 16 minutes and consisted of eight 1-minute dual-tasks, performing for 2 minutes then pausing for 2 minutes. The auditory vigilance task lasted 30 minutes (from time 18 to 48 minutes), and was followed by a 5- to 6-minute breath test. The dual-task activity then lasted 16 minutes, and was followed by 8-9 minutes of rest. This same sequence was repeated another two times for a total exposure duration of 240 minutes (4 h). The target MC concentration (200 ppm) was essentially obtained (195 ppm) within the first 30 minutes of exposure/testing and was maintained through the 4-h period. A pre- and post-exposure finger-prick blood sample was collected for COHb analysis. Alveolar breath samples were collected every hour during and after exposure for COHb analysis, for a total of 5 samples per 4-h exposure.

An eye-hand coordination task with concurrent peripheral brightness monitoring was the main performance test and required manipulation of a small hand control lever to position an oscilloscope beam in the center of the scope face. The degree to which the subject tracked the beam by compensatory movements of the control lever served as the index of eye-hand coordination. Monitoring peripheral stimuli for the occurrence of a signal was the second task which created the dual-task along with the beam tracking task. More specifically, subjects were

to press a response switch when the intensity of two peripherally mounted lights increased, requiring sustained attention between two tasks. A MC-induced decrease in alertness or arousal would cause a decreased ability to divide attention and perform one or both of the dual-task activities. The auditory vigilance task entailed listening to pulses of white noise (0.4 second duration, 2 seconds apart) by earphones. At random intervals with a probability of 0.2, a slightly more or less intense pulse was inserted. A hand-held button was to be pressed in response to a less intense pulse (the signal). A computer automated both the dual-task and auditory vigilance task. Arousing aspects of the testing situation were minimized as direct subject-experimenter interaction was eliminated through use of the computer interface and the selected tests could be more-or-less conducted continuously. Low arousal in the testing environment, a longer testing period, and tests designed to load the behavioral system and reduce spare compensatory capacity are conducive to the identification of subtle functional deficits (Putz et al. 1979).

Study results indicate that exposure to MC significantly decreased visual and psychomotor performance and auditory function. In the dual-task test, MC induced decrements of 36% and 17% in the eye-hand coordination and visual-periphery components, respectively. In the auditory test, MC exposure produced decrements of approximately 16-20% for various indices (e.g., consistent decline in percent correct during exposure, signal detectability, average response time). These performance decrements are indications of CNS depression (ATSDR 2000, Putz et al. 1979). No subjective symptoms, such as headache, nausea, or irritation of the nose and throat, were reported. The time-progression of effects was as follows:

- A progressive deterioration in visual-peripheral performance (i.e., an increase in peripheral light response time) was reported as a function of time, and significant decreases in peripheral-visual performance were noted after 1.5 h of exposure.
- Increasing decrements in tracking and auditory monitoring performance were observed after longer exposure durations (2-4 h).
- In the final hour of exposure, the level of CO in exhaled breath had risen to 50 ppm and the level of COHb had risen to 5%. Blood COHb levels rose from 1.35% pre-exposure to 5.1% post-exposure.

*Deterioration in peripheral-visual performance was the CNS/neurological effect that occurred at the exposure duration of 1.5 h and exposure concentration of 195 ppm, and this lowest-observed-adverse-effect-level (LOAEL) will be used as the point-of-departure (POD) for derivation of the acute ReV and ESL.*

#### **3.1.1.2.1.2 Supporting Study - Stewart et al. (1972)**

Stewart et al. (1972) conducted a series of four exposure chamber experiments. Subjective and objective responses were recorded upon entrance to the exposure chamber and every 15 minutes thereafter. A venous blood sample was collected before exposure, every 30 minutes during exposure, and after exposure for COHb analysis. Breath samples were collected for MC and CO analysis, and 24-h urine samples were collected for urobilinogen analysis. For experiments 2 and



3, visual evoked response (VER) was obtained for each subject before exposure and after 1 and 2 h of exposure. Generally, VER evaluates the conduction of electrical impulses from the optic nerve to the occipital cortex of the brain (the center of the visual perception system) in response to flashes of light. While alterations in VER concern changes in sensory processes and accompany the initial phases of CNS depression, beyond this it is unclear as to the extent the limited VER observations in this study should be considered adverse. Regardless, VER changes are not the critical effect used by TD based on this study.

In experiment 1, the male subject first noted in a previous experiment to have elevated COHb was exposed to 213 ppm of MC for 1 h. Blood COHb rose from 0.4% pre-exposure to 1.75% after the 1-h exposure, and 2.4% three hours after exposure had ended. *Data indicate that COHb levels  $\geq 2\%$  result in decreased time to onset of exercise-induced angina and time to ischemic electrocardiographic changes in nonsmoking coronary artery disease patients (ACGIH 2001, CalEPA 1999a,b), a potentially sensitive subpopulation to COHb resulting from MC metabolism.* Angina pectoris, whether induced by physical exertion or otherwise, is characterized by severe pain (typically radiating down the left arm) and a sensation of heart constriction due to a deficiency of oxygen to the heart (Davis 1989). Ischemic electrocardiographic changes are changes in the electrical activity of the heart due to ischemia, a deficiency of blood supply (oxygen) to the heart due to obstruction (Davis 1989). Additionally, the 2.4% COHb level reported 3-h following exposure approaches the level ( $\geq 2.5\%$ ) which produces a significant decrease in strenuous exercise capacity in healthy nonsmokers (ACGIH 2001). Twenty hours after exposure ended in Stewart et al. (1972), COHb had declined to 1.5%. No adverse symptoms were noted during or within 24 h following exposure.

In experiment 2, three male volunteers were exposed to 986 ppm of MC for 2 h. Odor was reported to be moderately strong but not particularly objectionable. After 1 h of exposure, two of the subjects reported light-headedness which remained throughout the rest of the exposure and cleared within five minutes of cessation of exposure. One subject noted a “thick” tongue and difficulty enunciating. After 1 h of exposure, the VERs for two subjects showed alterations, and after 2 h, all subjects had alterations of their VERs. The study did not provide degrees of relative VER change (e.g., percent increase in peak-to-peak amplitude), only a figure. The VERs of all three subjects shifted toward control levels 1 h after termination of exposure. One hour into the exposure, blood COHb rose to 10.1%, and declined to 3.9% seventeen hours after exposure had ended.

In experiment 3, three male volunteers were exposed to 514 ppm of MC for 1 h, followed by 868 ppm for a second hour. While no adverse subjective symptoms occurred during the first hour, one subject developed definite light-headedness when the concentration was increased to 868 ppm, which persisted until five minutes after exposure had ended. Based on Figure 4 in Stewart et al. (1972), COHb rose from the 0.6% baseline to as much as approximately 3% after 1 h of exposure, as much as about 6% after 2 h of exposure, and as much as about 8% one hour after exposure had ended. Twenty-four hours after exposure had ended, COHb levels had not yet returned to normal. The VER of all subjects was altered after 1 h of exposure to 514 ppm, and

after the second hour of exposure to 868 ppm. The study did not provide degrees of relative VER change (e.g., percent difference in peak-to-peak amplitude) or a figure for this experimental condition.

In experiment 4, eight male subjects were exposed to 515 ppm of MC for 1 h. No adverse symptoms were noted. However, effects on VER were not assessed. Blood COHb rose to 2.6% after 1 h of exposure, and to 3.4% one hour after exposure had been terminated. At twenty-one hours post exposure, it was still slightly elevated. As mentioned previously, the BEI recommended by ACGIH for CO is 3.5% COHb (at the end of the worker shift) based on preventing adverse neurobehavioral changes, maintaining cardiovascular exercise capacity, and protecting pregnant workers (i.e., the fetus) and workers with chronic cardiovascular and respiratory disease (ACGIH 2001). In addition, ACGIH (2001) concludes that persons with coronary artery disease are particularly sensitive to the effects of COHb, with decreased exercise time to onset of angina or ischemia observed at COHb levels less than the 3.4% COHb level reported in this study 1 h after exposure to 515 ppm had ended.

Exposure to MC in this study produced signs of CNS depression (e.g., light-headedness, VER alterations) and elevations in COHb levels. The COHb generated from MC metabolism may pose an additional human health burden for persons with existing cardiovascular disease, smokers, etc. Based on experiment 3 in Stewart et al. (1972), 514 ppm was considered the 1-h LOAEL based on VER alterations in all subjects after a 1-h exposure. *Based on experiment 1, the 213 ppm MC exposure level is considered the 1-h LOAEL for increases in blood COHb levels (i.e., 2.4% blood COHb level 3 h after exposure to 213 ppm MC for 1 h) associated with aggravation of angina in a sensitive subpopulation (i.e., those with coronary artery disease), and will be used as the POD for derivation of supporting values.* Although experiment 1 utilized a single male volunteer, the LOAEL is reasonable for use in supporting calculations given: (1) the relative increase in COHb and exposure level in experiment 4, where exposure of eight males produced a COHb of 2.6% after 1 h of exposure to 515 ppm (compared to 1.75% COHb after 1 h of exposure to 213 ppm in experiment 1), rising to 3.4% 1 h later; (2) it is based on measured COHb and exposure concentration values which likely provide a similar but slightly more conservative LOAEL than physiologically-based pharmacokinetic (PBPK) modeling results (e.g., CalEPA 1999a used a PBPK model to predict that a 1-h exposure to 340 ppm would be associated with a 2% COHb level); and (3) exposure to this study LOAEL (213 ppm) for 1 h resulted in a COHb level (2.4% at 3 h after exposure) just above the lower end of the COHb level of concern for those with coronary artery disease ( $\geq 2\%$  COHb). Given the TCEQ's role in protecting public health, the blood COHb level (2.4%) which serves as basis for the LOAEL (213 ppm) is somewhat more conservative than the BEI (3.5% blood COHb) for occupational workers, who are typically more healthy as a group than the general population and less likely to contain as large of a proportion of particularly sensitive individuals and/or subpopulations.

#### ***3.1.1.2.1.3 Supporting Study - Winneke (1974)***

Winneke (1974) is used as a supporting study for the adverse CNS/neurological effects of MC. In Winneke (1974), 6-20 female volunteers were exposed to 317, 470, or 751 ppm MC

(analytical concentrations) or filtered air for 3-4 h. Subjects were tested with standard neurobehavioral tests measuring critical flicker fusion frequency (visual), auditory vigilance performance, and performance on psychomotor tasks. These higher-order functions involve complex visual and CNS processes that are assumed to be influenced by the level of “cortical alertness” mediated by subcortical structures, especially the reticular formation (Fodor and Winneke 1971 as cited in ATSDR 2000), and are sensitive indicators of CNS-related depression, drowsiness, or narcosis (ATSDR 2000).

The auditory vigilance test entailed listening to pulses of white noise (0.36 second duration, 2 seconds apart) by earphones. A slightly less intense pulse was inserted at random intervals with a probability of 0.03. A key was to be pressed in response to a less intense pulse (the signal). The test included four observation periods of about 45 minutes each. There was a 5-minute interruption between any two observation periods for determination of visual critical flicker fusion (CFF) frequency. CFF is the frequency at which a flickering stimulus is perceived to be steady, with higher values suggesting greater perceptual accuracy. Each CFF value was the average of eight single CFF determinations. Auditory vigilance and CFF were tested at approximately 50-minute intervals in 20 females exposed to 317, 470, or 751 ppm MC or filtered air. CNS depressants decrease CFF and impair vigilance performance (Winneke 1974).

Psychomotor tests included: (1) hand tapping for speed without hand-eye coordination (at maximum speed, the average number of taps during four test periods of 15 seconds each); (2) two-plate tapping for speed with some hand-eye coordination (at maximum speed with one arm alternating taps between two plates 40 cm apart, the average number of taps during four test periods of 20 seconds each); (3) steadiness for the static control of hand/arm (attempting to hold a stylus in the middle of an opening, the average number of contacts with the opening edge and the cumulative contact time during four test periods of 30 seconds each); (4) Purdue Hand-Precision Test for hand-eye coordination and precision under a paced working condition (the average number of hits, errors, and cumulative contact time of errors when using a stylus to tap on three targets appearing in succession on a disk rotating 50 rpm during three test periods of 60 seconds each); (5) visual pursuit tracking for visual-motor control of larger muscle groups (the average time-on-target when using a stylus to contact a target appearing on a disk rotating 50 rpm during three test periods of 40 seconds each); and (6) visual test of reaction time (over at least 30 trials, the average time to switch off 1 of 2 lights or 1 of 5 lights by pressing the appropriate button with the right index finger). Psychomotor testing, along with auditory vigilance and CFF, were tested in 18 females exposed to 800 ppm MC or filtered air. Psychomotor testing began at 127 minutes into the 4-h exposure. See Figure 1 of Winneke (1974) for more details regarding the temporal structure of the testing experiments.

All exposure concentrations produced a statistically significant depression in CFF frequency compared to controls. The magnitude of CFF frequency depression was similar at 317 and 470 ppm, and was larger at 751 ppm. It appears from Figure 4 of Winneke (1974) that after 77 minutes of exposure, only the 751 ppm exposure group (N=6) was significantly different than controls (N=20) in CFF, and that the 317 ppm exposure group (N=12) was significantly different

than controls at 177 minutes (2.95 h) and 227 (3.78 h) minutes. Auditory vigilance performance was significantly impaired at 317 ppm (N=12), in one experiment of two at 470 ppm (N=14), and at 751 ppm (N=6). Psychomotor task performance, such as motor speed (tapping), reaction time, hand precision, and steadiness, was decreased at 751 ppm, the only concentration tested (see Figure 2 of Winneke 1974). Although data indicate the impairment of auditory vigilance performance at all three exposure concentrations, the lack of a consistently significant response at 470 ppm causes the pooled data at 470 ppm to show less of a response than at 317 ppm. The result is not only a non-monotonic dose-response, but a dose-response inversion at the two lowest doses (i.e., exposure to 470 ppm results in less of an impairment than exposure to 317 ppm). For this reason, the Toxicology Division (TD) did not consider 317 or 470 ppm as a clear LOAEL for impairment of auditory vigilance performance. The 751 ppm exposure level produced a much greater response/impairment of auditory vigilance, the interpretation of which is not confounded by an inverted dose-response at lower concentrations and inconsistent results at 500 ppm, and was considered by TD as the LOAEL for this endpoint. *Therefore, decreased CFF frequency was the most sensitive CNS response of the neurological indicators tested in this study, and was associated with a LOAEL of 317 ppm MC.* Given the known narcotic properties of MC (e.g., it was once used as a narcotic), it may be logical to describe these CNS effects observed at low MC concentrations as a result of a state of reduced cortical arousal, or a pre- or sub-narcotic state (Winneke 1974).

While Winneke (1974) is useful as a supporting study for the adverse CNS/neurological effects of MC reported in Putz et al. (1979), calculations for Winneke (1974) are not carried forward in this document since: (1) the LOAEL (317 ppm) is somewhat higher than that from the Putz et al. (1979) key study (195 ppm) and the Stewart et al. (1972) supporting study (213 ppm); and (2) adjustment of the LOAEL to a 1-h duration either by PBPK modeling or default methods would result in an even higher human point-of-departure (POD<sub>HEC</sub>) (e.g.,  $POD_{HEC} = C_2 = [(C_1)^3 \times (T_1 / T_2)]^{1/3} = [(317 \text{ ppm})^3 \times (2.95 \text{ h} / 1 \text{ h})]^{1/3} = 455 \text{ ppm}$ ).

#### ***3.1.1.2.1.4 Developmental/Reproductive Effects in Humans***

ATSDR (2000) cites no LOAELs or no-observed-adverse-effect-levels (NOAELs) for developmental/reproductive effects in humans. Although some human studies have suggested potential effects (e.g., reduced sperm count and abnormal morphology, increased spontaneous abortion), the studies are limited by multiple concurrent exposures, lack of adequate MC exposure data, small sample size, inconsistent findings (e.g., oligospermia), and in some cases, a lack of a control group or not controlling for confounding factors (e.g., smoking, alcohol). See Section 3.1.1.2.2 for a discussion of developmental/reproductive effects in animals.

### 3.1.1.2.2 Animal Studies

Human data are available and preferred over animal studies for calculation of the acute ReV and acute<sup>ESL</sup> for MC. Therefore, with the exception of a brief discussion of developmental/reproductive effects in animal studies below, this document focuses on relevant human studies (see above).

#### ***Developmental/Reproductive Effects in Animals***

ATSDR (2000) cites no LOAELs for reproductive effects in animals, and provides the following information on developmental effects in animals following short- and long-term exposure (*italics* added for emphasis). A draft 2010 toxicological review of MC by USEPA (2010) does not provide more current information than ATSDR (2000) on developmental/reproductive studies, as such studies were conducted for MC some time ago. Ultimately, ATSDR concludes that data suggest developmental toxicity due to MC exposure is not a major area of concern. See ATSDR (2000) for the cited references.

A study in rats demonstrated that methylene chloride crosses the placental barrier (see Section 2.3.2.1; Anders and Sunram 1982). No treatment-related visceral abnormalities were reported in fetuses of mice and rats exposed to 1,250 ppm of methylene chloride during gestation, but an increase in the incidence of minor skeletal variants (e.g., delayed ossification of sternbrae or extra sternbrae) was observed in both species; rats also exhibit an increased incidence of dilated renal pelvis. A maternal effect of increased liver weight was observed (Schwetz et al. 1975). When rats were exposed to 4,500 ppm, maternal liver weights increased and fetal body weights decreased, but teratogenic effects were not observed and viability and growth were not affected (Bornschein et al. 1980; Hardin and Manson 1980). Wheel running activity and avoidance learning were not affected in rats born to dams exposed prior to and/or during gestation to methylene chloride at 4,500 ppm (Bornschein et al. 1980). Longer-term exposure (for two generations) to concentrations of 1,500 ppm of methylene chloride did not affect neonatal survival or neonatal growth in rats (Nitschke et al. 1988b). *Although fetal body weights were decreased, the absence of other fetotoxic effects, major skeletal variants, or significant embryolethality suggests that developmental toxicity is not a major area of concern following exposure to methylene chloride.*

In regard to short-term exposure, Schwetz et al. (1975) reported a LOAEL of 1,250 ppm for minor skeletal variants in Swiss-Webster mice and Sprague-Dawley rats exposed 7 h/day on gestational days 6-15. This LOAEL for developmental effects is lower than the NOAEL (1,500 ppm) for developmental/reproductive effects reported in a long-term (two generation) rat study (Nitschke et al. 1988a), and is a potential POD. However, at 1,250 ppm MC, it is significantly higher than the human LOAEL of 195 ppm from the key Putz et al. (1979) study, and from a less relevant species. Additionally, CalEPA (1999a) considered the effects reported in Schwetz et al. (1975) as reflective of developmental variation as opposed to representing adverse developmental effects. Nevertheless, the human equivalent concentration (POD<sub>HEC</sub>) that results

based on the LOAEL from Schwetz et al. (1975) is considered here for use as a POD. See Appendix 1 for additional details.

Comparison of  $POD_{HEC}$  values provides a reasonable method for determining the likely critical effect of a chemical in humans based on scientific knowledge of chemical-specific effects levels. As the  $POD_{HEC}$  of 1,250 ppm based on Schwetz et al. (1975) (Appendix 1) is significantly higher than that based on the human study (223 ppm derived in Section 3.1.5 below), derivation of an acute ReV and <sup>acute</sup>ESL based on the human study is expected to be protective of developmental effects. This conclusion remains unchanged even considering the uncertainty in using a laboratory animal study since dividing this  $POD_{HEC}$  (1,250 ppm) by an animal-to-human uncertainty factor ( $UF_A$ ) of 3 for potential pharmacodynamic species differences (the pharmacokinetic/dosimetric adjustment from rats to humans has already been made in Appendix 1) would result in a value (417 ppm) higher than that based on the human study (223 ppm). Please refer to ATSDR (2000) for additional discussion of short-term animal inhalation studies.

### **3.1.2 Metabolism and Mode-of-Action Analysis**

#### ***3.1.2.1 Metabolism and Related Potentially Sensitive Subpopulations***

ATSDR (2000) provides the following discussion of MC metabolism. See ATSDR (2000) for the cited references.

Methylene chloride can be detected in blood. Because it is cleared from blood very rapidly, this method is only useful for monitoring recent exposures. A plasma half-life of inhaled methylene chloride in humans is estimated to be 40 minutes (DiVincenzo et al. 1972).

Available data suggest that there are two pathways by which methylene chloride is metabolized. One pathway utilizes the mixed function oxidase (MFO) enzymes and produces carbon monoxide (CO) (Figure 2-3, Pathway 1). The other pathway involves the glutathione transferase (GST) and produces carbon dioxide (CO<sub>2</sub>) (Figure 2-3, Pathway 2). It has been postulated that CO<sub>2</sub> can also be produced by the MFO pathway if the reactive intermediate in this pathway (postulated to be formyl chloride) reacts with a nucleophile prior to elimination of the chloride ion and formation of CO (Figure 2-3, Pathway 3) (Gargas et al. 1986).

The MFO pathway seems to be the preferred pathway for methylene chloride metabolism following inhalation exposures. Human subjects exposed by inhalation to 500 ppm or greater for 1 or 2 hours experienced elevated COHb concentrations indicating that methylene chloride was metabolized to CO by the MFO pathway (Stewart et al. 1972). The COHb concentrations rose to an average of 10.1% saturation 1 hour after the exposure of 3 subjects to 986 ppm of methylene chloride for 2 hours. The mean COHb concentration at 17 hours-post exposure remained elevated (3.9% saturation) above the preexposure baseline value (1–1.5% saturation). The exposure of 8 subjects to 515 ppm

of methylene chloride for 1 hour increased the COHb level, which remained elevated above baseline for more than 21 hours. The half-life of COHb is normally approximately 5.3 hours but this can be reduced to 60–90 minutes with inhalation of 100% oxygen. The half-life of COHb following methylene chloride exposure is twice that following exposure to carbon monoxide (NIOSH 1974; Stewart and Hake 1976). Because carbon monoxide is generated metabolically, this often necessitates a longer duration of oxygen therapy after methylene chloride poisoning than with carbon monoxide poisoning.

In human subjects exposed by inhalation to 50–500 ppm of methylene chloride for up to 5 weeks, COHb concentrations could be predicted from methylene chloride exposure parameters (Peterson 1978). However, the exhaled breath concentrations of methylene chloride correlated better with exposure parameters than did COHb concentrations. No differences in methylene chloride metabolism between male and female subjects were detectable and there was no induction of metabolism to CO during 5-weeks exposure to concentrations ranging from 100 to 500 ppm of methylene chloride (Peterson 1978). Metabolism of methylene chloride in animals has been shown to be similar to that in humans in an experiment by Fodor et al. (1973).

Thier et al. (1991) investigated whether the human metabolism of methylene chloride was similar to monohalogenated methanes; these compounds are metabolized via a glutathione-dependent pathway in human erythrocytes in a subgroup of the human population (called “conjugators”), whereas another subgroup does not exhibit erythrocyte glutathione-mediated metabolism (called “nonconjugators”). Blood samples were taken from 10 volunteers who had previously been determined to be either “conjugators” (subgroup B) or “nonconjugators” (subgroup A) of monohalogenated methanes. The samples were exposed to radiolabeled methylene chloride, incubated, centrifuged to separate blood plasma from cellular fraction, and the distribution of radioactivity between the different blood compartments measured. For individuals from subgroup B, radioactivity in blood plasma increased over time, reaching 30% in the low-molecular weight fraction and 5% in the high-molecular weight fraction after 9 hours. For individuals in subgroup A, almost no radioactivity was found in either blood plasma molecular fraction. In all samples from both groups, no radioactivity was found in either erythrocyte cytoplasm or membranes. Thus, although dihalogenated methylene chloride does not appear to undergo metabolism in erythrocytes, some metabolic transformation of methylene chloride appears to have occurred in the plasma of all individuals classified as “conjugators” (subgroup B), whereas this metabolism did not occur in “nonconjugators” (subgroup A). The authors concluded that these data provide evidence for enzyme polymorphism in humans with respect to methylene chloride metabolism.

An extremely detailed analysis of these two metabolic pathways was conducted in mice, rats, hamsters, and humans, both *in vivo* and *in vitro*. These studies provide evidence for the concentration-dependent behaviors of the two pathways and compare the metabolic rates by each pathway in the four species (Bogaards et al. 1993; Green 1991, 1997; Reitz

et al. 1989). *In vivo*, the cytochrome P-450 MFO pathway was the major route of metabolism at low-concentration inhalation exposures to methylene chloride. In both rats and mice, saturation of the MFO pathway occurred at concentration levels of 500 ppm and above, with maximum COHb levels reported to be 12–15%. *In vitro* studies verified that this pathway was similar in all four species. In contrast, the glutathione S-transferase pathway was the major metabolic pathway at exposure concentrations used in the rodent cancer bioassays, and showed a linear concentration response. Furthermore, metabolic activity in mouse tissue was more than 10-fold greater than metabolic activity in rat tissue. The glutathione-mediated metabolic rates in hamster and human tissues were even lower than those observed in the rat (Green 1997). In human tissues, metabolic rates in the lung were about 10-fold lower than those in the liver (Green 1997). Although the  $\Theta$  (theta) class glutathione S-transferase (enzyme 5-5 in rat) has a high specific activity for metabolizing methylene chloride (Meyer et al. 1991; Sheratt et al. 1997), the  $\mu$ -class GSTs (enzymes 3-3, 3-4, and 4-4) are, as a group, 800-fold more abundant in rat liver (Blocki et al. 1994); in rat liver cytosol, the  $\Theta$ -class enzyme (5-5) and the  $\mu$ -class group of enzymes (3-3, 3-4, and 4-4) are each responsible for half of the metabolism of methylene chloride to formaldehyde (Blocki et al. 1994). In addition, another  $\Theta$ -class enzyme (12-12) is present in rat liver, but its lability during isolation has prevented analysis of its specific activity (Meyer et al. 1991). Although Schroder et al. (1996) demonstrated that the human erythrocyte GSTT1-1 is polymorphic, from N-terminal modification, and differs from liver and lung GSTT1-1, it is not yet known whether liver and lung human GSTT1-1 are polymorphic. Thus, the enzymatic basis for methylene chloride metabolism in different tissues in different species is not completely elucidated.

Nelson et al. (1995) examined the distribution of erythrocyte GSTT1-1 polymorphisms among five different ethnic groups: North American Caucasians, African-Americans, Mexican-Americans, Chinese, and Koreans. Polymerase chain reaction (PCR)-based genotyping of erythrocyte GSTT1-1 demonstrated that significant ethnic variations occur. The prevalence of the nonfunctional genotype (i.e., the one lacking the ability to metabolize methylene chloride) was highest among Chinese (64%), followed by Koreans (60%), African-Americans (22%), Caucasians (20%), and Mexican-Americans (10%). These data suggest that there are ethnic differences in metabolizing capacity; additionally, substantial variations in GSTT1-1 polymorphisms also occur within ethnic groups (Nelson et al. 1995).

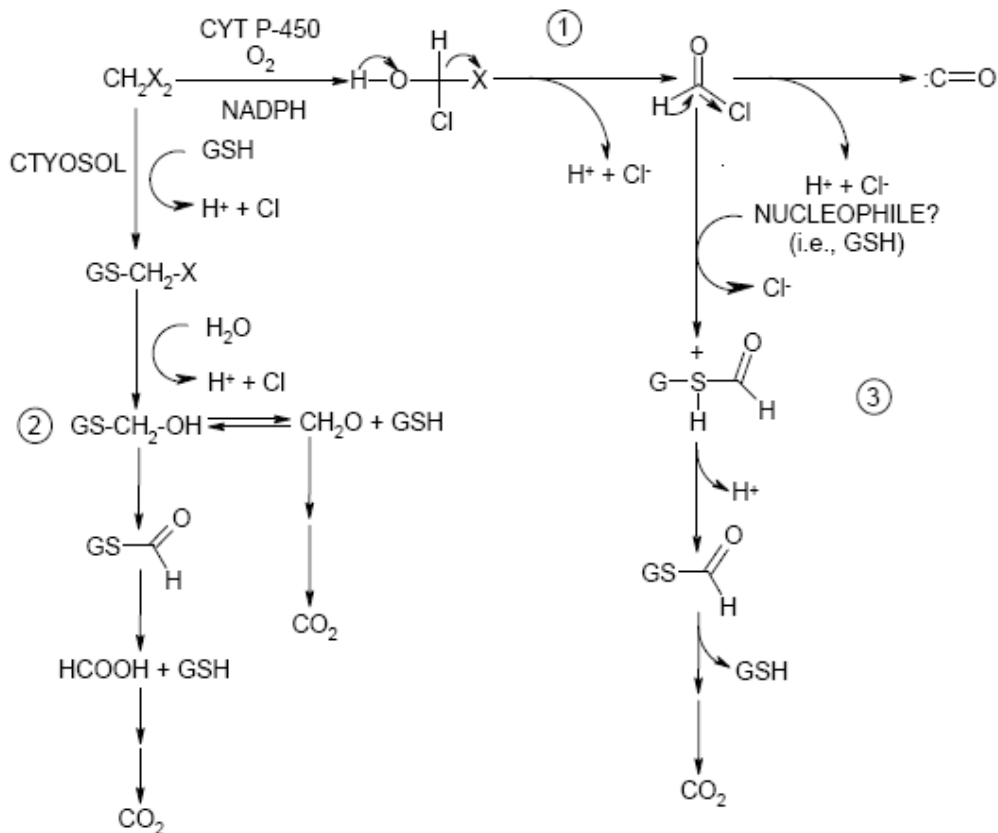
More specifically, the high affinity, low-capacity MFO pathway (microsomal) is mediated by CYP2E1, while GSTT1-1 has been identified as the isozyme that metabolizes MC via the lower affinity, higher capacity GST pathway (cytosolic). Other human liver GSTs appear to be largely unable to substitute for GSTT1-1 in MC metabolism, although a minor contribution from the GSTM1 isozyme cannot be completely ruled out (Jonsson and Johanson 2001, El-Masri et al. 1999, Burek et al. 1984).



In regard to metabolism as it relates to potentially sensitive subpopulations for the carcinogenic effects of MC, there are genetic differences in ethnic groups regarding the ability to metabolize MC via the putative carcinogenic GST pathway. A polymorphism in GSTT1-1 has been characterized and varies with race. Asian-americans appear to have the highest frequency of lacking GSTT1-1 (62% are homozygous (-/-) for the null gene allele), while hispanics have the highest frequency (47%) of being homozygous (+/+) for the positive allele, and Caucasians (49%) and African-americans (50%) have the highest percentages of being heterozygous (+/-). Heterozygotes (+/-) have half the GSTT1-1 activity as positive allele homozygotes (+/+), who may represent a sensitive subpopulation to the carcinogenic effects of MC as the GST pathway is the putative carcinogenic pathway (Haber et al. 2002, David et al. 2006). The null GSTT1-1 allele genotype (-/-) is considered protective as the enzyme is necessary for metabolic bioactivation of MC via the GST pathway, although there could be some contribution by GSTM1 (El-Masri 1999). Polymorphisms have also been detected in CYP2E1, and one such polymorphism leads to a higher rate of transcription, a higher level of protein, and higher enzyme activity. The frequency of the allele was 16% in a group of 203 Mexican-Americans, considerably higher than the 1–5% frequency measured in Caucasians. This polymorphism would presumably result in higher rates of MC metabolism and possibly more severe toxic effects (ATSDR 2000).

Regarding MC metabolism and children, a subpopulation commonly considered to be potentially sensitive to carcinogenicity, neonatal children up to 5 years of age may be less likely than adults to be exposed to the carcinogenic metabolites of MC based on the pharmacokinetics of blood MC and metabolism to reactive carcinogenic metabolites via the GST pathway (Clewell et al. 2004 as cited by David et al. 2006). To illustrate age-specific pharmacokinetic differences in metabolism, Clewell et al. (2004) report that at an oral MC exposure of 1 µg/kg-day, the estimated glutathione (GSH) conjugation rate per kg of liver (proportional to the cancer risk assessment dose metric of GST metabolites/L or kg tissue-day) increases consistently until age 25, where it essentially remains stable and is over 30 times that of an infant. Even at age 15, the analysis in Clewell et al. (2004) suggests that the metabolism of MC via the GST pathway is only about half that at age 25 (see Figure 2C in Clewell et al. 2004). In other words, adults are expected to experience higher doses of the putative carcinogenic (i.e., GST pathway) metabolites. The implication is that based on age-specific pharmacokinetic differences in metabolism via the putative carcinogenic GST pathway, children may be reasonably expected to be no more sensitive (and may be less sensitive) to the potential carcinogenic effects of MC due to childhood exposure. However, study limitations (e.g., limited age-specific GST data, need for age-specific PBPK model validation) preclude a quantitative use of reported results (e.g., derivation of age-specific adjustment factors). See Section 4.2.7 of the *Carcinogenic Potential* section for additional information and discussion on evaluating the potential for early-life susceptibility. Significant differences in pharmacokinetics were not demonstrated in a comparison of men and women (Clewell et al. 2004 as cited by David et al. 2006).

Figure 1 was taken from ATSDR (2000) and depicts the metabolism of MC. See Section 2.3.3 of ATSDR (2000) for additional information regarding the metabolism of MC.



Source: Gargas et al. 1986

- 1 Mixed Function Oxidase Pathway
- 2 Glutathione Transferase Pathway
- 3 Nucleophile Pathway

**Figure 1 Proposed Metabolic Pathways for MC**

### 3.1.2.2 Mode of Action (MOA) Analysis

A MOA is generally defined as a sequence of key events and processes (starting with interaction of an agent with a cell and proceeding through operational and anatomical changes) resulting in toxicity (USEPA 2005). ASTDR (2000) provides the following information on the MOA for MC-induced neurotoxicity (i.e., CNS effects).

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

monoxide (CO) associated mainly with metabolism via the MFO pathway. CO binds to hemoglobin, and can cause COHbemia.

In summary, the mechanism of MC-induced CNS/neurological effects is not clear. Putz et al. (1979) attributed the observed CNS/neurological effects to the accumulation of COHb, which may be the primary mediator of these effects at lower acute exposure levels (USEPA 2010). However, Winneke (1974) concluded that these effects were primarily due to the parent compound properties of MC, which could also play a role, especially at relatively high exposure levels (USEPA 2010). The anesthetic effects of the parent compound and the hypoxic effects of its metabolite CO may both contribute to the observed symptoms. Therefore, CNS/neurological effects observed in the key Putz et al. (1979) study and the supporting Winneke (1974) study may be caused by a combination of the anesthetic properties of MC and accumulation of COHb which forms as a result of MC metabolism (ATSDR 2000). See Sections 4.5.4 and 4.6.3.3 of USEPA (2010) for additional available information regarding the MOA for MC-induced neurotoxicity.

### 3.1.3 Dose Metric

In the key study (Putz et al. 1979) and supporting study (Stewart et al. 1972), data on MC air concentration are available. Exposure concentration of the parent chemical will be used as the default dose metric for the key study because there may be two MOAs involved in CNS effects (i.e., the anesthetic properties of MC and accumulation of COHb), resulting in uncertainty regarding the use of any single dose metric other than air concentration as the most appropriate (i.e., use of peak concentration of MC in brain tissue would ignore the potential contribution of blood COHb, and vice versa), and data on other dose metrics are not readily available. MC exposure concentration was also used as the dose metric for the supporting study as it is available, the LOAEL corresponds to a COHb level (2.4%) slightly above the lower end of the range (2-3% COHb) reported to be associated with effects (e.g., aggravation of angina) in a sensitive subpopulation (i.e., those with coronary artery disease), and use of a slightly lower target COHb level (2%) as a dose metric to derive a corresponding air concentration  $POD_{HEC}$  using PBPK is unlikely to produce a significantly lower value. In fact, CalEPA (1999a) used a PBPK model to predict the 1-h MC exposure level associated with a 2% COHb level and the predicted value (340 ppm) is actually higher than that demonstrated in the supporting study (213 ppm) to produce a 2.4% COHb level. Consequently, TD considers air concentrations demonstrated to produce certain COHb levels as potentially more predictive (associated with less uncertainty) for use as the dose metric.

### 3.1.4 Points-of-Departure (PODs) for the Key and Supporting Studies

The LOAEL of 195 ppm (analytical concentration) based on CNS effects (deterioration in peripheral-visual performance) from the Putz et al. (1979) key study will be used as the  $POD_{HEC}$  in calculation of the acute ReV and <sup>acute</sup>ESL. The LOAEL of 213 ppm based on increase in COHb level (2.4 %) from the Stewart et al. (1972) supporting study will be used as the  $POD_{HEC}$

in supporting calculations. Data from these studies were not amenable to benchmark dose modeling.

### 3.1.5 Dosimetric Adjustments

The LOAEL (195 ppm) from the Putz et al. (1979) key study is for a 1.5-h exposure duration, so an exposure duration adjustment to 1 h must be considered. Time-series data from Putz et al. (1979) indicate that MC-induced CNS effects are both concentration- and time-dependent (e.g., see Figure 3 of Putz et al. 1979). Therefore, exposure duration adjustment is appropriate for the Putz et al. (1979) key study (TCEQ 2006). Default procedures discussed in TCEQ (2006) with  $n = 3$  are used to adjust to a 1-h exposure duration for acute studies where both concentration and duration play a role in toxicity. Since the LOAEL (213 ppm) from the Stewart et al. (1972) supporting study is for 1 h, no exposure duration adjustment is needed.

Putz et al. (1979) key study:  
$$POD_{HEC} = C_2 = [(C_1)^3 \times (T_1 / T_2)]^{1/3} = [(195 \text{ ppm})^3 \times (1.5 \text{ h} / 1 \text{ h})]^{1/3} = 223 \text{ ppm (LOAEL)}$$

Stewart et al. (1972) supporting study:  
$$POD_{HEC} = 213 \text{ ppm (LOAEL)}$$

### 3.1.6 Critical Effect and Adjustments of the $POD_{HEC}$

#### 3.1.6.1 Critical Effect

CNS effects are considered the most sensitive endpoint for acute human inhalation exposure to MC. These effects are likely caused by a combination of the anesthetic properties of MC and accumulation of COHb which forms as a result of MC metabolism (ATSDR 2000). The specific critical effect for the Putz et al. (1979) key study is CNS depression as indicated by decreased peripheral-visual performance. As the accumulation of COHb due to MC-metabolism is another possible concern since levels > 2% are associated with the aggravation of angina in some individuals, this endpoint was evaluated through use of the Stewart et al. (1972) supporting study.

#### 3.1.6.2 Uncertainty Factors (UFs)

The MOA by which MC may produce CNS effects is discussed in Section 3.1.2.2. CNS depression as indicated by decreased peripheral-visual performance is the critical effect of short-term MC exposure in the key study and a threshold effect. For noncarcinogenic effects which exhibit a threshold, nonlinear MOA, a  $POD_{HEC}$  is determined and appropriate UFs are applied to derive a ReV (TCEQ 2006).

##### 3.1.6.2.1 Putz et al. (1979)

The duration-adjusted LOAEL from Putz et al. (1979) of 223 ppm was used as the  $POD_{HEC}$  and divided by the following uncertainty factors (UFs): 6.3 for extrapolation from a LOAEL to a NOAEL ( $UF_L$ ), 10 for intrahuman variability ( $UF_H$ ), and 1 for database uncertainty ( $UF_D$ ) (total

UF = 63). A  $UF_L$  of 6.3 was used per Section 3.5.2 of TCEQ (2006) as the effect was judged to be mild. It is not abundantly clear based on the adverse endpoint itself (i.e., decreased peripheral-visual performance) where it should fit into the spectrum of neurological effect severity (Chou and Johnson 1998, TCEQ 2006). This determination requires consideration of study-specific information regarding the degree of CNS depression, which appears to be relatively mild at the 1.5-h exposure point. The  $UF_L$  used by TD is consistent with that used by CalEPA (1999a) for the same study ( $UF_L$  of 6).

A  $UF_H$  of 10 was used for intrahuman variability since the study population was comprised of healthy adults and not known to include potentially sensitive subpopulations (e.g., those with pre-existing cardiovascular disease, children), and there are known polymorphisms in the human population which affect the ability to metabolize MC and may confer increased susceptibility to the toxic effects of MC (e.g., CYP2E1, GSTT1). For example, polymorphisms have been detected in CYP2E1, and one such polymorphism leads to higher enzyme activity. This allele would presumably result in higher rates of MC metabolism and possibly more severe toxic effects (see Section 3.1.2.1). A  $UF_H$  of 10 was also used by ATSDR (2000) in deriving the acute inhalation minimal risk level (MRL) for MC to account for data that indicates smokers, persons with existing cardiovascular disease, alcoholics, fetuses, and exercising individuals may be more susceptible. ATSDR (2000) indicates that one basis for a concern that certain subgroups of the general population may be more susceptible to MC-induced effects is the potential effect of COHb generated from MC metabolism, which is expected to be additive to COHb from other sources. Of particular concern are smokers (who maintain significant constant levels of COHb) and persons with existing cardiovascular disease (who are more sensitive to increases in COHb). Additionally, higher levels of COHb may result when alcoholics are exposed to MC since ethanol increases the expression and activity of CYP2E1, the high affinity of fetal hemoglobin for CO suggests that fetuses may be at greater risk to COHb-induced effects than pregnant females following maternal exposures to MC (ATSDR 2000), and exercising individuals have been shown to have increased MC blood levels, metabolism to CO, and resulting COHb (DiVincenzo and Kaplan 1981a).

A  $UF_D$  of 1 was used because the acute toxicological database for MC is sufficient and there are numerous studies which assess the most sensitive effects (i.e., CNS/neurological) (ATSDR 2000).

#### **3.1.6.2.2 Stewart et al. (1972)**

The following UFs were applied to the  $POD_{HEC}$  (213 ppm) from the Stewart et al. (1972) supporting study: A full  $UF_L$  of 10 was used as angina is considered a severe adverse effect (CalEPA 1999a, TCEQ 2006). A  $UF_D$  of 1 was used because the acute toxicological database for MC is sufficient. The  $UF_H$  was reduced from the full value of 10 as the LOAEL is based on effects in a sensitive subgroup (e.g., aggravation of angina in persons with coronary artery disease) that are associated with the COHb level (2.4%) reported in the study. However, a  $UF_H$  of 3 was maintained as it is unclear to what extent use of this study  $POD_{HEC}$  may account for the sensitivity of other subpopulations considered likely more sensitive to the toxic effects of MC

due to characteristics known to be relevant to the degree of susceptibility to COHb-induced effects from MC metabolism (e.g., CYP2E1 polymorphisms, smokers and others with higher COHb levels, alcoholics, fetuses). These subpopulations have known attributes different than persons with coronary artery disease, the consideration of which leads to a reasonable conclusion that they may also experience greater sensitivity to MC-induced effects as a result of metabolism to CO. For example, fetal hemoglobin has a high affinity for CO, endogenous CO production by pregnant women may be elevated as much as 2.3-fold, pregnant women may smoke (further increasing COHb), and the fetus may be very sensitive to the hypoxic effect of COHb (WHO 1979, ACGIH 2001). The unknown magnitude of the difference in sensitivity between persons with coronary artery disease and these other subgroups with known factors likely to increase susceptibility (e.g., fetuses) justifies the use of a  $UF_H$  of 3. Again, a  $UF_D$  of 1 was used because the acute toxicological database for MC is sufficient. Therefore, the total UF for this supporting study is 30.

### 3.1.7 Health-Based Acute ReV and <sup>acute</sup>ESL

As discussed in the previous section, UFs are applied to the key study (Putz et al. 1979)  $POD_{HEC}$  to derive the acute ReV.

Putz et al. (1979) key study:

$$\text{acute ReV} = POD_{HEC} / (UF_H \times UF_L \times UF_D) = 223 \text{ ppm} / (10 \times 6.3 \times 1) = 3.54 \text{ ppm}$$

Stewart et al. (1972) supporting study:

$$\text{acute ReV} = POD_{HEC} / (UF_H \times UF_L \times UF_D) = 213 \text{ ppm} / (3 \times 10 \times 1) = 7.10 \text{ ppm}$$

The acute ReV based on the Putz et al. (1979) key study is somewhat lower than the supporting acute ReV based on the Stewart et al. (1972) study. However, the values are similar (differ by a factor of only 2) and both studies provide useful, complementary information. The acute ReV was rounded to two significant figures at the end of all calculations. The rounded acute ReV was then multiplied by 0.3 to calculate the <sup>acute</sup>ESL, which was also rounded to two significant figures. Rounding to two significant figures, the 1-h acute ReV for MC is 3.5 ppm, or 3,500 ppb (12,000  $\mu\text{g}/\text{m}^3$ ). At the target hazard quotient of 0.3, the <sup>acute</sup>ESL is 1,100 ppb (3,600  $\mu\text{g}/\text{m}^3$ ) (see Table 4).

**Table 4. Derivation of the Acute ReV and acute ESL**

Parameter	Values and Descriptions
Study	Putz et al. (1979)
Study population	12 healthy adult volunteers
Study quality	High
Exposure Methods	4-h exposure chamber study with testing at various exposure time intervals
LOAEL	195 ppm
NOAEL	N/A
Critical Effects	CNS depression as indicated by decreased visual-peripheral performance in humans
POD <sub>HEC</sub>	195 ppm
Exposure Duration	1.5 h at time of effects noted
Extrapolation to 1 h	TCEQ (2006) default procedures with n=3
Extrapolated 1 h concentration	223 ppm
Total UFs	63
<i>Interspecies UF</i>	<i>Not applicable (NA)</i>
<i>Intraspecies UF</i>	<i>10</i>
<i>LOAEL-to-NOAEL UF</i>	<i>6.3</i>
<i>Incomplete Database UF</i>	<i>1</i>
<i>Database Quality</i>	<i>High</i>
<b>Acute ReV [1 h] (HQ = 1)</b>	<b>12,000 µg/m<sup>3</sup> (3,500 ppb)</b>
<b>Acute ESL [1 h] (HQ = 0.3)</b>	<b>3,600 µg/m<sup>3</sup> (1,100 ppb)</b>

### 3.1.8 Comparison to other State and Federal Values

The acute ReV of 3,500 ppb is similar to the CalEPA (1999a) acute Reference Exposure Level (REL) value of 4,000 ppb. It is somewhat higher than the ATSDR (2000) acute inhalation MRL value of 600 ppb. However, much of this difference is due to ATSDR using an acute exposure duration of 24 hours, which decreases the LOAEL/POD and the resulting acute MRL by a factor of 5.

## **3.2. Welfare-Based Acute ESLs**

### **3.2.1 Odor Perception**

MC has a sweet, pleasant odor (ATSDR 2000). Nagata (2003), Leonardos et al. (1969), and May (1966) have odor threshold information for MC and have been approved by TCEQ as sources for acceptable odor threshold values:

- Nagata (2003) lists a 50% odor detection threshold of 160,000 ppb ( $564,800 \mu\text{g}/\text{m}^3$ );
- Leonardos et al. (1969) lists a 100% recognition threshold of 730,000  $\mu\text{g}/\text{m}^3$  (206,799 ppb); and
- May (1966) lists a 100% recognition threshold of 790,000  $\mu\text{g}/\text{m}^3$  (223,796 ppb), and a 50% odor detection threshold of 550,000  $\mu\text{g}/\text{m}^3$  (155,807 ppb).

The Interim *Guidelines for Setting Odor-Based Effects Screening Levels* (TCEQ 2010), sets interim guidelines to be followed until the *Guidelines to Develop Effects Screening Levels, Reference Values, and Unit Risk Factors* (RG-442; TCEQ 2006) are revised to incorporate these guidelines. According to these interim guidelines, only Nagata (2003) reports an odor detection threshold of the highest quality (Level 1). Leonardos et al. (1969) and May (1966) are Level 3 (i.e., lesser quality) odor thresholds. Therefore, the Level 1 odor threshold from Nagata (2003) of 160,000 ppb ( $560,000 \mu\text{g}/\text{m}^3$ ) (rounded to two significant figures) will be used as the  $^{\text{acute}}\text{ESL}_{\text{odor}}$ . Since odor is a concentration-dependent effect, the same  $^{\text{acute}}\text{ESL}_{\text{odor}}$  is assigned to all averaging times.

### **3.2.2 Vegetation Effects**

No data were found regarding adverse effects observed in plants due to air exposure to MC. The only information found was in regard to adverse plant effects not being observed. WHO (1996) indicates that no effects were found in plants after exposure for 14 days to  $100 \text{ mg}/\text{m}^3$  (approximately 28.3 ppm). Therefore, no acute vegetation-based ESL ( $^{\text{acute}}\text{ESL}_{\text{veg}}$ ) was derived.

## **3.3. Short-Term ESL and Values for Ambient Air Monitoring Evaluation**

The acute evaluation resulted in the derivation of the following values:

- acute ReV =  $12,000 \mu\text{g}/\text{m}^3$  (3,500 ppb)
- $^{\text{acute}}\text{ESL} = 3,600 \mu\text{g}/\text{m}^3$  (1,100 ppb)
- $^{\text{acute}}\text{ESL}_{\text{odor}} = 560,000 \mu\text{g}/\text{m}^3$  (160,000 ppb)

For the evaluation of air monitoring data, the acute ReV of 3,500 ppb ( $12,000 \mu\text{g}/\text{m}^3$ ) will be used as the short-term, health-based AMCV, and the  $^{\text{acute}}\text{ESL}_{\text{odor}} = 560,000 \mu\text{g}/\text{m}^3$  (160,000 ppb) will be used as the odor-based AMCV (Table 1). The critical short-term ESL applicable to air permit reviews of MC is the health-based  $^{\text{acute}}\text{ESL}$  of 1,100 ppb ( $3,600 \mu\text{g}/\text{m}^3$ ) as it is lower than the



odor-based <sup>acute</sup>ESL<sub>odor</sub> (Table 2). The health-based <sup>acute</sup>ESL is only used for air permit reviews, and not for the evaluation of ambient air monitoring data.

## Chapter 4 Chronic Evaluation

### 4.1 Noncarcinogenic Potential

#### 4.1.1 Physical/Chemical Properties, Relevant Data, and Key Study

Physical/chemical properties of MC are discussed in Section 3.1.1.1.

Available data indicate that the liver is the primary target of chronic inhalation exposure (USEPA 2010, ATSDR 2000, Haber et al. 2002, Burek et al. 1984). While human data are preferred for derivation of a chronic noncarcinogenic ReV and ESL (i.e., <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub>), information on the long-term toxicity of inhaled MC in humans is limited. No adequate human study was identified by TD for derivation of the chronic ReV and <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub>, which is consistent with a recent draft USEPA toxicological review of MC (USEPA 2010). For example, DiVincenzo and Kaplan (1981b) is an occupational worker study that was utilized by CalEPA (1999b) to calculate the chronic REL. This study also included volunteer chamber exposures (e.g., 7.5-h exposures for 5 days). However, the exposure duration for workers was unspecified and exposure estimates were limited (i.e., only 5 days of air sampling data, range of 0-250 ppm MC for exposed workers with mean of 40 ppm, personal monitors for only 3 of the 19 exposed workers). More importantly, the end of workday COHb levels measured in this study and used by CalEPA are considered by TD to represent a short-term endpoint (e.g., COHb levels from the previous day's exposure did not accumulate in chamber-exposed volunteers). Thus, CalEPA essentially used a short-term endpoint (> 2% COHb as a LOAEL) following workday exposure to calculate the chronic REL. TD more appropriately addresses this short-term endpoint (> 2% COHb) with the acute ReV, more specifically, use of the Stewart et al. (1972) supporting study. Regardless, as will be discussed in Section 4.1.8, TD use of long-term endpoint data from a well-conducted animal study (Nitschke et al. 1988b) results in a chronic noncarcinogenic ReV that is very similar to CalEPA's chronic REL. Refer to ATSDR (2000) for the limited information available regarding the potential health effects of long-term MC inhalation exposure in humans.

In regard to animal data, important information on the long-term toxicity of MC is available from a two-year inhalation toxicity and oncogenicity rat study. Nitschke et al. (1988b) will be used as the key study in the derivation of a chronic noncarcinogenic ReV and <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub>. This study was also used by ATSDR (2000) for derivation of the chronic inhalation MRL.

#### **Key Study – Nitschke et al. (1988b)**

The objective of the Nitschke et al. (1988b) study was to investigate the toxicity of inhaled MC at lower concentrations than those used in other bioassays to determine a NOAEL for both toxicity and carcinogenicity. Target exposure concentrations of 0, 50, 200, and 500 ppm of MC were selected since a NOAEL was not identified in a previous oncogenicity study which used

exposure levels of 500-3,500 ppm (Burek et al. 1984). Groups of 90 male and 108 female Sprague-Dawley rats were exposed to MC at 0 (controls), 50, 200, or 500 ppm for 6 h/day, 5 days/week for 2 years. Actual mean analytical exposure concentrations were 50, 199, and 499 ppm. Two satellite groups of 30 female rats were also exposed (clean air for the first 12 months, 499 ppm for the next 12 months) to assess the temporal relationship between MC exposure and evidence of toxicity. All rats were examined after each exposure for signs of toxicity. Subgroups of 5 rats/sex/exposure level in the main study were sacrificed after 6, 12, 15, and 18 months of exposure. The following endpoints were evaluated: body weight, food consumption rates, organ weights, hematology, clinical chemistry, urinalysis, liver DNA synthesis, gross pathology, histopathology, and blood COHb levels (ATSDR 2000).

Mortality rates of MC-exposed rats were comparable to controls. No exposure-related gross or histopathologic changes were observed in animals from interim sacrifice groups. Blood COHb levels were dose-related and ranged from approximately 6-18% in the exposed animals. At terminal sacrifice, no pathologic or histopathologic non-tumor findings were reported except in the liver. *The incidences of both hepatocellular cytoplasmic vacuolization consistent with fatty changes and multi-nucleated hepatocytes were statistically elevated in female rats exposed to 499 ppm of MC.* A slight increase in the incidence of hepatocellular vacuolization was also observed in male rats exposed to 499 ppm. ATSDR considers hepatocellular vacuolization as a less serious adverse hepatic effect, although fatty changes are considered serious, and this degenerative change (histopathological lesion) has been selected as the critical endpoint in deriving multiple MRLs (Pohl and Chou 2005). Multi-nucleated hepatocytes are considered histopathological lesions, and may be associated with tumorigenesis in some cases (IARC 2006, Burek et al. 1984, Nitschke et al. 1988b, Ohbayashi et al. 2007). Histopathological changes in the liver were not found in female or male rats exposed to 50 or 199 ppm of MC, although multi-nucleated hepatocytes were non-statistically increased in female rats exposed to 199 ppm. No other pathologic or histopathologic non-tumor findings were reported. *Therefore, TD determined 499 ppm to be the LOAEL based on hepatic (liver histopathology) effects in female rats (more sensitive sex), and 199 ppm to be the NOAEL.* This is also consistent with USEPA's (2010) interpretation of results for this key study. [ATSDR (2000) incorrectly indicates that the study LOAEL and NOAEL for these effects are 200 and 50 ppm, respectively.]

The results of this study are consistent with the body of data on MC toxicity and toxicokinetics (ATSDR 2000). For example, Burek et al. (1984) complements the Nitschke et al. (1988b) study by demonstrating these same critical effects (i.e., critical effect concordance) at a LOAEL of 510 ppm (analytical concentration), although Burek et al. (1984) does not provide a NOAEL as 500 ppm was the lowest exposure concentration tested.

#### **4.1.2 MOA Analysis**

The MOA by which MC may produce neurotoxicity/CNS effects is discussed in Section 3.1.2.2. In summary, the roles of the parent compound, metabolites of the CYP2E1 pathway, metabolites of the GST pathway, or some combination of the parent compound and metabolites in MC-induced noncancer liver effects in rodents have not been determined (USEPA 2010). Therefore,

although it likely involves the metabolism of MC to reactive intermediates/metabolites, the MOA for hepatotoxic effects is yet to be elucidated (ATSDR 2000).

#### 4.1.3 Dose Metric

Data on exposure concentration of the parent chemical are available from the key study. The draft USEPA toxicological assessment (USEPA 2010) utilizes PBPK modeling estimates of internal liver dose of metabolites through the CYP2E1 (MFO) pathway (mg MC metabolized through the CYP/MFO pathway/L of liver/day) as the dose metric based on this metric providing adequate model fits to both the oral and inhalation key study data. However, USEPA (2010) acknowledges that there are no data to support the role of a specific metabolite in the development of noncancer liver lesions in the inhalation and oral studies, and that multiple internal dose metrics provide adequate model fit to the inhalation data (GST metabolites, CYP metabolites, AUC for the parent compound). While the model-fitting exercise performed by USEPA (2010) lends some support for use of CYP metabolites, TD does not consider it deterministic or adequately compelling to confidently inform selection of a specific alternate dose metric given: (1) the admitted data gap in MOA information as to the putative culpable metabolite(s); (2) that several dose metrics provided adequate model fits to the inhalation data; and (3) the uncertainties in the interspecies extrapolation performed by USEPA (2010) for MC (e.g., USEPA acknowledges that the modeling for the draft reference concentration (RfC) may not accurately describe the CYP2E1-catalyzed oxidation of MC serving as the basis of the dose metric). Consistent with TCEQ (2006), because of a lack of MOA information (e.g., putative hepatotoxic form: parent chemical/metabolite(s)) precluding use of another dose metric as most appropriate, exposure concentration of the parent chemical will be used as the default dose metric.

#### 4.1.4 PODs for Key Studies

The NOAEL of 199 ppm from the Nitschke et al. (1988b) key study will be used as the POD in calculation of the chronic noncarcinogenic ReV and  $^{chronic}ESL_{nonlinear(nc)}$ .

TD considered benchmark dose (BMD) modeling analysis for determining a POD based on the critical effect of hepatic (liver histopathology) effects in female rats. Some of the advantages of BMD modeling are to utilize the full dataset on the dose-response curve and to estimate a threshold concentration when a NOAEL cannot be established. However, Nitschke et al. (1988b) established a clear NOAEL of 199 ppm for the critical effect. Additionally, only at the highest dose of 499 ppm was there any increase in the critical effect over controls (e.g., 41 of 70 animals had the critical effect in both the controls and the 199 ppm exposure group), so there are no data between the NOAEL and LOAEL to inform the shape of the dose-response curve and support selection of a benchmark response (e.g., BMR of 10% increase in incidence). In other words, BMD modeling utilizing the full dataset on the dose-response curve does not appear to be an advantage in this case as the “curve” is actually defined by only two data points (i.e., the NOAEL and LOAEL). Nevertheless, per TCEQ (2006), BMD modeling was conducted using USEPA BMD software (version 2.1) and goodness-of-fit was evaluated by goodness-of-fit p

values > 0.1, visual inspection, and scaled residuals less than an absolute value of 2. While three models (i.e., Weibull, log-probit, gamma) provided adequate fit based on these criteria (data not shown), the lower 95% confidence limits on the benchmark concentrations corresponding to a 10% increased incidence (BMCL<sub>10</sub>) were far below (39.1-69.7 ppm) the NOAEL established for this study, with the low end of the BMCL<sub>10</sub> range being below even the dose (50 ppm) below the clear NOAEL (199 ppm). This appears to be a case where BMD modeling does not offer an advantage over the NOAEL/LOAEL approach as the data do not inform the shape of the dose-response curve (i.e., two points define it) or support the BMR, and there is a clear study NOAEL. Therefore, as indicated above, the POD based on the study NOAEL was used to derive the chronic noncarcinogenic ReV and <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub>.

#### 4.1.5 Dosimetric Adjustments

Because Nitschke et al. (1988b) is a discontinuous exposure animal study, is it necessary to adjust the animal exposure regimen to a continuous exposure.

$$\text{POD}_{\text{ADJ}} = \text{POD} \times (\text{D}/24 \text{ h}) \times (\text{F}/7 \text{ days})$$

where:

POD = POD from animal study based on discontinuous exposure regimen

D = exposure duration (h per day)

F = exposure frequency (days per week)

Nitschke et al. (1988b):

$$\text{POD}_{\text{ADJ}} = 199 \text{ ppm} \times (6/24) \times (5/7) = 35.5 \text{ ppm}$$

For Nitschke et al. (1988b), as the critical effect is a systemic effect (liver histopathology in female rats), MC will be treated as a Category 3 gas. For category 3 gases:

$$\text{POD}_{\text{HEC}} = \text{POD}_{\text{ADJ}} \times [(\text{H}_{\text{b/g}})_{\text{A}}/(\text{H}_{\text{b/g}})_{\text{H}}]$$

where:

$\text{H}_{\text{b/g}}$  = ratio of the blood:gas partition coefficient

A = animal

H = human

The blood:gas partition coefficients for Sprague-Dawley rats ( $(\text{H}_{\text{b/g}})_{\text{A}}$ ) and humans ( $(\text{H}_{\text{b/g}})_{\text{H}}$ ), are 19.4 and 8.94 respectively (ATSDR 2000). Other  $(\text{H}_{\text{b/g}})_{\text{H}}$  values (6.5-9.7) are available (CalEPA 2000), but would not affect calculation of the  $\text{POD}_{\text{HEC}}$  since the  $(\text{H}_{\text{b/g}})_{\text{A}}$  is greater than the  $(\text{H}_{\text{b/g}})_{\text{H}}$  no matter what value is used. Therefore, a default value of 1 is used for the RGDR (USEPA 1994).

Nitschke et al. (1988b):

$$\text{POD}_{\text{HEC}} = \text{POD}_{\text{ADJ}} \times [(\text{H}_{\text{b/g}})_{\text{A}}/(\text{H}_{\text{b/g}})_{\text{H}}] = 35.5 \text{ ppm} \times 1 = 35.5 \text{ ppm}$$

#### 4.1.6 Critical Effect and Adjustments of the $POD_{HEC}$

##### 4.1.6.1 Critical Effect

Statistically significant increases in the incidence of hepatocellular cytoplasmic vacuolization and multi-nucleated hepatocytes (i.e., liver histopathology) in female rats are the critical effects identified from the Nitschke et al. (1988b) study. Hepatocellular vacuolization and multi-nucleated hepatocytes are considered histopathological lesions (Pohl and Chou 2005, IARC 2006, Ohbayashi et al. 2007). In the absence of sufficient scientific information to the contrary (e.g., alpha-2 $\mu$ -globulin being recognized as an abnormality specific for male rat kidneys which does not have significance for human health), adverse effects observed in laboratory animals are assumed to be relevant to humans (TCEQ 2006). Regarding MC specifically: (1) MC-induced hepatotoxicity likely involves the metabolism of MC to reactive intermediates/metabolites and mammals metabolize MC via the same metabolic pathways (MFO, GST); (2) although there are some species differences in GST pathway metabolic rates, such differences are expected based on allometric considerations (e.g., allometry would predict a difference of approximately 7 between mice and humans); and (3) MFO pathway metabolic rates are similar between humans and rats (ATSDR 2000).

##### 4.1.6.2 UFs

Section 4.1.2 indicates that limited data is available regarding the MOA by which MC may produce hepatotoxicity. Determining a  $POD$  and applying appropriate UFs (i.e., assume a threshold/nonlinear MOA) is the default for noncarcinogenic effects with an unknown MOA. Therefore, UFs were applied to the  $POD_{HEC}$  value from the key study in deriving the chronic noncarcinogenic ReV. The  $POD_{HEC}$  (35.5 ppm) from Nitschke et al. (1988b) was divided by a  $UF_A$  of 3, a  $UF_H$  of 10, and a  $UF_D$  of 3, for a total UF of 100:

- A  $UF_A$  of 3 was used for potential pharmacodynamics differences between rats and humans since pharmacokinetic (dosimetric) adjustments from rats to humans have already been made;
- A  $UF_H$  of 10 was used since there are known polymorphisms in the human population which affect the ability to metabolize MC and may confer increased susceptibility to the hepatotoxic effects of MC (e.g., GSTT1, CYP2E1);
- A  $UF_D$  of 3 was used because the chronic inhalation database for MC is somewhat limited (e.g., very little published information on the hepatic effects of chronic MC exposure in humans, lack of neurodevelopmental inhalation studies, potential for immunological effects) (ATSDR 2000, USEPA 2010).

#### 4.1.7 Health-Based Chronic ReV and $^{chronic}ESL_{nonlinear(nc)}$

As discussed in the previous section, UFs are applied to the  $POD_{HEC}$  value from the key study in deriving the chronic noncarcinogenic ReV.

Nitschke et al. (1988b):

$$\text{chronic noncarcinogenic ReV} = \text{POD}_{\text{HEC}} / (\text{UF}_{\text{H}} \times \text{UF}_{\text{A}} \times \text{UF}_{\text{L}} \times \text{UF}_{\text{D}})$$

$$= 35.5 \text{ ppm} / (10 \times 3 \times 1 \times 3) = 0.355 \text{ ppm or } 355 \text{ ppb}$$

Rounding to two significant figures at the end of all calculations for the Nitschke et al. (1988b) key study yields a chronic noncarcinogenic ReV of 360 ppb ( $1,300 \mu\text{g}/\text{m}^3$ ). At the target hazard quotient of 0.3, the  $^{\text{chronic}}\text{ESL}_{\text{nonlinear(nc)}}$  is 110 ppb ( $390 \mu\text{g}/\text{m}^3$ ) (Table 5).

**Table 5. Derivation of the Chronic ReV and  $^{\text{chronic}}\text{ESL}_{\text{nonlinear(nc)}}$**

Parameter	Values and Descriptions
Study	Nitschke et al. (1988b)
Study Population	90 male and 108 female Sprague-Dawley rats per exposure group
Study Quality	High
Exposure Levels	0 (controls), 50, 199, or 499 ppm of MC
Critical Effects	Significant increases in the incidence of hepatocellular cytoplasmic vacuolization and multi-nucleated hepatocytes (i.e., liver histopathology) in female rats
POD (NOAEL)	199 ppm
Exposure Duration	6 h per day, 5 days per week for 2 years
Extrapolation to continuous exposure ( $\text{POD}_{\text{ADJ}}$ )	35.5 ppm
Extrapolation to humans ( $\text{POD}_{\text{HEC}}$ )	35.5 ppm
Total UFs	100
<i>Interspecies UF</i>	3
<i>Intraspecies UF</i>	10
<i>LOAEL-to-NOAEL UF</i>	NA
<i>Subchronic-to-Chronic UF</i>	NA
<i>Incomplete Database UF</i>	3
<i>Database Quality</i>	Medium
<b>Chronic ReV (HQ = 1)</b>	<b><math>1,300 \mu\text{g}/\text{m}^3</math> (360 ppb)</b>
$^{\text{chronic}}\text{ESL}_{\text{nonlinear(nc)}}$ (HQ = 0.3)	<b><math>390 \mu\text{g}/\text{m}^3</math> (110 ppb)</b>

### **4.1.8 Comparison of Results**

As mentioned previously, ATSDR (2000) incorrectly indicates that the NOAEL for the liver effects observed in Nitschke et al. (1988b) is 50 ppm. Examination of Table 3 of the study and the study's results section identifies 200 ppm (199 ppm analytical concentration) as the NOAEL for these effects. Additionally, the methodology used by ATSDR to derive MRLs does not incorporate the consideration of a  $UF_D$ , while TD applied a  $UF_D$  of 3. Despite these differences, the chronic inhalation MRL of 300 ppb is very similar to the chronic noncarcinogenic ReV of 360 ppb. The chronic noncarcinogenic ReV of 360 ppb is somewhat higher than the CalEPA chronic REL of 100 ppb, which is based on COHb levels greater than 2% in workers at the end of the workday, considered by TD to be a short-term effect (DiVincenzo and Kaplan 1981b, CalEPA 1999b). Additionally, the chronic noncarcinogenic ReV of 390  $\mu\text{g}/\text{m}^3$  is somewhat higher than the USEPA (2010) draft RfC of 200  $\mu\text{g}/\text{m}^3$ .

## **4.2 Carcinogenic Potential**

### **4.2.1 Relevant Human and Animal Data**

Although some human epidemiological studies are suggestive of an association between occupational exposure to MC and elevated cancer risk (e.g., liver/biliary tract, prostate, astrocytic brain cancer), any dose-response relationships are not clear, and epidemiologic evidence as a whole does not demonstrate a strong, statistically significant cancer risk associated with MC exposure. MC epidemiology studies do not have adequate information to perform a quantitative cancer risk assessment (USEPA 2010). While occupational epidemiological studies do not provide compelling evidence that inhalation exposure to MC presents a human cancer risk, they provide some support for animal bioassay inhalation (and ingestion) studies that indicate a risk for lung and liver carcinogenesis (CalEPA 2000, OSHA 1997). As it is reasonable to suspect that substances that cause cancer in multiple animal species and at multiple sites may be carcinogenic in humans in the absence of compelling epidemiological or mechanistic information to the contrary, agencies often rely on well-conducted animal bioassays as the primary basis for hazard identification and quantitative risk assessment (OSHA 1997). Data from animal (e.g., mouse) studies showing a clear dose-response relationship following inhalation exposure to MC (NTP 1986) are considered the best available for quantitative cancer risk assessment (OSHA 1997; USEPA 1987a; CalEPA 2000; USEPA 2010).

ATSDR (2000) provides the following information on the carcinogenic potential of MC:

A significant increase in bile-duct cancer was observed within a cohort of workers who had been exposed to methylene chloride (at  $\leq 1,700$  ppm, 8-h TWA) for up to 28 years (Lanes et al. 1990). Epidemiology studies have not revealed a causal relationship between deaths due to cancer and occupational exposure to methylene chloride at lower levels (475 ppm or less) (Friedlander et al. 1978; Hearne et al. 1987, 1990; Ott et al. 1983b). It should be noted that these latter studies had limited power to detect very small increases in cancer and are not sufficient to rule out a carcinogenic potential of methylene chloride.

Studies in animals exposed via inhalation have demonstrated that methylene chloride can increase the incidence of naturally-occurring tumors. When administered by inhalation, methylene chloride (2,000 ppm or greater) increased the incidence of alveolar/bronchiolar neoplasms in mice of both sexes (NTP 1986). Concentrations of 500 ppm or greater of methylene chloride increased the incidence of benign mammary gland tumors per animal in females and male rats (Burek et al. 1984; Nitschke et al. 1988a; NTP 1986). The incidence of liver tumors increased over concurrent control levels in male mice and female rats administered methylene chloride (50–250 mg/kg/day) in drinking water; however, the incidence of lesions in treated groups were within the historical range of control values and showed no dose response (Serota et al. 1986a, 1986b). The results of recent toxicokinetics studies suggest that the parent compound and/or reactive metabolites produced by the GST pathway are the source of methylene chloride-induced tumor increases.

See ATSDR (2000) for references for the studies cited above and Section 2.2.1.8 of that document for additional information on the findings of inhalation cancer studies. CalEPA (2000) also provides a summary of major epidemiological MC inhalation studies (pp. 80-91).

#### **4.2.2 Carcinogenic Weight-of-Evidence (WOE)**

IARC (1987,1999) has classified MC in Group 2B (possibly carcinogenic to humans) based on inadequate evidence of carcinogenicity in humans and sufficient evidence in experimental animals. IARC (1987) states (*italics added for emphasis*):

Seven cohort studies have examined the risk of cancer among populations exposed to dichloromethane. Two studies observed an excess of pancreatic cancer, but the three others which reported on this tumour did not. One study observed an excess of liver and biliary tract cancers among longer-term employees. One study observed an excess of prostate cancer that appeared to increase with level of exposure. One study observed an excess of breast cancer and gynaecological cancers among women with the highest likelihood of exposure and another study observed an excess of cervical cancer. With the exception of the prostate cancer excess observed in one study, all the excesses were based on small numbers. No estimates of exposure levels were available for two of the six studies.

Three case-control studies have examined the risk of cancer associated with dichloromethane exposure and provided data adequate for evaluation. One observed an association between estimated intensity, probability and duration of exposure and the risk of astrocytic brain tumours. A second, which focused on female breast cancer, observed an elevated risk in the highest exposure category but no association with probability of exposure. The third indicated an increased risk of rectal cancer and possibly lung cancer.

*For no type of cancer was there a sufficiently consistent elevation of risk across studies to make a causal interpretation credible.*



Dichloromethane was tested by oral administration in the drinking-water in one study in mice and one study in rats, by inhalation exposure in two studies in mice, three studies in rats and one study in hamsters and by intraperitoneal injection in a lung adenoma assay in mice. In the study in mice by oral administration, no increase in tumour incidence was observed. The study in rats by oral administration gave inconclusive results. In the two inhalation studies in mice, increased incidences of benign and malignant lung and liver tumours were observed in both sexes. In the three inhalation studies in rats, the incidence of benign mammary tumours was increased in one study in females of a strain in which the incidence of spontaneous mammary tumours is low, and the multiplicity was increased in two studies in females of a high-incidence strain. In one study, in males, the incidence of mammary gland adenomas and fibroadenomas was increased. Negative results were obtained in the lung adenoma test in mice and in the inhalation study in hamsters.

The Department of Health and Human Services (NTP 1999) has determined that MC may reasonably be anticipated to be a human carcinogen. OSHA (1997) determined that MC is a potential occupational carcinogen based primarily on positive carcinogenic findings in chronic inhalation bioassays in rodents (MC is a multi-species, multi-site carcinogen). The American Conference of Governmental Industrial Hygienists (ACGIH) classifies MC as a suspected human carcinogen, while the National Institute for Occupational Safety and Health (NIOSH) classifies it as a potential occupational carcinogen (CalEPA 2000).

USEPA (1989) classified MC as a Group B2 carcinogen (i.e., probable human carcinogen) based on:

- inadequate human data and sufficient evidence of carcinogenicity in animals;
- increased incidence of hepatocellular neoplasms and alveolar/bronchiolar neoplasms in male and female mice; and
- increased incidence of benign mammary tumors in both sexes of rats, salivary gland sarcomas in male rats and leukemia in female rats.

No recent information was identified in the USEPA screening-level literature review which would affect the USEPA WOE classification (USEPA 2002), although the cancer guidelines have been updated. Consistent with USEPA (1989), the draft USEPA (2010) assessment indicates that MC is considered “likely to be carcinogenic to humans” under the most recent cancer guidelines (USEPA 2005a). *TD also considers the WOE for MC as consistent with “likely to be carcinogenic to humans” under USEPA (2005a) (see pages 2-54 and 2-55). TD performs carcinogenic dose-response assessments for chemicals considered “likely to be carcinogenic to humans” (TCEQ 2006).*

### 4.2.3 Animal-to-Human Extrapolation

Mouse lung and liver tumors are the primary tumors of interest with regard to MC-induced tumorigenesis (ATSDR 2000). The available data suggest a plausible mechanism for the development of liver and lung tumors which occur in mice but not in rats exposed to MC. There are clear species differences in the putative carcinogenic pathway of MC metabolism (GST metabolic pathway) consistent with humans being less sensitive to MC-induced carcinogenesis than other species (e.g., mice) (Long et al. 1994). Mechanistic studies have established that glutathione S-transferase-mediated metabolism of MC is responsible for its genotoxicity and carcinogenicity in mice. The glutathione S-transferase responsible for the metabolism of MC (GSTT1-1) is expressed in significantly greater levels in mouse tissues as compared to rat, hamster, or human tissues (IARC 1987). Data from some *in vitro* studies suggest that humans are unlikely to be more susceptible than rodents to MC-induced liver cancer (e.g., DNA strand break differences). In addition, the human lung has little capacity to activate MC to biologically reactive intermediates through the isozyme (i.e., the  $\Theta$  (theta) class GSTs) associated with carcinogenicity in mice. GSTT1-1 is present in moderate quantities in the mouse lung but has been detected only at very low levels in human lung and liver tissue samples. These data suggest that the human lung and liver are likely to have little capacity to activate MC into its reactive metabolites (ATSDR 2000). While there is evidence that the MFO pathway metabolic rates (oxidation by cytochrome P-450) are similar among mice, rats, hamsters, and humans, GSH-mediated metabolism (glutathione conjugation; associated with carcinogenicity in mice) is more than an order of magnitude greater in mice than in rats with human and hamster rates being even lower than in rats. *These studies appear to demonstrate that species differences in glutathione-mediated metabolism are correlated with species differences in MC carcinogenicity. The mouse is the most sensitive species to metabolic activation of MC by glutathione metabolism, whereas humans appear to be the least (ATSDR 2000, Health Canada 1993).*

Since humans are more similar to rats than mice in regard to MC metabolism via the GST pathway, humans are believed to be more similar to rats in regard to MC-induced carcinogenesis. No significant increases were found in rats exposed to 100 ppm (4 h per day, 5 days per week for seven weeks, and 7 h per day, 5 days per week for 97 weeks) over their lifetime (Maltoni et al. 1988 as cited by CalEPA 2000). Additionally, MC is not known to cause cancer even in workers exposed to high MC concentrations (e.g., no excess risk of death from malignant neoplasms has been detected in workers exposed to MC at levels up to 475 ppm; ATSDR 2000) within or exceeding the range where the MFO metabolic pathway is likely saturated (CalEPA 2000), much less at significantly lower, long-term environmental ambient air concentrations (ppb range). See Section 4.2.2 for additional information on human cancer study findings.

*While humans appear to be less sensitive than mice to MC-induced carcinogenicity, there is nevertheless evidence that MC may be carcinogenic to humans. For example, the immunodetection of localized high concentrations of GSTT2-2 enzyme in human bile-duct epithelial cells is potentially significant, considering the increased incidence of biliary cancer following chronic exposure to MC (Lanes et al. 1990). Lanes et al. (1990) reported excess*

mortality associated with cancer of the buccal cavity and pharynx (combined), and liver and biliary passages (combined) in workers occupationally exposed to MC ( $\leq 1,700$  ppm) in the cellulose fiber production industry for more than 20 years. The excess mortality for the combined liver/biliary cancer cases was statistically significant with a standard mortality rate (SMR) of 5.75. The SMR was 20 for biliary cancer alone. However, an extended mortality follow-up (Lanes et al. 1993) did not confirm the excess liver/biliary tract cancer (ATSDR 2000, CalEPA 2000). Additionally, despite that Mainwaring et al. (1996) found that rates of MC metabolism were low in the human lung, higher than background amounts of GSTT1-1 mRNA were detected in some Clara cells and ciliated cells of the alveolar/bronchiolar junction in one of four human lung samples. *Therefore, despite the generally low level of GSTT1-1 and GSTT2-2 in human tissue, it is possible that specific cell types within the human liver (bile duct) and/or lung might produce genotoxic, reactive intermediates of MC metabolism in some individuals.* Heineman et al. (1994) reported an increase in the incidence of mortality due to astrocytic brain cancer associated with exposure to MC, carbon tetrachloride, tetrachloroethylene, and trichloroethylene. Importantly, the strongest association was found with MC exposure. Risk of astrocytic brain cancer increased with increasing exposure in occupations judged to be associated with MC exposure, and these trends could not be explained by exposures to the other solvents (although quantitative exposure information and workplace use records were lacking and there was a high potential for exposure misclassification) (ATSDR 2000). *OSHA and NIOSH agreed that Heineman et al. (1994) strongly suggests a possible association between MC and human cancer (OSHA 1997).*

Various literature reviews have come to different conclusions in regard to whether the epidemiological evidence is consistent (Stayner and Bailer 1993, Tollefson 1990,1988) or inconsistent (Hearne 1991) with positive animal bioassay results for carcinogenicity. USFDA (1989) indicates the possibility that MC causes cancer in humans as inferred from rodent studies cannot be ruled out, and consistent with USEPA (1987a), OSHA (1997) indicates that the epidemiological data are insufficient to rule out the cancer risk estimates derived from animal data (CalEPA 2000). *Consistent with TD's evaluation of the WOE (see Section 4.2.2) under USEPA guidelines (USEPA 2005a) and in the interest of public health, TD classifies MC as likely to be carcinogenic to humans.* However, based on GSTT enzyme distributions and concentrations, the carcinogenic risk from MC in humans appears to be low as in rats rather than high as in mice (ATSDR 2000). *Therefore, unless such considerations (e.g., species metabolic differences) are adequately accounted for through PBPK modeling, for example, estimation of the carcinogenic potential of MC in mice for application to humans may be unduly conservative (see Section 4.2.5 for more discussion).*

#### **4.2.4 Carcinogenic MOA**

A MOA is generally defined as a sequence of key events and processes (starting with interaction of an agent with a cell and proceeding through operational and anatomical changes) resulting in toxicity (USEPA 2005). This section discusses two subjects important to an understanding of the

putative carcinogenic MOA for MC-induced liver and lung tumors in mice: metabolic pathways and data supporting a mutagenic carcinogenic MOA.

#### **4.2.4.1 Metabolic Pathways**

As previously indicated, two principal pathways of MC metabolism have been identified: the MFO pathway (occurring in the microsomal fraction) and the GST pathway (localized primarily in the cytosolic fraction of several organs such as the liver and lung) (Long et al. 1994, OSHA 1997). The MFO pathway accounts for most of the metabolized MC at concentrations less than 500 ppm, but as exposure concentrations increase above the MFO saturation level, increases in the amount of MC metabolized by the secondary GSH-GST pathway are observed (ATSDR 2000). While data indicate the rate of MFO pathway metabolism is similar among mice, rats, hamsters, and humans, the rate of GST pathway metabolism is exceptionally high in the mouse (almost two orders of magnitude higher than humans). The GST pathway is the major pathway in mice when the MFO pathway is saturated, which occurs at relatively low experimental MC concentrations *in vivo* at around 500 ppm or less (Long et al. 1994), and does not appear to be saturated for any of the species investigated at doses up to 4,000 ppm (OSHA 1997).

Although one recent study raises some questions about the importance of the GST pathway (Evans and Caldwell 2010), the weight of available data strongly indicate that the GST metabolic pathway is responsible for MC-induced tumorigenesis, and that it is very unlikely that the MFO pathway is culpable for these tumors (e.g., neither MC nor the MFO pathway correlate with tumor incidence across doses or species) (USEPA 1987a, Anders et al. 2010). For example, the concentration dependency of the two metabolic pathways is consistent with the tumor results obtained in long-term rodent inhalation and drinking water cancer bioassays of MC and supports the assertion that GSH-GST-mediated metabolism is responsible for MC-induced tumorigenicity in mice (ATSDR 2000). *The assessment of cancer risk in Section 4.2.5 is based on the key role of GST metabolites in the MOA for MC-induced carcinogenesis.* There are several lines of independent evidence which support the key role of GST metabolites in the carcinogenic MOA (CalEPA 2000, ATSDR 2000):

- The dose dependency of MC-induced lung and liver tumors correlates with the lack of saturation kinetics expected of the GST pathway (at the relevant experimentally produced concentrations);
- The relative species tumor sensitivity correlates with measured GST activity (i.e., mice > rats > hamsters);
- There is a dependence on glutathione for mutagenicity of MC in the Ames *Salmonella typhimurium* test and for DNA damage in Chinese hamster ovary (CHO) cells;
- Increased mutagenicity is seen in bacteria transfected with multiple copies of 5,5-GST;
- The cellular localization of 5,5-GST in the mouse nucleus correlates with the higher sensitivity of the mouse (no nuclear localization is seen with MFO enzymes);
- Two MC metabolites of the GST pathway are capable of direct interaction with DNA;

- There is no evidence to suggest that MC is a direct acting carcinogen. For example, the marked species differences in carcinogenicity induced by MC are not typical behavior of direct-acting compounds, and MC does not exhibit the chemical reactivity towards nucleophiles normally associated with direct action.

#### **4.2.4.2 Data Supporting a Mutagenic MOA**

Mutations in somatic cells play a key, early role in cancer initiation and may also affect other stages of the carcinogenic process. Mutation induction or acquisition can be key events at some stage in all cancers since all cancer cells acquire multiple mutations during carcinogenesis. However, there are two important considerations in assessing evidence for a mutagenic MOA for carcinogenesis: (1) when the mutation occurs among the events that lead to cancer in relationship to other key events; and (2) whether the action of the carcinogen as a mutagen is a key event in its carcinogenic process. Mutagenicity is an early obligatory action of a chemical (or its metabolite) acting through a mutagenic MOA which produces heritable changes in DNA that initiate the carcinogenic process. *In other words, for a chemical to act by a mutagenic MOA, the chemical (or its direct metabolite) is the agent inducing the mutation(s) which act as the key event in initiating cancer.* By contrast, in other MOAs (e.g., cytotoxicity-induced regenerative cell proliferation) mutations are acquired subsequent to other key events in the carcinogenic process. The determination that a chemical carcinogen can induce mutation in one of a number of mutation assays is not sufficient to conclude that it causes specific tumors by a mutagenic MOA or that mutation is the only key event in the pathway to tumor induction (USEPA 2007).

Generally, the following information on the carcinogenic MOA of MC was taken almost verbatim from USEPA (2010), with *italics* provided for emphasis. The italicized section headings (e.g., *strength, consistency, and specificity of association*) are those recommended by USEPA (2007) for discussion of the experimental mutagenicity data. Please see USEPA (2010) for references of the cited studies.

The hypothesized MOA for MC-induced liver tumors is through a mutagenic MOA. MOA data indicate that MC-induced DNA damage can be produced in the cancer target tissues of mice (liver, lung) by reactive GST metabolites. Evidence of mutagenicity (or genotoxicity) includes *in vitro* bacterial assays and mammalian assays (e.g., increased hypoxanthine phosphoribosyl-transferase (HPRT) mutations in CHO cells incubated with mouse liver cytosol preparations with GST activity) as well as *in vivo* mammalian system assays (e.g., DNA single strand breaks (SSBs), sister chromatid exchanges, or DNA-protein cross-links in the mouse liver or lung). *However, mutational events in critical genes (tumor suppressor genes or oncogenes) leading to MC-induced tumor initiation and tumor promotion have not been established.* The GST metabolic pathway has been found in human tissues, albeit at lower levels than in mouse tissues; therefore, the cancer results in animals are considered relevant to humans.

#### ***Strength, consistency, and specificity of association***

It is hypothesized that mutagenic events lead to the development of liver and lung tumors following MC exposure. Several observations from experimental studies support the

mutagenicity of MC and the key role of GST metabolism and the formation of DNA-reactive GST-pathway metabolites. The GST pathway produces two metabolites of MC, S-(chloromethyl)glutathione and formaldehyde, which are potentially reactive with DNA and other cell macromolecules. Enhanced MC genotoxicity in bacterial and mammalian *in vitro* assays with the introduction of GST metabolic capacity provides support that GST metabolism and metabolites are involved (DeMarini et al. 1997, Graves and Green 1996, Graves et al. 1996, 1995, 1994b, Thier et al. 1993).

In bacterial strains where GST activity was not present (e.g., TA1535, TA1538), mutagenic effects were not reported following MC exposure (Oda et al. 1996, Simula et al. 1993, Osterman-Golkar et al. 1983, Gocke et al. 1981). Further tests of GST-dependent mutagenicity were evaluated by transfecting GST into non-GST bacterial strains or decreasing GST activity in GST bacterial strains (e.g., TA100). When GST-T1 was cloned into bacterial strain TA1535, MC treatment resulted in reverse mutations in this new GST+ TA1535 strain, and these mutations were independent of rat S9 metabolic activation (DeMarini et al. 1997, Pegram et al. 1997, Thier et al. 1993). Similarly, TA100/NG-11, a bacterial strain with decreased GST activity in comparison to the wild-type TA100 strain, showed significantly decreased mutagenicity (reverse mutations) following MC treatment (Graves et al. 1994a).

*In vitro* mammalian genotoxicity studies also support the importance of the GST pathway. Positive results in the *in vitro* assays were limited to experiments with the presence of GST in the cell system. When mouse liver cytosol was added to hamster cell lines, MC induced DNA-protein cross-links, DNA SSBs, and HPRT gene mutations (Graves and Green 1996, Graves et al. 1996, 1994b). Additionally, in mouse Clara cells (GST is localized in the lung cells of mice), DNA SSBs were reported following MC treatment, and the extent of DNA damage was significantly decreased when the cells were pretreated with a glutathione depletor (Graves et al. 1995). Other studies evaluating similar genotoxic endpoints in rat or CHO cells without modification of the low GST activity in the test system generally reported no evidence of genotoxic events (Graves et al. 1995, Andrae and Wolff 1983, Garrett and Lewtas 1983, Thilagar and Kumaroo 1983, Jongen et al. 1981). A study evaluating the genotoxic effects of MC (up to 6 mM) in freshly isolated mouse, rat, hamster, and human hepatocytes provides additional supporting evidence of the influence of GST activity on mutagenicity (Casanova et al. 1997). Positive results were only observed in hepatocytes from B6C3F1 mice; the interspecies variability in effects correlated proportionally with the enhanced GST metabolic capacity in mice (Reitz et al. 1989). In studies with human cell lines or isolated cells, positive results were reported for sister chromatid exchanges, chromosomal aberrations, DNA damage, and in the micronucleus test. Negative results were obtained with human cells in unscheduled DNA synthesis assays (Jongen et al. 1981, Perocco and Prodi 1981) and MC was not demonstrated to be genotoxic in studies of human hepatocytes (Casanova et al. 1997, Graves et al. 1995).

Two of three *in vivo* genotoxicity studies in insects reported positive results. Genotoxicity was observed in *Drosophila* for the gene mutation assay (Gocke et al. 1981) and the somatic assay (Rodriguez-Arnaiz 1998) when MC was administered through the food. When *Drosophila* were

exposed to MC via inhalation, genotoxic effects were negative as measured through gene mutation assays (sex-linked recessive lethal, somatic mutation and recombination) (Kramers et al. 1991).

*In vivo* genotoxicity studies reported DNA-protein cross-links, DNA SSBs, chromosomal aberrations, and sister chromatid exchanges in liver cells of B6C3F1 mice following acute inhalation exposure to concentrations producing liver tumors with chronic exposure (Casanova et al. 1996, 1992, Graves et al. 1995, 1994b). The formation of DNA SSBs was suppressed when the mice were pretreated with a GSH depletor (Graves et al. 1995), providing additional support for the involvement of GST metabolism. Increased sister chromatid exchanges and chromosomal aberrations were found in the lungs of mice exposed to MC for 2 weeks to 8,000 ppm or for 12 weeks to 2,000 ppm. In this study, however, there was evidence of damage at other sites too: sister chromatid exchanges were also seen in peripheral lymphocytes, chromosomal aberrations were seen in bone marrow, and micronuclei were seen in peripheral red blood cells under the same exposure protocol (Allen et al. 1990). As was seen in the liver, DNA SSBs were seen in lungs of B6C3F1 mice following acute inhalation exposure to concentrations producing lung tumors with chronic exposure, and this effect was suppressed with pretreatment with a GSH depletor, buthionine sulfoximine (Graves et al. 1995). Other studies of sister chromatid exchange (Allen et al. 1990) or DNA damage detected by the comet assay (Sasaki et al. 1998) also provide evidence of genotoxic effects specifically in lung cells of mice. These *in vivo* mammalian genotoxicity studies demonstrate site-specific effects correlating to the MC-induced tumors in animals. Additional evidence for site specificity comes from a study in which DNA damage (detected by the comet assay) was enhanced in liver tissue but not stomach, kidney, brain, or bone marrow 24 hours after oral administration of 1,720 mg/kg MC to CD-1 mice (Sasaki et al. 1998).

*DNA reaction products (e.g., DNA adducts) produced by GST metabolites, such as S-(chloromethyl)glutathione, have not been identified by in vivo studies (Watanabe et al. 2007).* The authors speculated that these results are due to the instability of the reaction products (Hashmi et al. 1994). DNA adducts, however, have been observed in *in vitro* studies in which calf thymus DNA was incubated with MC and GST or was incubated with S-(1-acetoxymethyl)glutathione, a compound structurally similar to S-(chloromethyl)glutathione (Marsch et al. 2004, Kayser and Vuilleumier 2001). These findings indicate that the S-(chloromethyl)glutathione intermediate formed by GSH conjugation has mutagenic potential and is likely responsible, at least in part, for the mutagenic response observed following MC exposure. However, other studies (Hu et al. 2006, Casanova et al. 1996) provide evidence of formaldehyde-related DNA-protein cross-links in relation to MC exposure. These results show that, while most studies indicate the importance of the S-(chloromethyl)glutathione intermediate in mediating genotoxic damage following MC exposure, DNA damage resulting from formaldehyde formation should also be considered. [Although formaldehyde might play a role in MC genotoxicity, its weak mutagenicity and the absence of MC-induced DNA-protein cross-linking in the CHO/HPRT assay suggests that MC-induced DNA damage and resulting

mutations are likely produced by its glutathione conjugate, putatively S-(chloromethyl)glutathione (Graves and Green 1996).]

*Mutagenic data in critical genes leading to the initiation of MC-induced liver or lung tumors are not available. In vivo* assays evaluating mutations in tumor suppressor genes and oncogenes reported similar frequencies of activated *H-ras* genes and inactivation of the tumor suppressor genes, *p53* and *Rb-1*, in the liver tumors seen in the nonexposed and MC-exposed B6C3F1 mice (Devereaux et al. 1993, Hegi et al. 1993). There were too few lung tumors (n = 4) in controls to provide a conclusive comparison of mutation patterns between exposed and nonexposed tumors.

#### ***Dose-response concordance***

Statistically significant increases in liver tumor incidences in male and female (2,000 and 4,000 ppm) mice were observed in the inhalation bioassay in B6C3F1 mice (NTP 1986). Several studies provide evidence of an association between mutagenic events mediated by GST-pathway metabolites and the exposure levels inducing liver tumors in B6C3F1 in this study, and concentration-dependent increases in genotoxicity have been observed in *in vitro* and *in vivo* assays.

*In vitro* mammalian genotoxicity studies were positive and demonstrated a dose-response relationship for DNA-protein cross-links, DNA SSBs, and DNA damage as measured by the comet assay at concentrations ranging from 2.5 to 60 mM when mouse liver cytosol was added or if mouse GST-T1 was transfected into hamster cell lines (Hu et al. 2006, Graves et al. 1996, 1994b). In mouse hepatocytes, DNA-protein cross-links were observed following MC exposures ranging between 0.5 and 6.0 mM (Casanova et al. 1997). DNA-protein cross-links were detected in mouse hepatocytes incubated with 1.9 mM MC (Casanova et al. 1997), a concentration chosen based on its correspondence to the time-weighted average (TWA) liver concentration of MC that was predicted by the Andersen et al. (1987) PBPK model for mice exposed by inhalation to 4,000 ppm for 6 h (a dose that resulted in increased liver tumor incidence in the 2-year bioassay reported by NTP 1986). Consistent with the relative lack of liver tumor responses in Syrian golden hamsters (Burek et al. 1984) and F344 rats (NTP 1986) with chronic exposure to 3,500 or 4,000 ppm, hepatocytes from these strains of animals did not form detectable DNA-protein cross-links when incubated with 1.9 mM MC (Casanova et al. 1997).

DNA-protein cross-links were not detected in livers of mice exposed to 146 ppm 6 h/day for 3 days, but a concentration-dependent increase in DNA-protein cross-links was observed in DNA from livers of mice exposed to several concentrations between 500 and 4,000 ppm (Casanova et al. 1996). Following exposure under similar conditions (concentrations of 498, 1,553, or 3,923 ppm), DNA-protein cross-links were not detected in the livers of Syrian golden hamsters, a species that did not develop tumors after chronic inhalation exposure to MC (Casanova et al. 1996, 1992). Increased DNA SSBs were detected in liver tissue of B6C3F1 mice immediately following a 6-h inhalation exposure to MC at concentrations ranging from 2,000 to 8,000 ppm (Graves et al. 1995), and in mouse hepatocytes after a 3-h exposure to 4,000 (but not 2,000) ppm (Graves et al. 1994b).



Statistically significant increases in the incidence of lung tumors were observed in the inhalation chronic bioassay in male and female B6C3F1 mice exposed to 2,000 or 4,000 ppm MC (Menear et al. 1988, NTP 1986). Evidence of mutagenicity at these exposure levels comes from two inhalation studies (Graves et al. 1995, Allen et al. 1990). Increased DNA SSBs were detected in lung tissue of B6C3F1 mice immediately following a 6-h inhalation exposure to MC at concentrations ranging from 2,000 to 8,000 ppm (Graves et al. 1995). In the study by Allen et al. (1990), increased presence of sister chromatid exchanges was observed in mouse lung cells following a 12-week exposure at 2,000 ppm; shorter durations of exposure (2 weeks) were positive for measures of sister chromatid exchange and chromosome aberrations at 8,000 ppm, but not at 2,000 or 4,000 ppm.

DNA adducts were observed and increased with dose in an *in vitro* preparation of calf thymus DNA when treated with MC (5–60 mM) and bacterial, rat, or human GST (Marsch et al. 2004).

### ***Temporal relationship***

MC-induced liver and lung tumors first appeared in mice after 52 weeks of exposure (Maronpot et al. 1995, Kari et al. 1993). The detection of DNA-protein cross-links in the livers of B6C3F1 mice following short-term inhalation exposures to MC concentrations that induced tumors with chronic exposure (Casanova et al. 1996, 1992) provides temporal support for the proposed mutagenic MOA. Additional supporting evidence comes from observations that increased levels of DNA SSBs were detected in the liver and lungs of B6C3F1 mice immediately following 3-h inhalation exposure to 2,000–8,000 ppm MC (Graves et al. 1995, 1994b). Single dose and inhalation exposure studies of  $\leq 6$  hours did not detect an effect on DNA synthesis (Lefevre and Ashby 1989) or unscheduled DNA synthesis (Trueman and Ashby 1987) in mouse liver cells.

### ***Biological plausibility and coherence***

Bioactivation of a parent compound into a mutagenic metabolite resulting in cancer is a plausible MOA of carcinogenicity in humans and is a generally accepted MOA. MC-induced carcinogenicity is hypothesized to be due to metabolism of the parent compound by the GST pathway (GST-T1) to a metabolite that is tumorigenic. The GST metabolite, S-(chloromethyl)glutathione, formed from MC, has been characterized as labile and highly reactive through *in vitro* evaluation of MC metabolism in hepatocytes using [ $^{13}\text{C}$ ]-NMR techniques (Hashmi et al. 1994) and through an enzyme digestion assay using calf thymus DNA and GST-T1 enzyme (Marsch et al. 2004). *The hypothesis that the formation of a mutagenic metabolite is a preliminary step resulting in carcinogenicity is based on evidence that malignant tumors are primarily located in areas where MC is highly metabolized by GST-T1, such as the liver and the lung, and on mutagenicity studies indicating the importance of the GST pathway and that the lung and liver are more prone to mutagenic effects of MC (Sasaki et al. 1998, Casanova et al. 1996, 1992, Graves et al. 1995, 1994b).* The site selectivity of the mutagenicity in liver and lung tissue as evidenced by several studies suggests that the GST reactive metabolite remains in the tissue where it is formed. *Collectively, the studies support the hypothesis that MC-mediated carcinogenicity results from a GST metabolite that produces selective DNA damage in the tissues where the metabolite is formed, but this hypothesis is based in part on assumptions*

*regarding metabolite clearance and reactivity. DNA damage in the liver and lung, as well as the increased incidence of tumor formation resulting from MC exposure, indicates coherence of the mutagenic and carcinogenic effects and is evidence supporting a mutagenic MOA.*

*Differences in GST activity in mice compared with other species, and the interspecies variability in genotoxic effects corresponding to interspecies variability in tumor response, support the MOA hypothesis. DNA SSBs were not detected in liver or lung cells in rats exposed to similar inhalation exposures that induce strand breaks in mice (Graves et al. 1995, 1994b) and were detected at much lower in vitro concentrations in isolated hepatocytes from B6C3F1 mice (0.4 mM) than in hepatocytes from Alpk:APfSD rats (30 mM) (Graves et al. 1995, Figure 3). The difference in susceptibility to carcinogenic response between mice and rats likely reflects differences in GST metabolism. Toxicokinetic studies indicate that with increasing exposure levels, increasing amounts of MC are metabolized via GST metabolism.*

Data are not available to adequately support other possible MOAs for the liver and lung tumors in rodents. Although the database is lacking *in vivo* evidence of specific mutagenic events following chronic exposure to MC, there is sufficient evidence indicating the involvement of GST metabolism in the lung and liver carcinogenicity of MC in mice and supporting a mutagenic MOA. *The weight of the available evidence indicates that MC is acting through a mutagenic MOA (USEPA 2010).*

#### **4.2.4.3 TD Conclusions Regarding the Carcinogenic MOA**

MC is likely to produce a carcinogenic response via GST-mediated metabolism. For a mutagenic MOA, mutation is the first step which initiates a cascade of other key events such as cytotoxicity or cell proliferation that are key to the carcinogenesis process (USEPA 2007). The carcinogenic MOA for MC has not been fully elucidated. For example, mutational events in critical genes leading to MC-induced tumor initiation have not been identified, much less identified as the key event(s) leading to carcinogenesis, and therefore cannot be directly and definitively attributed to the various genotoxic effects demonstrated thus far for GST metabolites by *in vitro* and *in vivo* tests. USEPA (2010) acknowledges that *in vivo* evidence of specific mutagenic events following chronic exposure to MC is lacking. *However, TD agrees that the weight of the available evidence supports a mutagenic MOA under the USEPA framework for determining a mutagenic MOA (USEPA 2007).*

Please see USEPA (2010) and ATSDR (2000) for additional information relevant to the carcinogenic MOA.

#### **4.2.5 Key Study and Carcinogenic Dose-Response Assessments**

No human data were located which could be used to calculate an inhalation unit risk factor (URF) and carcinogenic-based ESL (i.e., <sup>chronic</sup>ESL<sub>linear(c)</sub>). There are no adequate epidemiologic studies to assess the carcinogenic potential of MC in humans (USEPA 2002, Long et al. 1994, USEPA 2010). However, animal carcinogenicity data are available for MC. Carcinogenicity data from inhalation animal studies are more relevant for human health risk assessment than those

from oral animal studies because inhalation is the principal route of human exposure to MC. NTP (1986) is a two-year inhalation mouse and rat study that has been used as the key study for calculation of URFs by regulatory agencies.

Based on information presented in previous sections, derivation of an inhalation URF and  $^{chronic}ESL_{linear(c)}$  based on the NTP (1986) mouse study for application to humans should take into account:

- The likely key role of GST metabolites (as opposed to the MFO metabolic pathway) in MC-induced murine carcinogenesis;
- Significantly greater GST-mediated metabolism of MC in mice as compared to humans (i.e., use of a species that is likely much more sensitive to MC-induced carcinogenesis);
- Operation of the high-affinity MFO metabolic pathway at environmentally-relevant exposure concentrations when this pathway was likely saturated (and the putative culpable GST-mediated pathway was operable) at the 2,000 and 5,000 ppm exposure levels which were carcinogenic in the mouse study (the MFO pathway is only saturated at or above approximately 200-500 ppm and the maximum annual level in Texas for 1999-2007 was about 1.6 ppb).

Unless these considerations are adequately accounted for, estimation of the carcinogenic potential of MC in mice for application to humans may be conservative and result in overestimation of the carcinogenic potential in human populations.

#### **4.2.5.1 NTP (1986) Key Study**

TCEQ, USEPA, and other regulatory agencies have determined that the NTP (1986) study provides the strongest evidence that MC is carcinogenic to rodents (CalEPA 2000), a clear dose-response (liver and lung tumors in mice), the best toxicological and statistical information on the carcinogenicity of MC for quantitative risk assessment, and is of the highest data quality (USEPA 2010, OSHA 1997). Therefore, the NTP (1986) study is the key study for development of the URF and  $^{chronic}ESL_{linear(c)}$  for MC. Based on study results, NTP (1986) concluded that there was clear evidence of liver and lung carcinogenicity in B6C3F1 mice of both sexes, and only some evidence of benign mammary tumorigenesis in female rats (CalEPA 2000). As the rat tumors were not of a type with known malignant potential, the relevance of these tumors to human health is unclear. Only the mouse portion of this study is discussed in this document because it is the portion relevant to the development of a URF for MC-induced carcinogenicity since the response was unequivocally carcinogenic and dramatic (USEPA 1987a). The following is a brief summary of the mouse study.

Groups of 50 male and 50 female B6C3F1 mice were exposed to 0, 2,000, and 4,000 ppm of MC for 6 h per day, 5 days per week, for two years. Survival was decreased in a treatment-related fashion for male and female mice. The survival of male and female mice from the high dose

groups was significantly lower ( $p < 0.001$ ) than controls. Mice were sacrificed during week 112 to 113.

There were statistically significant, dose-related increases in the incidences of alveolar-bronchiolar adenomas ( $p < 0.001$ ) and carcinomas (generally  $p < 0.001$ ) among treated mice of both sexes. No lung tumors were found in the controls, while 70 percent of the high-dose males and 71 percent of high-dose females had multiple tumors. Additionally, there were significant dose-related increases in the number of lung tumors per animal (multiplicity) in both sexes. Male mice had an elevated incidence of hepatocellular adenomas ( $p = 0.001$ ) and carcinomas ( $p < 0.001$ ) of the liver at the high dose. Females had dose-related increases of both hepatocellular adenomas ( $p < 0.001$ ) and carcinomas ( $p < 0.001$ ). Adenomas and carcinomas were significantly increased alone as well as in combination. Other studies have confirmed lung and liver tumors in mice following two-year inhalation exposure to 2,000 ppm of MC (Kari et al. 1993, Maronpot et al. 1995), and pulmonary adenomas and/or carcinomas in mice exposed via other routes (e.g., Theiss et al. 1977, Maltoni et al. 1988). Refer to Table 6 for information on the incidences of mouse lung and liver cancer reported in NTP (1986).

**Table 6. Tumor Incidence Data from NTP (1986) <sup>a</sup>**

Gender	Target Tissue	Administered Dose (ppm)	Adenoma	Carcinoma	Combined
Female	Lung <sup>b</sup>	0	2/50	1/50	3/50
		2,000	23/48	13/48	30/48
		4,000	28/48	29/48	41/48
	Liver <sup>c</sup>	0	2/50	1/50	3/50
		2,000	6/48	11/48	16/48
		4,000	22/48	32/48	40/48
Male	Lung <sup>b</sup>	0	3/50	2/50	5/50
		2,000	19/50	10/50	27/50
		4,000	24/50	28/50	40/50
	Liver <sup>c</sup>	0	10/50	13/50	22/50
		2,000	14/49	15/49	24/49
		4,000	14/49	26/49	33/49

<sup>a</sup> from Health Canada (1993).

<sup>b</sup> all  $p < 0.001$  except for alveolar-bronchiolar carcinoma in males with  $p = 0.016$ .

<sup>c</sup> generally  $p \leq 0.001$  or  $p < 0.05$ .

#### **4.2.5.2 Physiologically-Based Pharmacokinetic (PBPK) Model Assessments**

The use of scientifically-accepted PBPK models that are available in the scientific literature is preferred over default dosimetry procedures (TCEQ 2006). PBPK compartmental models are used to characterize the pharmacokinetic (PK) behavior of a chemical. Available data on blood flow rates and metabolic and other processes which the chemical undergoes within each

compartment are used to construct a mass-balance framework. Data on the metabolism of MC in humans and other species have been acquired to serve as the basis for PBPK modeling (e.g., metabolic rate constants for the MFO and GST pathways for humans, mice, and other species) (Long et al. 1994). The advantage of the PBPK approach is that it allows for consideration of pertinent data (e.g., metabolic rate species differences, target tissue dose, dose-dependent differences in disposition and metabolism, nonlinearity between applied dose and target tissue dose of carcinogenic species) that cannot be taken into account in an applied dose approach (CalEPA 2000, USEPA 1987a). Risk assessments which properly consider the role PBPK should be significantly more reliable than those which do not (Andersen et al. 1987).

USEPA (1987a) used PBPK modeling to derive the inhalation URF for MC currently available on the Integrated Risk Information System (IRIS) (USEPA 1989). Uncertainty in deriving the URF is reduced by use of PBPK modeling (compared to extrapolation based on the applied dose) since the potential influence of PK factors is markedly restricted. There have been PBPK assessments conducted by other agencies or researchers since USEPA (1987a) (e.g., CalDHS 1989, OSHA 1997, Andersen and Krishnan 1994, CalEPA 2000, Health Canada 1993, David et al. 2006), including an updated (albeit draft) assessment by USEPA (2010) used to derive a draft URF. *Although a discussion of every assessment is beyond the scope of this document, for the reasons cited below, TD selected Health Canada (1993), USEPA (2010), David et al. (2006), USEPA (1987a), and CalEPA (2000) as the assessments to be discussed in some regard within this document.*

The assessments by USEPA (1987a) and CalEPA (2000) are discussed in Appendices 3 and 4, respectively, to provide information on historical regulatory PBPK risk assessments for MC and associated issues. Briefly, TD did not consider adopting CalEPA (2000) for purposes of deriving an inhalation URF and  $^{chronic}ESL_{linear(c)}$  as human PBPK modeling was not taken advantage of for cross-species extrapolation and scaling across species with body weight<sup>3/4</sup> may not adequately account for species-specific variation in MC metabolism via the putative carcinogenic metabolic (i.e., GST) pathway (see Appendix 4). TD did not consider USEPA (1987a) for adoption as it is outdated and used inappropriate allometric scaling (see Appendices 2 and 3).

*Health Canada (1993) is discussed below as it is considered by TD to employ the most appropriate PBPK dosimetric modeling for MC of the final regulatory carcinogenic assessments available.* It utilized both mouse and human PBPK models to extrapolate across species and no superfluous allometric scaling factor was used in conjunction with the PBPK modeling. Allometric scaling is considered by TD to be inappropriate when assessing the carcinogenic potency of MC using appropriate mouse and human PBPK models for interspecies extrapolation and appropriate dose metrics (i.e., equal lifetime cancer risk should be presumed if tissues experience equal average concentrations of the carcinogenic moiety over a lifetime). This is consistent with more recent USEPA cancer assessments (USEPA 2000) and recommendations cited in the Interagency Pharmacokinetics Group (USEPA, Food & Drug Administration, Consumer Product Safety Commission) consensus report by federal scientists on cross-species extrapolation of cancer (USEPA 1992) and elsewhere (e.g., Barton et al. 1998). See Appendix 2

for additional information on allometric scaling discussed in the context of the USEPA (2000, 1987a, 2010) PBPK assessments. To say that PBPK carcinogenic assessments require considerable time and significant agency resources should not be understated. *Health Canada (1993), supported by two more recent assessments, was the regulatory assessment (final) adopted by TD for the purpose of deriving an inhalation URF and <sup>chronic</sup>ESL<sub>linear(c)</sub> due to agency time and resource constraints.*

*Results from the David et al. (2006) PBPK model and the draft USEPA (2010) assessment (the latest regulatory risk assessment, albeit draft) are discussed below as they are more recent assessments which support the Health Canada (1993) PBPK assessment.* TCEQ contracted with ICF International (work order 582-9-90441) to review the strengths and weaknesses of the existing PBPK models for MC risk assessment. ICF International identified the David et al. (2006) PBPK model as the best model for derivation of human health criteria (i.e., URF) in their August 31, 2009 report to TCEQ. However, both TD and ICF International identified some uncertainties of particular concern (e.g.,  $k_F$  value for GST metabolites, less-than-desirable level of model development detail documentation) which cannot be adequately addressed for use of David et al. (2006) as the key assessment at least partly due to agency time and resource constraints. *Therefore, use of David et al. (2006) was limited to supporting and demonstrating the probable conservativeness of the Health Canada (1993) assessment.* Although more elaborate and resource-intensive alternatives were presented as options for use of the David et al. (2006) PBPK model, one alternative identified by ICF International was to use the published human risk URFs provided by the study. TD adopted this alternative to provide human URFs from David et al. (2006) for support of, and comparison to, the URF from Health Canada (1993). Results from the draft USEPA (2010) assessment are not adopted by TD as they are draft and subject to some level of change. *Similar to David et al. (2006), use of results from the draft USEPA (2010) assessment is limited to supporting and demonstrating the conservativeness of the Health Canada (1993) PBPK assessment.*

#### **4.2.5.2.1 Health Canada (1993) Carcinogenic Assessment**

The Health Canada (1993) assessment was based on female mouse lung/liver adenoma and carcinoma data from NTP (1986) and used mouse and human PBPK modeling to account for species differences in PK (e.g., metabolism). The NTP (1986) data utilized by Health Canada (1993) are provided in Table 6 (Section 4.2.5.1).

The Health Canada (1993) assessment adequately addresses the considerations discussed in Section 4.2.5. Health Canada (1993) extrapolates risk across species and from high-to-low doses based on the amount of MC metabolism by the GST pathway at the sites of carcinogenic action (i.e., lung, liver). Unlike the USEPA (1987a) assessment discussed in Appendix 3, the Health Canada (1993) assessment does not incorporate an allometric scaling factor (i.e., surface area and/or body weight correction) for interspecies toxicodynamic differences. This is consistent with more recent USEPA assessments (e.g., vinyl chloride carcinogenic assessment in USEPA 2000) as well as considerations in a USEPA report on use of a cross-species scaling factor for cancer risk assessment (USEPA 1992). The consensus group report (USEPA 1992) does not

recommend a pharmacodynamic adjustment factor, but rather indicates that equal lifetime cancer risk should be presumed if tissues experience equal average concentrations of the carcinogenic moiety over a lifetime.

Similar to other PBPK model assessments of MC (e.g., David et al. 2006, Marino et al. 2006, OSHA 1997), the Health Canada (1993) assessment utilized the structure of the Andersen et al. (1987) PBPK model. The model groups tissues of the body into five compartments: lungs, liver, fat, and slowly and richly perfused tissues. The model incorporates metabolism of MC through both the MFO pathway and GST pathway (putative carcinogenic pathway) in the liver and lung. See Figure A-1 of the study for more information on the structure of the PBPK model. The dose metric is that typically used for MC risk assessments, internal dose of MC metabolized through the GST pathway (glutathione conjugate) in the liver and lung (mg GST-mediated metabolite/L of tissue/day). The required model inputs of physiological parameters (e.g., tissue volumes, blood flow rates, alveolar ventilation rates, cardiac output), partition coefficients (e.g., blood:air, tissue:air, tissue:blood), and metabolic rate constants for the GST and MFO pathways in mouse and human liver and lung were obtained from *in vivo* and *in vitro* experiments or from the scientific literature. For example, the assessment used updated human metabolic  $k_F$  and  $V_{max}$  values, re-optimized mouse PK parameters, and updated mouse partition coefficients based on the most recent data. See Health Canada (1993) and Table A-2 of that assessment for more details on the selection of model input values.

The tumor doses corresponding to a 5% excess tumor rate ( $TD_{0.05}$ ) for humans based on the liver and lung tumors in mice were calculated by Health Canada (1993) in five basic steps:

1. The NTP (1986) mouse exposure concentrations were converted to internal doses of MC metabolized through the GST pathway in the liver and lung (mg/L of tissue/day) through PBPK modeling (Andersen et al. 1987 model).
2. The multistage model was used to fit dose-response curves for internal dose (step 1) and the liver and lung tumor incidence data.
3. The dose-response curves (step 2) were used to determine the internal doses corresponding to the  $TD_{0.05}$  values for liver and lung tumors.
4. The  $TD_{0.05}$  values for liver and lung tumors were assumed to be equivalent in mice and humans when expressed on an internal dose basis for the amount of MC metabolized by the GST pathway (i.e., the putative carcinogenic pathway), consistent with USEPA (1992).
5. The  $TD_{0.05}$  values (step 3) are converted from mg/L of tissue/day to environmental concentrations for humans using PBPK modeling.

This procedure resulted in the following environmental  $TD_{0.05}$  values (ppm) for liver and lung tumors in humans:

**Table 7. Environmental TD<sub>0.05</sub> Values for Humans Based on Mouse Liver/Lung Tumor Data<sup>a</sup>**

<b>Tumor Type/ Target Tissue</b>	<b>Female TD<sub>0.05</sub> (ppm)</b>	<b>Male TD<sub>0.05</sub> (ppm)</b>
Adenoma, Lung	1155	1634
Carcinoma, Lung	2651	5257
<b>Combined, Lung</b>	<b>645</b>	902
Adenoma, Liver	4092	5590
Carcinoma, Liver	2965	4467
<b>Combined, Liver</b>	<b>2408</b>	4106

<sup>a</sup> From Health Canada (1993).

Table 7 shows that lung cancer (combined) in female mice is the most sensitive cancer endpoint/gender combination for extrapolation to humans as it is associated with the lowest TD<sub>0.05</sub> value of 645 ppm. For liver cancer, female mice were also more sensitive with the lowest TD<sub>0.05</sub> value of 2408 ppm. The URF for lung tumors based on the TD<sub>0.05</sub> of 645 ppm is 7.75E-05 per ppm, whereas the URF for liver tumors based on the TD<sub>0.05</sub> of 2408 ppm is 2.08E-05 per ppm.

So as not to underestimate total risk, the TD<sub>0.05</sub> values for female mice were used by TD to calculate a combined lung/liver tumor URF for MC assuming independence:

$$\text{URF} = 0.05 / \text{TD}_{0.05} (\text{lung}) + 0.05 / \text{TD}_{0.05} (\text{liver}) - [0.05 / \text{TD}_{0.05} (\text{lung}) \times 0.05 / \text{TD}_{0.05} (\text{liver})]$$

where:

[0.05 / TD<sub>0.05</sub> (lung) x 0.05 / TD<sub>0.05</sub> (liver)] accounts for the probability of liver and lung cancer occurring in the same individual (Hogg and Craig 1978).

$$\begin{aligned} \text{URF} &= 0.05 / 645 \text{ ppm} + 0.05 / 2408 \text{ ppm} - [0.05 / 645 \text{ ppm} \times 0.05 / 2408 \text{ ppm}] \\ &= 7.75\text{E-}05 \text{ per ppm} + 2.08\text{E-}05 \text{ per ppm} - [7.75\text{E-}05 \text{ per ppm} \times 2.08\text{E-}05 \text{ per ppm}] \\ &= 9.83\text{E-}05 \text{ per ppm} - 1.61\text{E-}09 \text{ per ppm} = 9.83\text{E-}05 \text{ per ppm} \\ &= 9.8\text{E-}08 \text{ per ppb} (2.8\text{E-}08 \text{ per :g/m}^3) \end{aligned}$$

This URF for the combined liver/lung tumors will be used in Section 4.2.6 to calculate an air concentration associated with a 1 in 100,000 excess lifetime risk.

#### **4.2.5.2.2 David et al. (2006) Carcinogenic Assessment (Supporting Study)**

Results from the more recent David et al. (2006) probabilistic PBPK assessment will be used to support the Health Canada (1993) assessment. As mentioned previously, while ICF International (TCEQ work order 582-9-90441) identified the David et al. (2006) PBPK model as the best model for derivation of human health criteria, both TD and ICF International identified some uncertainties of particular concern (e.g., k<sub>F</sub> value for GST metabolites, less-than-desirable level



of model development detail documentation) which cannot be adequately addressed for use of David et al. (2006) as the key assessment as least partly due to agency time and resource constraints. Therefore, David et al. (2006) will only be used as a supporting assessment to demonstrate the probable conservativeness of the Health Canada (1993) assessment. One alternative identified by ICF International for use of the David et al. (2006) was to use the published human risk URFs provided by the study. TD adopted this alternative to provide human URFs from David et al. (2006) for support of, and comparison to, the URF from Health Canada (1993).

The PBPK model used by David et al. (2006) is of the basic structure developed by Andersen et al. (1987,1991) and refined by Marino et al. (2006) for the mouse, calibrated by David et al. for humans using all appropriate human data and Markov chain Monte Carlo (MCMC) analysis. Two competing metabolic pathways (saturable MFO pathway, pseudo-first order GST pathway) are included in the model to describe the metabolism of MC in both the liver and the lung. Liver/lung carcinogenicity in mice is dependent upon a dose-dependent transition in metabolism from the saturable, high-affinity MFO pathway to the high-capacity, low-affinity GST pathway, above which increases in tumorigenesis occur in laboratory animals. This dose-dependent change in metabolism results in non-linear risk for MC and only minor risk impacts of GST-T1 polymorphisms at the low end of the dose-response (David et al. 2006). Extrahepatic/ extrapulmonary metabolism was also incorporated. See the study for more information on the refinements and structure of the PBPK model. Physiological parameters for input into the MCMC analysis were selected from multiple sources to represent the most current scientific evidence for each parameter. Distributions for the US population of the genetic polymorphisms in the GST metabolic pathway (putative carcinogenic pathway), which may confer increased (+/+) or decreased (-/-) susceptibility as discussed in Section 3.1.2.1, were also included in the MCMC analysis. Using calibrated metabolic parameters, the PBPK model was used to perform a probabilistic cancer risk assessment using the same female mouse tumor incidence and exposure concentration data (NTP 1986) relied upon for the current and draft USEPA URF. The basic steps included:

1. The NTP (1986) mouse exposure concentrations were converted to internal doses of MC metabolized through the GST pathway in the liver and lung (mg/L of tissue/day) through PBPK modeling (Andersen et al. 1987 model as refined by Marino et al. 2006).
2. Dose-response modeling consistent with USEPA methodology was performed using these internal dose metrics (step 1) for the liver and lung tumor incidence data.
3. The dose-response curves (step 2) were used to determine potency factors (i.e., 0.1/LED<sub>10</sub>) for liver and lung tumors.
4. The potency factors for liver and lung tumors were assumed to be equivalent in mice and humans when expressed on an internal dose basis for the amount of MC metabolized by the GST pathway (i.e., the putative carcinogenic pathway), consistent with USEPA (1992) (i.e., no interspecies surface area adjustment was made to the internal dose metric).

5. The potency factors (step 3) were combined with human PBPK modeling results for the distribution of the internal dose metric (accounting for GST-T1 polymorphisms in the US population) to produce a probabilistic distribution of URFs for the human population.

See the study for more detailed information on the various procedures, methodologies, and steps involved in calibrating the human PBPK model, conducting the probabilistic risk assessment, etc. Combined liver/lung tumor URF values estimated from the calibrated human model and distribution of risk from David et al. (2006) are shown in Table 8. The distribution is described as upper bound as it is based on the upper bound of the slope factor estimated from BMD modeling.

**Table 8. URF Estimate Descriptive Statistics <sup>a</sup>**

Distribution Statistic	Combined liver/lung tumor URF (per $\mu\text{g}/\text{m}^3$ )
Mean	1.05E-09
Median	9.33E-10
95 <sup>th</sup> Percentile	2.70E-09
99 <sup>th</sup> Percentile	3.75E-09

<sup>a</sup> From Table 6 of David et al. (2006).

David et al. (2006) report that these values represent the best estimates to date for MC cancer risk because all available human data sets were used and a probabilistic methodology was followed. Because the distribution is skewed, the median URF (as opposed to the mean) is reported to be most representative of an average individual, although risk would be zero for individuals homozygous (-/-) for the null gene allele (about 20% of the US population per Haber et al. 2002).

#### **4.2.5.2.3 Draft USEPA (2010) Carcinogenic Assessment (Supporting Study)**

The draft USEPA (2010) assessment used the human PBPK model from David et al. (2006) in derivation of the draft URF. USEPA revised some of the physiological and metabolic parameter distributions used in David et al. (2006), the discussion of which is outside the scope of this document (see Appendix B to USEPA 2010). Similar to other assessments, USEPA utilized mouse liver and lung tumor dose-response data from NTP (1986) and the tissue-specific GST metabolism dose metric. However, male mouse data were used as opposed to female. Use of female mouse data by USEPA (2010) for comparison resulted in the same URF as calculated for male mice based on liver and lung tumors (see Table F-4 of USEPA 2010). The basic steps included:

1. The NTP (1986) mouse exposure concentrations were converted to internal doses of MC metabolized through the GST pathway in the liver and lung (mg/L of tissue/day) through PBPK modeling (Andersen et al. 1987 model as refined by Marino et al. 2006).

2. The multistage model (BMDS version 2.0) was used to fit dose-response curves for internal dose (step 1) and the liver and lung tumor incidence data and determine the mouse BMD and BMD low values at the 10% response rate (BMD<sub>10</sub> and BMDL<sub>10</sub>).
3. USEPA (2010) indicates that because the dose metric is a rate of metabolism (i.e., per day is in the denominator), the mouse BMDL<sub>10</sub> (step 2) was multiplied by a body weight (BW<sup>0.75</sup>) allometric scaling factor ((BW<sub>human</sub>/BW<sub>mouse</sub>)<sup>0.25</sup> ≈ 7) to account for potential slower clearance per volume tissue in the human and determine the human BMDL<sub>10</sub>.
4. The human BMDL<sub>10</sub> values (step 3) were used to determine potency factors (i.e., 0.1/BMDL<sub>10</sub>) for liver and lung tumors.
5. The potency factors (step 4) were combined with human PBPK modeling results (revised David et al. 2006 model) for the distribution of the internal dose metric (accounting for GST-T1 polymorphisms in the US population) to produce a probabilistic distribution of URFs for the human population.

In regard to step 3 above, USEPA indicates that allometric scaling (BW<sup>0.75</sup>) was performed because the dose metric is a rate of metabolism (i.e., mg/L of tissue/day has “day” in the denominator). *However, such an adjustment was deemed “inappropriate” by USEPA (2000) for vinyl chloride which used the same dose metric (mg/L of tissue/day) (see page E-4 and Table B-12 of USEPA 2000).* Additionally, no such adjustment was made by Health Canada (1993) or David et al. (2006) which used the same dose metric. In these cases, to not use an allometric adjustment is consistent with USEPA (1992), which indicates that tissues experiencing equal average concentrations of the carcinogenic moiety over a full lifetime should be presumed to have equal lifetime cancer risk. Although USEPA (2010) describes the dose metric as a rate, it can also be described as the dose metric referred to in USEPA (1992) (i.e., average target tissue concentration of the carcinogenic moiety over a full lifetime), or how USEPA (2000) described the same dose metric (i.e., the steady-state concentration of the active metabolite per L liver tissue). With these dose metrics, an allometric scaling factor was deemed unnecessary or inappropriate.

USEPA (2010) selected the URF (1.3E-08 per µg/m<sup>3</sup> or 4.6E-08 per ppb) based on liver and lung tumors in male mice, allometric scaling, and the most sensitive GSTT1-1 genotype, those individuals homozygous (+/+) for the positive allele (about 32% of the US population according to Haber et al. 2002). However, the URF (7.4E-09 per µg/m<sup>3</sup> or 2.6E-08 per ppb) based on representative frequencies of the three genotypes for the US (20% -/-, 48% +/-, 32% +/+) would likely be more representative of risk to the general US population. USEPA (2010) applied age-dependent adjustment factors (ADAFs discussed in Section 4.2.7) to the rounded URF (1.0E-08 per µg/m<sup>3</sup> or 3.5E-08 per ppb) to derive an ADAF-adjusted URF of 1.7E-08 per µg/m<sup>3</sup> (6.0E-08 per ppb). USEPA calculated many other URFs (e.g., Table 5-22 of USEPA 2010). Of those based on female mouse data, the URF (1.0E-09 per µg/m<sup>3</sup> or 3.5E-09 per ppb) based on liver and lung tumors, without allometric scaling, and representative US genotype frequencies is probably most comparable to the URFs from Health Canada (1993) and David et al. (2006) (i.e., neither

used allometric scaling of internal dose and David et al. 2006 used representative US genotypic frequencies).

#### 4.2.6 URF and Calculation of the Carcinogenic-Based ESL

As previously mentioned, Health Canada (1993) was adopted by TD for the purpose of deriving an inhalation URF and  $^{chronic}ESL_{linear(c)}$  due to agency time and resource constraints as it is considered by TD to employ the most appropriate PBPK dosimetric modeling for MC of the final regulatory carcinogenic assessments available. From Section 4.2.5.2.1, the URF from Health Canada (1993) based on liver and lung tumors combined in female mice in NTP (1986) is 9.8E-08 per ppb (2.8E-08 per  $\mu\text{g}/\text{m}^3$ ).

An air concentration associated with a 1 in 100,000 excess lifetime risk can then be calculated based on this URF:

$$\begin{aligned} \text{1 in 100,000 excess lifetime risk air concentration} = \\ \text{target risk level} / \text{URF} = 1.0\text{E-}05 / 9.8\text{E-}08 \text{ per ppb} = 102 \text{ ppb} \end{aligned}$$

After rounding, the  $^{chronic}ESL_{linear(c)}$  is 100 ppb ( $350 \mu\text{g}/\text{m}^3$ ).

For comparison:

- The URFs presented in Table 8 based on the David et al. (2006) supporting study are associated with a 1 in 100,000 excess risk air concentration range from 2,667  $\mu\text{g}/\text{m}^3$  (756 ppb) (99<sup>th</sup> percentile URF) to 10,718  $\mu\text{g}/\text{m}^3$  (3,036 ppb) (median URF) (calculations not shown).
- Based on the range of URFs specifically discussed above from USEPA (2010), the 1 in 100,000 excess risk air concentration would range from 588  $\mu\text{g}/\text{m}^3$  (167 ppb) (ADAF-adjusted URF) to 10,000  $\mu\text{g}/\text{m}^3$  (2,833 ppb) (female mouse liver/lung tumor data without allometric scaling, representative US genotype frequencies) (calculations not shown).

*These supporting results strongly suggest that the 1 in 100,000 risk air concentration of 100 ppb used as the  $^{chronic}ESL_{linear(c)}$  based on the Health Canada (1993) assessment is conservative and health-protective. This is true even assuming increased susceptibility due to early life exposure (discussed below), as the  $^{chronic}ESL_{linear(c)}$  of 100 ppb is well below the 1 in 100,000 excess risk air concentration of 170 ppb (rounded) based on the USEPA (2010) ADAF-adjusted URF.*

#### 4.2.7 Evaluating Susceptibility from Early-Life Exposures

TD concurs with USEPA (2010) that the weight of evidence indicates MC acts through a mutagenic MOA (see Section 4.2.4). Determination of a mutagenic MOA for carcinogenesis invokes use of the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (USEPA 2005b). USEPA (2005b) indicates that chemical-specific risk estimates are developed incorporating life-stage susceptibility data when such data are available. In the absence of early-life studies on a chemical operating through a mutagenic MOA for

carcinogenesis, early-life susceptibility is assumed and USEPA (2005b) provides default ADAFs to account for potential increased susceptibility in children due to early-life exposure (USEPA 2007). *However, TD believes that supporting studies demonstrate the adequate health-protectiveness of the  $^{chronic}ESL_{linear(c)}$  for children without application of ADAFs and provide a strong rationale for not applying them in this particular case.*

As mentioned previously, the  $^{chronic}ESL_{linear(c)}$  (100 ppb) is well below the 1 in 100,000 excess risk air concentration (170 ppb) based on the USEPA (2010) ADAF-adjusted URF of 6.0E-08 per ppb. This ADAF-adjusted URF is very conservative not just because it applies ADAFs in consideration of the potential increased sensitivity of children, but also because USEPA (2010) used allometric scaling, PBPK models, and the most sensitive human GSTT1-1 genotype (i.e., the 32% of individuals homozygous (+/+) for the positive allele) to derive it. Additionally, the  $^{chronic}ESL_{linear(c)}$  is well below the 1 in 100,000 excess risk air concentration based on the 99<sup>th</sup> percentile URF from David et al. (2006) with ADAFs applied (756 ppb x 0.6 (overall ADAF adjustment) = 454 ppb). *These recent PBPK risk assessment results, with ADAFs incorporated, are associated with higher 1 in 100,000 excess risk air concentrations (170 and 454 ppb) than the  $^{chronic}ESL_{linear(c)}$  (100 ppb) and strongly suggest that the  $^{chronic}ESL_{linear(c)}$  is already adequately protective of children without further adjustment.*

Additionally, in regard to a qualitative evaluation of children as a potentially sensitive subpopulation, neonatal children up to 5 years of age may be less likely than adults to be exposed to the carcinogenic metabolites of MC based on the PK of blood MC and metabolism to reactive carcinogenic metabolites via the GST pathway (Clewell et al. 2004 as cited by David et al. 2006). Relevant to age-specific PK differences in MC metabolism and the potential effect on carcinogenic risk, Clewell et al. (2004) report that the GSH conjugation rate per volume of liver (the typical cancer risk assessment dose metric for MC) at an oral MC exposure of 1 µg/kg-day increases consistently until age 25, where it essentially remains stable and is over 30 times that of an infant. In other words, adults experience the highest dose of the putative carcinogenic (i.e., GST pathway) metabolites as this dose increases with age until about 25. *This suggests that children metabolize less of MC to carcinogenic metabolites than adults due to PK differences (e.g., the immaturity of metabolic enzyme systems).* For example, while the first 5 years of life represented about 7% of the exposure duration evaluated, Clewell et al. (2004) indicates that exposure during this period resulted in less than 1% of the cumulative lifetime internal dose of GST pathway carcinogenic metabolites (see Table 5 in Clewell et al. 2004), which would result in a disproportionately lower estimated risk for exposure during this period as compared to adult exposure based on PK/metabolic differences (e.g., 25-75 years of age represents 67% of the exposure duration but 84% of the cumulative internal dose of GST metabolites). USEPA (2005b) provides default ADAFs greater than 1 only for up to age 15. While the default USEPA ADAF is 10 for a 0-2 year old and would be 3 for a 2-5 year old, results from Clewell et al. (2004) suggest that a 0-5 year old would metabolize about 10 times less MC via the GST pathway as compared to a 25 year old (see Table 3 in Clewell et al. 2004). Even at age 15, the analysis in Clewell et al. (2004) suggests that the metabolism of MC via the GST pathway is only about half that at age 25 (see Figure 2C in Clewell et al. 2004). *The implication of this is that based on age-specific PK*

*differences in metabolism via the putative carcinogenic GST pathway, children may be reasonably expected to be no more sensitive (and may be less sensitive) to the potential carcinogenic effects of MC due to childhood exposure.* Study limitations such as limited age-specific GST data and the need for age- and chemical-specific PBPK model validation preclude a quantitative use of reported results such as derivation of age-specific adjustment factors. However, analyses from Clewell et al. (2004) are useful as part of a qualitative evaluation of potential susceptibility due to early-life exposures. *The results of supporting PBPK assessments with ADAFs incorporated (discussed above), in combination with the analyses of Clewell et al. (2004) which provide a biological rationale (PK differences), strongly suggest that the <sup>chronic</sup>ESL<sub>linear(c)</sub> is already adequately protective of children without further adjustment.* Therefore, ADAFs will not be applied at this time. This issue will be reevaluated periodically as new scientific information becomes available.

### **4.3. Welfare-Based Chronic ESL**

No data were found regarding adverse effects observed in plants due to air exposure to MC. The only information found was in regard to adverse plant effects not being observed (see Section 3.3). Therefore, no chronic vegetation-based ESL (<sup>chronic</sup>ESL<sub>veg</sub>) was derived.

### **4.4 Long-Term ESL and Values for Ambient Air Monitoring Evaluation**

The chronic evaluation resulted in the derivation of the following values:

- chronic ReV = 1,300 µg/m<sup>3</sup> (360 ppb)
- <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub> = 390 µg/m<sup>3</sup> (110 ppb)
- <sup>chronic</sup>ESL<sub>linear(c)</sub> = 350 µg/m<sup>3</sup> (100 ppb)

The <sup>chronic</sup>ESL<sub>linear(c)</sub> of 350 µg/m<sup>3</sup> (100 ppb) is the critical long-term, health-based AMCV for the evaluation of long-term ambient air data as it is lower than the chronic ReV (Table 1). The long-term ESL for air permit reviews is the <sup>chronic</sup>ESL<sub>linear(c)</sub> of 350 µg/m<sup>3</sup> (100 ppb) as it is slightly lower than the <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub> (Table 2). The <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub> is not used for the evaluation of ambient air monitoring data.

## Chapter 5 References

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## ***5.2 Other Studies and Documents Reviewed by the TD***

Other studies or documents reviewed by TD include, but are not limited to, the following:

Casanova M, Conolly RB, Heck HA. 1996. DNA-protein crosslinks (DPX) and cell proliferation in B6C3F1 mice but not Syrian golden hamsters exposed to dichloromethane: Pharmacokinetics and risk assessment with DPX as dosimeter. *Fundam Appl Toxicol* 31:103-116.

## Appendix 1: Consideration of Schwetz et al. (1975) for a Potential Acute Point-of-Departure (POD)

Schwetz et al. (1975) reported a LOAEL of 1,250 ppm for minor skeletal variants in Swiss-Webster mice and Sprague-Dawley rats exposed 7 h/day on gestational days 6-15. This LOAEL for developmental effects is lower than the NOAEL (1,500 ppm) for developmental/reproductive effects reported in a long-term (two generation) rat study (Nitschke et al. 1988a), and is a potential POD. However, at 1,250 ppm MC, it is significantly higher than the human LOAEL of 195 ppm from the key Putz et al. (1979) study, and from a less relevant species. Additionally, CalEPA (1999a) considered the effects reported in Schwetz et al. (1975) as reflective of developmental variation as opposed to representing adverse developmental effects. Nevertheless, the  $POD_{HEC}$  that results based on the LOAEL from Schwetz et al. (1975) is considered here for use as a POD.

TD does not exposure-duration adjust PODs based on developmental effects (TCEQ 2006), so the  $POD_{ADJ}$  would be set equal to the animal POD of 1,250 ppm. Adjustment of this animal  $POD_{ADJ}/LOAEL$  (e.g., rat) to a  $POD_{HEC}$  is therefore:

$$POD_{HEC} = POD_{ADJ} \times [(H_{b/g})_A / (H_{b/g})_H]$$

where:  $H_{b/g}$  = ratio of the blood:gas partition coefficient  
A = animal  
H = human

The blood:gas partition coefficients for Sprague-Dawley rats and humans are 19.4 and 8.94, respectively (ATSDR 2000). Other human blood:gas partition coefficient values (6.5-9.7) are available (CalEPA 2000), but would not affect calculation of the  $POD_{HEC}$  if used instead of the selected value (8.94). This is because if the animal blood:gas partition coefficient is greater than the human blood:gas partition coefficient, as with MC, a default value of 1 is used for the regional gas dose ratio (RGDR) (USEPA 1994).

$$POD_{HEC} = POD_{ADJ} \times [(H_{b/g})_A / (H_{b/g})_H] = 1,250 \text{ ppm} \times 1 = 1,250 \text{ ppm}$$

Comparison of  $POD_{HEC}$  values provides a reasonable method for determining the likely critical effect of a chemical in humans based on scientific knowledge of chemical-specific effects levels. As the  $POD_{HEC}$  of 1,250 ppm based on Schwetz et al. (1975) is significantly higher than that based on the human study (223 ppm), derivation of an acute ReV and <sup>acute</sup>ESL based on the human study is expected to be protective of developmental effects. This conclusion remains unchanged even considering the uncertainty in using a laboratory animal study since dividing this  $POD_{HEC}$  (1,250 ppm) by an  $UF_A$  of 3 for potential pharmacodynamic species differences (the pharmacokinetic/dosimetric adjustment from rats to humans has already been made) would result in a value of 417 ppm, which is higher than 223 ppm based on the human study.

## Appendix 2: Allometric Scaling In the Context of the USEPA (2000, 1987a, 2010) PBPK Modeling Assessments

Allometry concerns how features (e.g., metabolic rates) vary as a function of animal size. For example, volumes and capacities (e.g., blood volume, organ size, lung capacity, volumes of distribution) tend to be proportional to mammal body size, while rates (e.g., basal metabolic rate, glomerular filtration rate, cardiac output, minute volume, food/air/water consumption rates, anything with time in the units) tend to be proportional to body weight<sup>3/4</sup>. For example, when doses are given proportional to body weight, tissue exposures (area under the concentration curve) are larger in humans by the ratio of human-to-animal body weight<sup>1/4</sup> (e.g., the human area under the curve is about 7 times that of a mouse and 4 times that of a rat). If doses are given proportional to body weight<sup>3/4</sup>, the doses tend to be pharmacokinetically equivalent, yielding similar areas under the curve over time across species (USEPA 1992). These relationships apply to concentrations of the parent compound or metabolite(s) in the blood or in any organ or tissue (e.g., the area under the curve for the ultimate carcinogenic species at the target of carcinogenesis, presuming site concordance across species). The rates of individual metabolic transformation steps are also assumed to generally scale to body weight<sup>3/4</sup>, although if available, case-specific (e.g., species, chemical, dose) data regarding the metabolic transformation of a chemical should be used in preference to this general allometric relationship if deviation from the default is indicated (USEPA 1992). In addition to body weight<sup>3/4</sup>, surface area (e.g., dose expressed as mg/m<sup>2</sup> of body surface area or approximated by body weight<sup>2/3</sup>) has also been used historically for allometric scaling between species for certain features (e.g., rates of physiological processes such as the rate of chemical clearance (metabolism and non-metabolic)) (USEPA 1987a). The aim of the allometric approach to interspecies extrapolation is to adjust for the *pharmacokinetic* differences expected due solely to differences in body size (USEPA 1987a). Default allometric procedures are based on broad generalizations about carcinogen exposures that can be considered of equal risk in laboratory animals and humans in the absence of adequate chemical-specific information (e.g., pharmacokinetics, mechanism of action, identification of carcinogenic metabolite) to extrapolate risk across species. When available, applicable chemical-specific pharmacokinetic and mechanistic data should be used *in lieu* of simple general nonchemical-specific default predictions about extrapolation of toxicologically equivalent doses across species (USEPA 1992).

For inhalation exposure, humans will have a lower applied dose of the parent compound in units of mg/kg since the breathing rate per kg body weight is lower than that for rodents. However, as cardiac output, minute volume of breathing, and rate of metabolism all tend to scale allometrically, all species are expected to have equal blood concentrations upon reaching steady-state while breathing a given air concentration (USEPA 1987a). In other words, different species are expected to experience pharmacokinetically-equivalent doses at a given air concentration since relevant parameters tend to vary across species allometrically (e.g., with body weight<sup>3/4</sup>) (USEPA 1992).

### ***USEPA (2000) Assessment of Vinyl Chloride***

It is reasonable to modify risk extrapolation from experimental animals to humans by the degree to which the species differ in degree of metabolic activation of the applied dose at the site of carcinogenic action (USEPA 1987a). The USEPA (2000) *Toxicological Review of Vinyl Chloride* indicates that cross-species scaling of lifetime cancer risk can be performed without an allometric scaling factor (i.e., surface area (or body weight) adjustment) when risks are based on biologically-appropriate dose metrics (e.g., carcinogenic metabolite concentrations at the target organ) calculated with a validated PBPK model. In other words, when the carcinogenicity assessment is based on an appropriate PBPK model and dose metric, no allometric scaling is needed to account for potential pharmacodynamic differences (or pharmacokinetics of course). A significant majority of the expert peer reviewers for the vinyl chloride carcinogenic assessment (USEPA 2000) indicated that no allometric scaling factor (i.e., surface area correction factor) was required since a PBPK model was used. USEPA's response to the two reviewers which recommended the adjustment indicates that such a correction would be a *metabolic scaling factor* applied for the purpose of accounting for the faster metabolism and detoxification of a chemical by laboratory animals relative to humans, resulting in a reduced steady-state concentration at the target site and reduced susceptibility to a given dose per unit body weight for laboratory animals (see page E-4 of USEPA 2000). However, a *metabolic scaling factor* regards pharmacokinetics, and both the vinyl chloride and MC PBPK models themselves account for metabolic rate factors and predict active metabolite concentrations. *Thus, applying an allometric scaling factor (e.g., surface area<sup>2/3</sup>, body weight<sup>3/4</sup>) as a metabolic/pharmacokinetic scaling factor (the stated purpose per USEPA 2000) for MC is considered by TD, as with vinyl chloride, to be unnecessary and would likely render the carcinogenic potency results overly conservative as they are based on PBPK modeling.*

By definition, when appropriate PBPK modeling is used to extrapolate between species, no allometric scaling factor (i.e., surface area/body weight correction) is necessary to adjust for pharmacokinetic differences. However, a PBPK model is not developed to account for pharmacodynamic species differences, only pharmacokinetic ones (i.e., species-specific dosimetric variables such as metabolism and liver perfusion rates). In regard to potential pharmacodynamic differences, USEPA (2000) indicates that Barton et al. (1998) suggests use of a default pharmacodynamic adjustment value of 1 if *either* a PBPK model or body weight<sup>3/4</sup> is employed for cross-species scaling. PBPK modeling was utilized for cross-species scaling, for example, in USEPA (1987a), USEPA (2010), and Health Canada (1993). Additionally, the Interagency Pharmacokinetics Group (USEPA, Food & Drug Administration, Consumer Product Safety Commission) consensus report by federal scientists on cross-species extrapolation of cancer (USEPA 1992, cited in USEPA 2000) indicates that, "...tissues experiencing equal average concentrations of the carcinogenic moiety over a full lifetime should be presumed to have equal lifetime cancer risk." In other words, equal carcinogen (i.e., putative proximate metabolite) concentrations at the target tissue should be assumed to lead to equal degrees of impact at the cellular level, which if continued for a lifetime, yield equal lifetime cancer risks across species at the target organ. This means that pharmacokinetically-equivalent, average



lifetime carcinogen exposure at the target organ should be presumed to be equivalent in terms of lifetime cancer risk. Thus, a pharmacodynamic adjustment would be presumed to be unnecessary when performing cross-species extrapolation on the basis of pharmacokinetically-equivalent putative carcinogen exposure at the target organ. *While the reasons for approximate lifetime equivalence in the carcinogenic process among species of different body size and lifespan are not clear (USEPA 1992), the above further supports the conclusion by TD that an allometric scaling factor is not necessary in the carcinogenic assessment of MC when appropriate PBPK modeling has been performed for cross-species extrapolation.* Additionally, epidemiological studies do not support the idea that humans are more sensitive to MC-induced carcinogenesis than mice as they do not even clearly establish MC as a carcinogen in humans (i.e., they provide mixed results and one extensive study (Hearne et al. 1990) reported various cancer deficits), much less demonstrate greater sensitivity, and metabolic pathway/rate data strongly suggest humans to be less sensitive than mice to MC-induced carcinogenesis.

### ***USEPA (1987a) Assessment of MC***

In addition to PBPK modeling, USEPA (1987a) utilized interspecies surface area scaling (allometric scaling) in their carcinogenic assessment. Surface area scaling from smaller animals (e.g., mice) to humans results in theoretically slower parent chemical elimination in humans (i.e., slower metabolism and thus greater human sensitivity to the parent compound). As carcinogenicity in mice is associated with GST metabolism and mice have a higher metabolism rate via this pathway than humans, it might be argued that slower removal of the parent compound by GST metabolism in humans should be associated with lower human risk, and that surface area scaling has the opposite effect. However, as both metabolic and non-metabolic clearance scale with surface area, these surface area correction factors would cancel each other (USEPA 1987a). That is, while the rate of metabolic activation of the parent compound to the active metabolite may be slower in humans, clearance of the parent compound is also slower, resulting in the same fraction of an applied dose being metabolized in each species, although the process will take longer in humans. *As indicated in USEPA (1992), the proportion of a dose having any particular ultimate fate (e.g., metabolized by a particular pathway at a particular site, being excreted unchanged) is generally predicted to be the same regardless of species, although there may be case-specific species (e.g., metabolic processing) or other differences (e.g., pathway saturation due to high animal dosing) which cause deviation from this proportionality.* Generally, this principal (the same fraction of an applied dose being metabolized in each species) applies to inhalation exposure as well as other routes (USEPA 1987a). Because the balance of metabolic and non-metabolic clearance is assumed to lead to an equal proportion of an applied dose being metabolized in rodents and humans (in absence of data to the contrary), scaling based on metabolic rate constants alone without accounting for parent compound concentration would ignore an important element of the two interacting processes. While applied and internal dose (proportion metabolized) ought to be directly proportional across species all else being equal, species peculiarities in metabolic enzyme properties (e.g., rates, metabolic saturation, shifts among metabolic pathways) may cause deviation from allometric scaling, and rate constants of specific metabolic pathways may not scale in the same way as the overall

metabolic process (USEPA 1987a). *In other words, species differences in metabolic pathway rate constants which do not scale in the same way as the overall metabolic process due to a species metabolic peculiarity may result in species differences in the proportion of applied dose metabolized by a specific metabolic pathway (e.g., GST-mediated).*

Although, according to USEPA (1987a), the aim of the allometric approach is to adjust for *pharmacokinetic* differences, USEPA (1987a) attempts to justify use of interspecies surface area scaling by indicating that the adjustment may be viewed as a correction for *expected species differences in risk from a given internal dose (i.e., greater human responsiveness/tissue sensitivity)*. However, USEPA acknowledges that the interspecies difference in carcinogenic responsiveness (i.e., pharmacodynamics) is unknown and therefore problematic to address. Despite the admitted lack of knowledge and understanding of potential interspecies toxicodynamic differences (if any), USEPA uses an allometric *pharmacokinetic* scaling factor to adjust for “expected” *pharmacodynamic* differences, which are not adequately supported in USEPA (1987a). The interspecies surface area scaling adjustment results in an “expected” tissue sensitivity/responsiveness to internal dose (GST metabolites) for humans which is significantly greater than that of mice (i.e., humans are assumed to have an equal cancer risk from an internal dose which is 12.7 times lower per kilogram body weight than that in mice). *However, USEPA (1987a) does not provide support for this expectation.* It is also contrary to the Interagency Pharmacokinetics Group (USEPA, Food & Drug Administration, Consumer Product Safety Commission) consensus report by federal scientists on cross-species extrapolation of cancer (USEPA 1992), which indicates that, “...tissues experiencing equal average concentrations of the carcinogenic moiety over a full lifetime should be presumed to have equal lifetime cancer risk.”

USEPA (1987a) points out that as humans live about 35 times longer than mice, the total cumulative exposure leading to the same risk would still be higher in humans, even with the surface area adjustment. That is, since humans live and will be exposed about 35 times longer than mice, even if a surface area adjustment of 12.7 is applied assuming greater human sensitivity, since  $35/12.7$  equals 2.8, this is tantamount to humans actually being assumed to be about 3 times less sensitive. However, this attempted justification for surface area scaling concerns total lifetime cumulative dose differences between species at a given exposure concentration, not the features for which interspecies allometric scaling was developed (e.g., rates of physiological processes) or the greater expected human responsiveness basis given to justify the adjustment. This amounts to USEPA using consideration of a particular dose metric (cumulative lifetime dose) and *assumed toxicodynamic differences* (greater human tissue sensitivity) between species in an attempt to justify an allometric *toxicokinetic* adjustment not developed for this purpose. Additionally, despite lifespan differences, two years in mice is a lifetime of exposure for that laboratory animal, and the usual practice is to assume lifetime equivalence when projecting carcinogenesis across species (i.e., total lifetime cumulative dose differences are not adjusted for) (USEPA 1992). *The consensus report on cross-species extrapolation of cancer (USEPA 1992) indicates that despite 35 times longer exposure, pharmacodynamic considerations (e.g., physiological time) suggest that carcinogenesis proceeds*

*more slowly in humans than rodents in such a way that tends to be equivalent on a lifetime basis.* Again, that report emphasizes equal average lifetime target tissue exposure concentrations in regard to toxicological equivalence (not cumulative dose), indicating that "...tissues experiencing equal average concentrations of the carcinogenic moiety over a full lifetime should be presumed to have equal lifetime cancer risk." USEPA (1987a) should have specifically and directly adjusted for total cumulative dose species differences if a robust scientific justification could have been provided for this adjustment in the carcinogenic assessment.

Finally, USEPA (1987a) attempts to justify the surface area scaling adjustment on the basis of historical use (i.e., tradition) by USEPA, and because it has been used on chemicals that MC is compared to when a company considers chemical substitution. Although the document indicates that in practice, it has been a correction for the relative tumorigenicity across species for a given internal dose (i.e., greater potency or tissue responsiveness in humans compared to rodents), no support is provided. No support for surface area adjustment being used for pharmacodynamic differences is provided in USEPA (1992) either, which was published five years later. The consensus group report (USEPA 1992) does not recommend a pharmacodynamic adjustment factor, but rather indicates that equal lifetime cancer risk should be presumed if tissues experience equal average concentrations of the carcinogenic moiety over a lifetime. *In other words, assume toxicological (carcinogenic) equivalence (i.e., no pharmacodynamic adjustment is needed) if tissues in different species experience equal lifetime average concentrations of the carcinogenic moiety, or, pharmacokinetically equivalent lifetime exposures at the target may be presumed to be equivalent in the degree of lifetime cancer risk they engender.*

Other information regarding the USEPA (1987a) assessment argues against use of a surface area adjustment, which can be viewed as artificially reducing the human dose associated with a particular risk level. The PBPK model as used by USEPA predicts that approximately equal proportions of the applied doses are metabolized by the GST pathway in both mice and humans (based on the allometric scaling ( $BW^{0.7}$ ) of rodent data), as data showing significant species differences in GST metabolism were not available until after the model was developed. However, subsequent data showed that the first-order rate constant ( $k_F$ ) used for human GST metabolism by Andersen et al. (1987) may have been a good deal too high (USEPA 1987a). This data existed at the time USEPA (1987a) was written, and USEPA indicates that an estimate of the  $k_F$  for human GST metabolism based on *in vitro* data (not a direct estimate of the *in vivo*  $k_F$ ) was at least 7-fold lower than that used by Andersen et al. (1987), which would result in a similar reduction in human risk. This information argues against use of an additional surface area adjustment. Applying a surface area scaling factor of 12.7 only serves to further increase estimated upper-bound risk in humans when risk may already be overestimated by the PBPK model assumption of essentially equal proportions of the applied doses being metabolized by the GST pathway in both mice and humans. Referring to surface area scaling to account for possible species differences in responsiveness (pharmacodynamics), USEPA (1987a) concludes that, "the factor of 12.7 should continue to be applied to internal doses from the pharmacokinetic model because this corresponds to the assumption that has in effect been used all along," although it is

admitted in another section of the document that there is no clear basis for choosing to use surface area correction or not (p. 107 of that document).

*As no direct support was provided by USEPA (1987a) for assuming greater human sensitivity and USEPA had knowledge that mice likely produce more of the putative GST pathway metabolites involved in carcinogenesis than represented in the PBPK model (i.e., USEPA's assessment was already likely conservative), TD believes that the surface area correction factor for sensitivity was not justified or necessary. Additionally, in regard to interspecies extrapolation with surface area, Andersen et al. (1987) indicated that the PBPK model already incorporated appropriate factors for interspecies scaling. [A world-renowned PBPK modeler with the Hamner Institutes for Health Sciences concurs that the surface area adjustment was not justified (personal communication with Dr. Harvey Clewell, February 19, 2009).] Regardless of USEPA's reasons at the time, the surface area adjustment also appears to be inconsistent with recent USEPA practice (e.g., USEPA 2000) and language in the consensus report (USEPA 1992).*

### ***Draft USEPA (2010) Assessment of MC***

For the draft USEPA (2010) PBPK assessment of MC, the dose metric was internal dose of MC metabolized through the GST pathway in the liver and lung (mg/L of tissue/day). USEPA indicates that because the dose metric is a rate of metabolism (i.e., per day is in the denominator), a body weight ( $BW^{0.75}$ ) allometric scaling factor ( $(BW_{\text{human}}/BW_{\text{mouse}})^{0.25} \approx 7$ ) was used to account for potential slower clearance per volume tissue in the human. *However, such an adjustment was deemed "inappropriate" by USEPA (2000) for vinyl chloride which used the same dose metric (mg/L of tissue/day) (see page E-4 and Table B-12 of USEPA 2000).* Additionally, no such adjustment was made by Health Canada (1993) or David et al. (2006) which used the same dose metric. In these cases, to not use an allometric adjustment is consistent with USEPA (1992), which indicates that tissues experiencing equal average concentrations of the carcinogenic moiety over a full lifetime should be presumed to have equal lifetime cancer risk. Although USEPA (2010) describes the dose metric as a rate, it can also be described as the dose metric referred to in USEPA (1992) (i.e., average target tissue concentration of the carcinogenic moiety over a full lifetime), or how USEPA (2000) described the same dose metric (i.e., the steady-state concentration of the active metabolite per L liver tissue). With these dose metrics, an allometric scaling factor was deemed unnecessary or inappropriate.

*Based on TD's review of relevant information, allometric scaling appears to have been inappropriately applied in both the USEPA (1987a) and USEPA (2010) PBPK risk assessments for MC.*

### **Appendix 3: USEPA (1987a) Inhalation Carcinogenic Assessment**

*As the USEPA (1987a) assessment is outdated and used inappropriate allometric scaling (see Appendix 2 above), TD did not consider it for adoption for purposes of deriving an inhalation URF and <sup>chronic</sup>ESL<sub>linear(c)</sub> and only an overview is provided here. See the USEPA assessment for additional information and details. USEPA (1987a) derived an inhalation URF of 4.7E-07 per  $\mu\text{g}/\text{m}^3$  based on female mouse lung/liver adenoma and carcinoma data combined from NTP (1986). USEPA (1987a) used the PBPK model developed by Andersen et al. (1987) to extrapolate risk across species and from high-to-low doses based on internal dose estimates (i.e., the amount of MC metabolism by the GST pathway) in the liver and lung. Cross-species extrapolation of dose is an area of uncertainty in risk assessments utilizing animal data (USEPA 2000). While the use of animal and human metabolism and pharmacokinetic data reduces some of the uncertainty typically associated with dose-risk extrapolation, USEPA (1989) acknowledges that uncertainty exists in the estimates of internal dose generated by the Andersen et al. (1987) model. The uncertainties associated with the model, however, were considered to be less than those associated with traditional carcinogenic assessment methods. USEPA (1987a) corrected internal dose for potential interspecies differences in tissue sensitivity/dose potency (i.e., toxicodynamics) by using an allometric surface area correction factor (i.e., the relative surface-to-volume ratios of mice and humans). As indicated in Appendix 2, however, the surface area adjustment (approximated by body weight<sup>2/3</sup>) appears to be inconsistent with recent USEPA practice (USEPA 2000) and language in the Interagency Pharmacokinetics Group (USEPA, Food & Drug Administration, Consumer Product Safety Commission) consensus report by federal scientists on cross-species extrapolation of cancer (USEPA 1992). TD believes that the surface area adjustment was not scientifically justified by USEPA for reasons discussed in Appendix 2.*

No recent studies were identified in the USEPA screening-level literature review which would affect the basis of the existing Integrated Risk Information System (IRIS) URF (USEPA 2002), although the cancer guidelines under which the URF was developed have been updated (USEPA 2005a). The draft USEPA (2010) assessment uses the same study. In regard to the NTP (1986) study, USEPA (1989) indicates that adequate numbers of animals were observed and tumor incidences were significantly increased in a clearly dose-dependent manner. Additionally, excluding animals that died before observation of the first tumors produced similar risk estimates, as did time-to-tumor analysis. The existing URF (4.7E-07 per  $\mu\text{g}/\text{m}^3$ ) on USEPA's IRIS (USEPA 1989) incorporates information on pharmacokinetics and metabolism of MC (discussed in Section 4.2.5), and is approximately nine times lower than the previous URF (4.1E-06 per  $\mu\text{g}/\text{m}^3$ ) derived in 1985 based on applied dose (USEPA 1985,1987a,b). As an additional comparison, the USEPA URF currently on IRIS (4.7E-07 per  $\mu\text{g}/\text{m}^3$ ) is about 36 times higher than the draft USEPA (2010) URF of 1.3E-08 per  $\mu\text{g}/\text{m}^3$  based on liver/lung tumors in male mice, PBPK modeling, allometric scaling ( $\text{BW}^{0.75}$ ), and the most sensitive human GSTT1-1 genotype (+/+).

## Appendix 4: CalEPA (2000) Carcinogenic Assessment

CalEPA (2000) calculated both inhalation and oral cancer slope factors (CSFs) for MC. However, as inhalation is the exposure route of interest in this document, discussion will be limited to the inhalation carcinogenic assessment. CalEPA (2000) derived several human CSFs for MC based on lung and liver tumors observed in the same mouse inhalation study (NTP 1986) utilized by USEPA (1987a), and selected two CSFs to be used (in conjunction with CSFs based on an oral exposure study) to evaluate MC inhalation risk associated with household use of drinking water as part of calculating a drinking water public health goal concentration for MC. While the two selected CSFs based on inhalation data are expressed as oral CSFs (risk per mg/kg-day) for purposes of calculating the public health goal for MC in drinking water, the CSFs are also given as inhalation unit risk factors (URFs in risk per ppm or  $\mu\text{g}/\text{m}^3$ ) in Table 21 (at the top of page 117) of CalEPA (2000). The URF associated with one of the inhalation CSFs used is  $1.6\text{E}-06$  per ppb. This URF ( $1.6\text{E}-06$  per ppb or  $4.5\text{E}-07$  per  $\mu\text{g}/\text{m}^3$ ) is based on mouse “prior mean” PBPK model parameters, and is very similar to the USEPA Integrated Risk Information System (IRIS) URF ( $4.7\text{E}-07$  per  $\mu\text{g}/\text{m}^3$ ). The URF associated with the other inhalation CSF selected is  $2.4\text{E}-06$  per ppb. This URF ( $2.4\text{E}-06$  per ppb or  $6.8\text{E}-07$  per  $\mu\text{g}/\text{m}^3$ ) is based on mouse “posterior median” PBPK parameters, and is somewhat more conservative than the IRIS URF. A general discussion of the derivation of these CSFs/URFs is provided below. Refer to CalEPA (2000) for additional details.

As mentioned above, CalEPA (2000) used the NTP (1986) mouse study to assess inhalation cancer risk. The NTP (1986) study found significantly elevated increases in liver and lung tumors in mice exposed to 2,040 or 4,052 ppm MC. This same study was used by USEPA (1987a) for derivation of the URF on IRIS. The dose metric used for the CalEPA (2000) CSFs/URFs was the amount (mass) of GST conjugates formed per day per liver (or lungs) from the mouse PBPK model on a body weight basis (i.e., mg GST metabolites/L tissue-day  $\times$  L tissue/kg BW = mg GST metabolites/kg-day). This dose metric is a target tissue-specific metabolized dose derived by PBPK modeling (as opposed to the applied dose) and is referred to as the PBPK tissue dose. It is considered an appropriate dose metric as the carcinogenic MOA appears to be related to the generation of metabolites (e.g., S-chloromethylglutathione) via the GST pathway (see Section 4.2.4). Cancer potency factors based on the pharmacokinetic tissue dose (i.e., mg GST metabolites/kg-day as opposed to applied dose) are preferred because they are closest to the site of carcinogenic action and involve the fewest adjustments or other manipulations. The inhalation doses of 2,040 and 4,052 ppm (6 h per day) from the NTP (1986) study were used to determine the PBPK tissue doses (i.e., the amount of GST pathway metabolites generated per day by the mouse liver and lungs). The relationships between applied dose and PBPK tissue doses were linear. PBPK tissue doses were adjusted to a continuous daily exposure (see Table 18 of CalEPA 2000).

The mouse PBPK model used in CalEPA (2000) was essentially that used by OSHA (1997). It is based on the PBPK model of Clewell (1993, 1995), as modified by Bois and Smith (1995) and Hattis (1995). However, CalEPA (2000) used central estimates of the mouse PBPK model

parameters while OSHA (1997) used upper bound estimates. A human PBPK model was also evaluated in CalEPA (2000) but was not selected in consideration of pharmacokinetic uncertainties, including metabolic parameters and polymorphisms (see CalEPA 2000 for more details). As a mouse PBPK model was not used in conjunction with a human PBPK model, PBPK modeling was not used to perform a full cross-species extrapolation as human pharmacokinetics were not accounted for through modeling. The mouse model PBPK parameters used are listed in Table 19 of CalEPA (2000). Two sets of central estimate mouse parameters were used in the modeling: (1) the geometric mean values from the prior distributions used in the Bayesian analysis from Table VI-5 of OSHA (1997), termed “prior mean” parameters; and (2) the median values of the posterior distributions from Table VI-9 of OSHA (1997), termed “posterior median” parameters. There was little difference between the two sets of mouse PBPK model parameters in goodness-of-fit to the quantal tumor incidence data from NTP (1986). *Considering that data indicate mice are likely more susceptible than humans to the carcinogenic effects of MC due to metabolic differences, TD believes the use of central parameter estimates in a mouse PBPK model is more likely to result in cancer risk estimates which do not exaggerate potential cancer risk to humans.*

Carcinogenic potency values were determined for each set of mouse model parameters using two methods of low dose extrapolation: the linearized multistage (LMS) model and the lowest effective dose (LED<sub>10</sub>) linear method (LED<sub>10</sub> = 95% lower bound on the dose giving a 10% tumor response). The LMS model (Tox\_Risk version 3.5 software) produces a q<sub>1</sub>\* value, which is the upper 95% confidence limit on the cancer potency slope calculated by the LMS model. The 0.1/LED<sub>10</sub> method gives a CSF derived from the lower 95% confidence limit on the 10% tumor dose (CSF = 0.1/LED<sub>10</sub>), and is the method of calculating a URF discussed in TCEQ (2006). Carcinogenic potency estimates (q<sub>1</sub>\* and CSF values) were adjusted for human equivalence with body weight<sup>3/4</sup> scaling (see Tables 16 and 17 of CalEPA 2000), which corresponds to a mouse-to-human scaling factor of 7.3 (i.e., (70 kg/0.025 kg)<sup>1/4</sup> = 7.3). Unlike the USEPA (1987a) assessment which used interspecies scaling to account for “expected” *toxicodynamic* differences since *toxicokinetic* differences in GST metabolism had already been accounted for by PBPK modeling, scaling to calculate human equivalents in the CalEPA (2000) assessment may be viewed as done in consideration of *toxicokinetic* species differences since PBPK modeling was not used to account for species differences in GST metabolism but to calculate internal GST metabolite tissue doses in mice. However, the rate of GST pathway metabolism in the mouse being reported to be almost two orders of magnitude higher than in humans (Long et al. 1994) strongly suggests that metabolism of MC via the GST pathway does not scale well allometrically, which would only predict about a seven times higher rate in mice. *Therefore, CalEPA (2000) scaling across species with body weight<sup>3/4</sup> may not adequately account for species-specific variation in MC metabolism via the putative carcinogenic metabolic pathway, which in this case does not appear to adhere well to allometric scaling, a principal reason for deviation from default allometric assumptions (USEPA 1992).*

Although based on the NTP (1986) inhalation study, potency factors in CalEPA (2000) are presented on a risk per mg/kg-day basis. Potency values based on the two sets of model

parameters (prior mean versus posterior median) were fairly similar, as were those based on the LMS model and 0.1/LED<sub>10</sub> methods. The URFs (risk per  $\mu\text{g}/\text{m}^3$ ) selected by CalEPA (2000) and referred to above were derived from the inhalation NTP (1986) study slope factors expressed on a risk per mg/kg-day basis using regressions of GST metabolite concentrations against applied doses (Table 20 of CalEPA 2000). To derive these URFs, CalEPA (2000) used the central estimates of GST metabolites generated but the lower 95% confidence limit on the GST metabolite dose yielding a 10% tumor response (LED<sub>10</sub>). This combination of central metabolite estimates with a 95% lower confidence limit on the dose is likely more representative of how TD would likely conduct a MC carcinogenic assessment under TCEQ (2006) than OSHA's assessment (95<sup>th</sup> percentile of the GST metabolite distribution with the maximum likelihood estimate of extra risk). However, as indicated in the previous paragraph, CalEPA (2000) scaling across species with body weight<sup>3/4</sup> may not adequately account for species-specific variation in MC metabolism via the putative carcinogenic metabolic pathway as the rate of GST pathway metabolism does not appear to scale well allometrically (i.e., the rate in mice has been reported to be almost two orders of magnitude higher than in humans versus the allometric prediction of only about seven times). *Therefore, deviation from default allometric assumptions for cross-species extrapolation (body weight<sup>3/4</sup>) appears to be justified in this case (USEPA 1992). However, as the CalEPA (2000) assessment did not account for this (or take advantage of human PBPK modeling for cross-species extrapolation), TD did not consider it for adoption for purposes of deriving an inhalation URF and <sup>chronic</sup>ESL<sub>linear(c)</sub>.*

The CalEPA (2000) URFs (4.5E-07 per  $\mu\text{g}/\text{m}^3$  based on mouse "prior means" and 6.8E-07 per  $\mu\text{g}/\text{m}^3$  based on "posterior medians") are similar to the USEPA IRIS URF (4.7E-07 per  $\mu\text{g}/\text{m}^3$ ). As an additional comparison, these URFs are about 35-52 times higher than the draft USEPA (2010) URF of 1.3E-08 per  $\mu\text{g}/\text{m}^3$  based on liver/lung tumors in male mice, allometric scaling (BW<sup>0.75</sup>), and the most sensitive human GSTT1-1 genotype (+/+).