TECHNICAL SUPPORT DOCUMENT

PROPOSED IDENTIFICATION OF METHYLENE CHLORIDE AS A TOXIC AIR CONTAMINANT

Part B Report

$$CH_2Cl_2$$

Degreasing

Paint Stripping

Aerosols

Electronics

Chemical Production and Processing

Pharmaceuticals
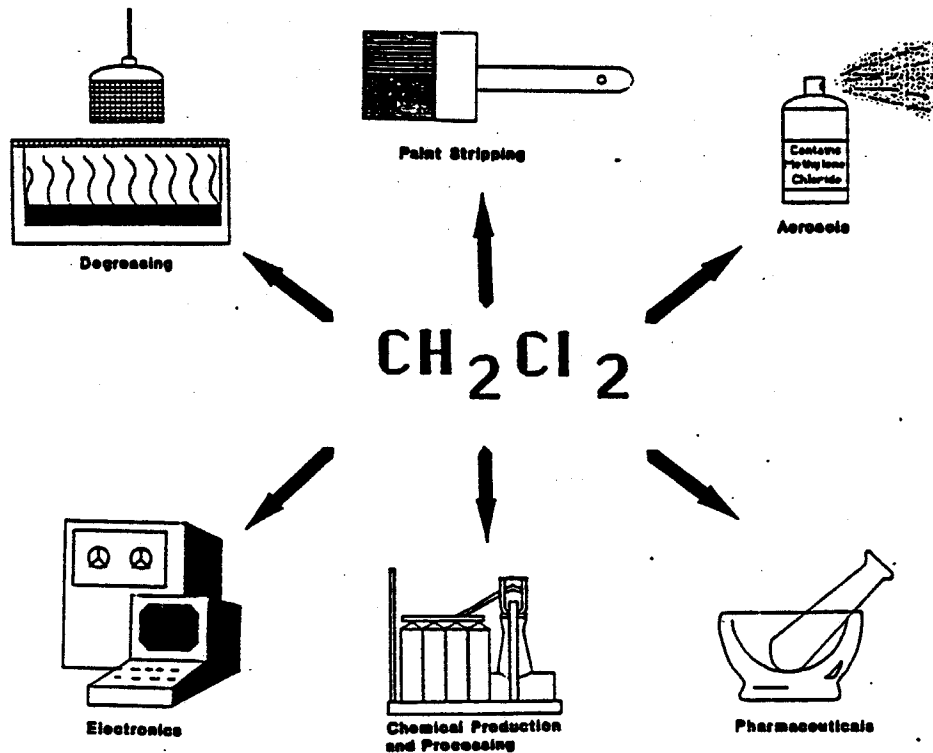
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PART B - HEALTH EFFECTS OF METHYLENE CHLORIDE

Principal Editor:

Norman Gravitz, Ph.D., MPH, Staff Toxicologist
California Department of Health Services

Reviewed and Edited by:

George V. Alexeeff, Ph.D.
Peter C. Flessel, Ph.D.
Irva Hertz-Picciotto, M.P.H., M.A.
Michael J. Lipsett, M.D.
Lauren Zeise, Ph.D.

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Executive Summary

Methylene chloride (dichloromethane, CH_2Cl_2) is a solvent with a variety of uses in industry and food processing. It is rapidly absorbed through the lungs and is distributed throughout the body. It can cross the blood-brain barrier and the placental barrier to the fetus. Although it is not highly bioconcentrated, it reaches equilibrium in fat at concentrations six to eight times greater than in other tissues.

Two pathways have been identified for CH_2Cl_2 metabolism in rats and mice. A cytochrome P450 oxidative dehalogenation pathway yields primarily carbon monoxide (CO), which has also been identified as a metabolite in humans. A second pathway involves glutathione and glutathione-S-transferase. Each pathway theoretically involves the formation of metabolically active intermediates that may be capable of binding to cellular macromolecules, including DNA. The oxidative pathway appears to be saturated at CH_2Cl_2 inhalation exposure concentrations above 500 ppm, while even at 4,000 ppm there is no evidence of saturation of the glutathione pathway.

Methylene chloride has a relatively low acute toxicity. Animal and human studies indicate that the central nervous system (CNS) is the principal target organ, resulting in CNS depression at inhalation concentrations greater than 1000 ppm. Chronic and subchronic effects on lung and liver (other than cancer) are observed in laboratory animals at concentrations of 100 ppm or greater. The most sensitive animal toxicity study suggests an oral no-observed-adverse-effect level (NOAEL) of 5 mg/kg-day. Assuming

equivalency of toxicity by the oral and inhalation exposure routes, a calculated NOAEL for humans is 5 ppm. Since these levels are at least three to four orders of magnitude greater than ambient air concentrations (approximately 2 ppb), adverse health effects other than cancer are not expected from acute or chronic exposures to methylene chloride in ambient air.

Inhalation of maternally toxic concentrations of methylene chloride (1250-4500 ppm) by female rodents throughout pregnancy can result in fetotoxicity. CH_2Cl_2 has low teratogenic potential in rodents, but experimental data are inadequate to make inferences about effects on human reproduction.

The genotoxicity of CH_2Cl_2 has been shown in several assay systems, including bacteria (S. typhimurium and E. coli), yeast (Saccharomyces cerevisiae), and fruit flies (Drosophila). Methylene chloride induced sister chromatid exchange and chromosomal aberrations in cultured mammalian cells. These responses, albeit positive, were weak.

The U.S. Environmental Protection Agency (EPA) concluded that CH_2Cl_2 is capable of inducing mutations in exposed human cells. The International Agency for Research on Cancer (IARC) reviewed short-term tests of DNA damage for CH_2Cl_2 and concluded that there was sufficient evidence to classify CH_2Cl_2 as genetically active. IARC also concluded that CH_2Cl_2 causes cell transformation in mammalian cells cultured in vitro. Staff of the California Department of Health Services (DHS) concur with IARC's and EPA's evaluations but stress that the high concentrations of CH_2Cl_2 necessary to

induce mutagenic and cellular transformations demonstrate that CH_2Cl_2 should be considered weakly genotoxic.

A series of epidemiological studies by Ott and co-workers compared the mortality experience of workers exposed to CH_2Cl_2 in one fiber production plant to nonexposed workers in another. No effects on mortality were attributable to CH_2Cl_2 exposure, but the studies had low statistical power and confounding factors; thus increased health risks cannot be excluded.

The mortality experience of a male cohort of Eastman-Kodak employees exposed to low levels of CH_2Cl_2 was compared both to Kodak employees not exposed to CH_2Cl_2 and to the general New York State male population. This study demonstrated an increase in pancreatic cancer compared to the nonexposed Kodak cohort and compared to the state male population. The authors attributed the increase in pancreatic cancer to an artifact of multiple statistical testing. DHS staff conclude that the epidemiological data are inadequate either to establish or to rule out carcinogenicity of methylene chloride in humans.

Several chronic rodent bioassays have shown that CH_2Cl_2 increases tumor rates in some organs, primarily the mouse liver and lung and the rat mammary gland. A lifetime inhalation bioassay was conducted in 1986 by the National Toxicology Program (NTP) in which both male and female rats and mice were exposed to 1,000 to 4,000 ppm of CH_2Cl_2 in air. The NTP concluded, "Under the conditions of these inhalation studies, there was some evidence of carcinogenicity of dichloromethane for male F344/N rats...and clear evidence

of carcinogenicity ... for female F344/N rats (and) ... for male and female B6C3F$_1$ mice."

The EPA concluded that for CH_2Cl_2 the evidence for carcinogenicity in experimental animals is sufficient, and the human evidence is inadequate. Overall, the EPA assigned CH_2Cl_2 to category B2, meaning that methylene chloride should be considered a probable human carcinogen. IARC recently reviewed the carcinogenicity data and concluded that methylene chloride is carcinogenic in animals although there is inadequate evidence that it is a human carcinogen. IARC assigned CH_2Cl_2 a rating of 2B, a category comprised of possible human carcinogens. DHS staff concur with EPA's and IARC's conclusions. Since the staff found no evidence of a carcinogenic threshold level and because there are several short-term tests suggesting that CH_2Cl_2 is mutagenic, the staff recommends that CH_2Cl_2 be considered as not having a threshold for carcinogenicity.

DHS staff used female mouse lung tumors (the most sensitive sex, species and tumor site of the 1986 NTP inhalation bioassay) to calculate the low-dose risk from exposure to CH_2Cl_2. The mean environmental concentration measured by Air Resources Board staff is approximately 2 ppb (0.002 ppm), while the animal experimental concentrations were 2,000 to 4,000 ppm. The mice were exposed to these experimental concentrations for six hours per day, five days per week. Adjusting for lifetime daily exposure, the experimental concentrations correspond to environmental exposures of approximately 357 ppm to 714 ppm. The low-dose extrapolation for human exposure from the animal bioassays spans five to six orders of magnitude.

As shown below, DHS staff fit several low-dose risk assessment models to the mouse lung tumor data, including the multistage (Global 82 and Global 86), time-dependent multistage (Weibull 82), probit, logit, Weibull, gamma multihit, and two-stage models. DHS staff also applied a physiologically based pharmacokinetic model to estimate the internal dose. This model adjusts the expected exposure concentration and suggests lower human risks than predicted by an unadjusted or applied dose approach. The NTP bioassay results are consistent with an applied dose as well as a pharmacokinetic approach. The application of the pharmacokinetic approach to risk assessment is based on information developed by the EPA and the U. S. Consumer Product Safety Commission. DHS staff recommend that the range of risks for ambient exposures to CH_2Cl_2 be based on the upper 95% confidence limit predicted from fitting either the multistage (Global 82) model or the time-dependent multistage (Weibull 82) model to the animal data. These approaches suggest that the upper bound of excess carcinogenic risk from a lifetime exposure to a range of CH_2Cl_2 concentrations from 1.1 to 2.4 ppb is 1 to 24 cases per million persons exposed. The unit risk for a lifetime of continuous exposure to 1 ppb of CH_2Cl_2 is 1 to 10 x 10^{-6} (lifetime exposure to 1 $\mu g/m^3$ of CH_2Cl_2 is 0.3 to 3 x 10^{-6}). The most likely estimate of the upper limit of risk, based on application of the PBPK high-to-low dose adjustment, is 4 x 10^{-6} per ppb. These calculations are for the upper range of plausible excess cancer risks: the actual risk, which cannot be calculated, may be insignificant.

The California Air Resources Board estimates a range of CH_2Cl_2 concentrations from 1.5 to 3.1 in the South Coast air basin. Assuming that there are about 10.09 million residents of this area, this suggests that the

upper bound for the excess number of additional lifetime cases of cancer in the South Coast air basin ranges from 20 to 300.

Based on the finding of methylene chloride-induced carcinogenicity and the results of the risk assessment, DHS staff finds that at ambient concentrations, methylene chloride is an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

METHYLENE CHLORIDE EVALUATION HIGHLIGHTS

I. <u>National and International Evaluation (Other Agencies' Evaluations).</u>

  A. <u>U.S. Environmental Protection Agency (EPA)</u>

    1. Short-Term Tests: <u>Sufficient evidence of genetic activity</u> and cell transformation.

    2. Animal carcinogenicity assays: <u>Sufficient evidence of animal carcinogenicity</u> by inhalation.

    3. Human Evidence: <u>Inadequate evidence of human carcinogenicity</u>.

    4. Conclusion: Methylene chloride is a <u>probable human carcinogen,</u> ranked B2.

  B. <u>International Agency For Research on Cancer (IARC)</u>

    1. Short-Term Tests: <u>Sufficient evidence of genetic activity</u> and cell transformation.

    2. Animal carcinogenicity assays: <u>Sufficient evidence of animal carcinogenicity</u> by inhalation.

    3. Human Evidence: <u>Inadequate evidence of human carcinogenicity</u>.

    4. Conclusion: Methylene chloride is a <u>possible human carcinogen</u> grouped under <u>IARC category 2B.</u>

  C. <u>Conclusions</u>: Both EPA and IARC concluded that there is ample evidence that methylene chloride is carcinogenic in animals (mice and rats) by inhalation and that it is genotoxic. Other than a slight increase in pancreatic cancer, there was no excess risk of death from malignancies in three epidemiological studies; however, both EPA and

IARC noted that the studies had a very limited power to detect excess risk.

II. Carcinogenic Threshold.

A. Shape of the dose-response curve

1. Animal: The carcinogenic dose-response curve for female mice lung tumors (adenomas and carcinomas combined) is consistent with a linear extrapolation. However, it should be noted that the dose-response curve consisted of only three data points, a control and two exposure concentrations.

2. Human: NA

B. Animal pharmacokinetic information: Moderate to good. No evidence for the presence of a carcinogenic threshold at low doses; both metabolic pathways appear to act at low doses.

C. Human pharmacokinetic information: Very limited. At low doses humans are likely to produce metabolic products by both pathways.

D. Conclusions: The positive short-term genetic activity tests, the observed animal carcinogenic dose-response curve, and the activity of metabolic activation at low doses lead the staff of DHS to conclude that methylene chloride is genotoxic and should not be considered to have a carcinogenic threshold.

III. Exposure Sources

A. Air levels

1. Ambient levels measured in the Los Angeles basin area: 1 to 5 ppb

2. Ambient levels measured in "hot spots": 17 to 28 ppb

3. Indoor air: Concentrations are variable depending on the size of the room, ventilation, quantity released, and time since release. Measured methylene chloride concentrations for a 90-minute average use in controlled studies using commercially available paint removers ranged from 50 to 2200 ppm. An Italian study reported that the mean methylene chloride concentration in 15 homes was 193 ppb with a high concentration of 1.5 ppm.

B. Reported Levels in Water

1. Methylene chloride may result from water chlorination; thus, finished water may have higher methylene chloride concentrations than raw water.

2. National Data

a. ambient waters: range 0 to 120 ppb, detected in 60 of 118 samples.

b. drinking water: range 1 to 3 ppb.

3. California drinking water: In 11 wells sampled in Southern California, methylene chloride concentrations ranged from 0.65 ppb to 10 ppb.

C. Reported levels in food: No information

IV. Quantitative Risk Assessment

A. Range of Extrapolation: Animal to human exposures in air for calculated lifetime daily exposures

1. Experimental to ambient: approximately 10^5

2. Experimental to hot spots: approximately 10^4

B. <u>Range of Risks from MLE to 95% UCL:</u>

Model	MLE to 95% UCL
Multistage	1.3 fold
Time-dependent multistage	2.6 fold

C. <u>Physiologically-Based Pharmacokinetic Adjustments</u>

1. CPSC high-to-low dose adjustment reduced applied dose risk estimate by approximately 2.2-fold.

2. EPA species-to-species dose adjustment reduced applied dose risk estimate by approximately 8-fold.

D. <u>Range of 95% Upper Bound Risk Estimate for Lifetime Excess Cancer Cases</u>

1. Per $\mu g/m^3$: 3×10^{-7} to 3×10^{-6}

2. Per ppm: 1×10^{-3} to 10×10^{-3}

3. Per ppb: 1×10^{-6} to 10×10^{-6}

E. <u>Comparison with other carcinogenic potencies:</u> Methylene chloride is in the <u>lower fourth quartile of 53 carcinogens</u> for which EPA has calculated potencies.

1.0 INTRODUCTION

Methylene chloride (dichloromethane, CH_2Cl_2) is a volatile, synthetic, halogenated hydrocarbon of low flammability. It has gained wide use in industry as a paint stripper, cleaning agent, process solvent, decaffeinating agent for coffee, and as a component of aerosol propellants. In acute exposures CH_2Cl_2 is toxic to humans and animals, with central nervous system depression as the major grossly observable effect. Subchronic and chronic exposures have been reported to cause behavioral changes, hepatic dysfunction, and, less frequently, lung lesions. Based on available data, the reproductive and teratogenic effects of CH_2Cl_2 cannot be evaluated. CH_2Cl_2 is mutagenic in bacteria test systems, but other mutagenicity tests are negative or equivocal; several tests for clastogenic activity have been positive. An International Agency for Research on Cancer (IARC) working group recently concluded that, although the evidence for carcinogenicity in animals is sufficient, there is inadequate evidence of its carcinogenicity in humans. IARC considers substances such as this to be potential human carcinogens. Applying the criteria of the EPA (1984) for evaluating the overall weight of evidence of carcinogenicity to humans, CH_2Cl_2 is most appropriately classified in Group B2, meaning that it is a probable human carcinogen.

This report reviews and evaluates the literature on the toxicity of CH_2Cl_2 and develops a risk estimate for its carcinogenic potency based on mathematical models. A risk estimate is also presented based on exposure adjusted to account for metabolism of CH_2Cl_2 to a proposed biologically active metabolite.

2.0 METABOLISM AND PHARMACOKINETICS

2.1 Absorption, Distribution, and Elimination

The route of exposure has been found to have a significant effect on the disposition of CH_2Cl_2 in rats and mice (Angelo et al. 1986a,b). Unlike the oral administration pattern, there was a dramatic retention of unmetabolized CH_2Cl_2 in tissues of mice following i.v. administration and the blood profile did not resemble the tissue profile (Angelo et al. 1986a).

2.1.1 Oral Absorption   Methylene chloride appears to be rapidly absorbed from the gastrointestinal tract following ingestion (EPA 1985a). Rats given a single oral dose of 1 or 50 mg/kg $^{14}CH_2Cl_2$ excreted 92% and 96%, respectively, as the combined metabolized and nonmetabolized dose (McKenna and Zempel 1981). Similarly, Angelo et al. (1986) found that 97% of an oral dose of 50 or 200 mg/kg was excreted within 24 hours. Peak blood levels of CH_2Cl_2 were attained in mice less than 10 minutes after oral administration of 50 mg/kg CH_2Cl_2 (Pritchard and Angelo 1982).

2.1.2 Dermal Absorption   The available information indicates that CH_2Cl_2 is absorbed very slowly across the skin (Stewart and Dodd 1964).

2.1.3 Pulmonary Uptake   Inhaled CH_2Cl_2 is rapidly absorbed in the lung. Pulmonary uptake is facilitated by the water and lipid solubilities of CH_2Cl_2 and by the large lung alveolar surface area (EPA 1985a). The actual rate of CH_2Cl_2 absorption depends upon several factors: concentration in inspired air, pulmonary ventilation, duration of exposure, diffusion rates into blood and

tissues, and solubilities in blood and tissues. The concentration of CH_2Cl_2 in alveolar air, which is in equilibrium with the concentration of CH_2Cl_2 in the pulmonary venous blood, asymptotically approaches the concentration in inspired air, at which point a steady-state condition is attained.

Data from Riley et al. (1966) can be used to illustrate the complex absorption of CH_2Cl_2 in humans. During a 2-hour inhalation exposure to air containing 100 ppm CH_2Cl_2, the alveolar air concentration could be described by an exponentially rising curve with three components. At the beginning of exposure, an initial rapid rate of uptake occurred (0 to 50 ppm alveolar air), followed by a second slower uptake (50 to 65 ppm alveolar air), and finally, a very slow rate of uptake as equilibrium was approached, but not reached, at 70 ppm alveolar air concentration (EPA 1985a). These observations can be interpreted as a function of both the tissue partition coefficients and the degree of tissue blood perfusion and suggest that there are three types of tissues serving as body stores for absorbed CH_2Cl_2: a rapidly equilibrating group of tissues (termed the "vessel-rich group") with high blood flows, an intermediate group (primarily muscles), and the slowly diffusing adipose tissues. Obese human subjects absorbed 30% more CH_2Cl_2 on a mg/kg basis than lean subjects from one hour exposure of 750 ppm (EPA 1985a).

Human exposure studies (Divincenzo and Kaplan, 1981b) indicate that exercise increased the CH_2Cl_2 blood concentrations, the carboxyhemoglobin levels, exhaled level of CO. Based on ventilation capacity in exercising individuals the percent CH_2Cl_2 absorbed decreased from 72 to 47%, but the net pulmonary uptake of CH_2Cl_2 actually increased up to 4-fold. Also reflective of the increased uptake, the extent of CO production increased from 26% during

sedentary conditions up to 40% during exercise. In an attempted follow-up study, Carlson and Kim (1986) examined whether a similar phenomenon could be demonstrated in rats. For rats exposed to 500 or 1000 ppm for 8 hours, exercise did not elevate carboxyhemoglobin levels and the CH_2Cl_2 blood levels were lower in the exercised animals than in the sendentary animals. The authors concluded that additional safety considerations are needed to include the influence of exercise which exacerbates CH_2Cl_2's effects in humans but not in rats.

2.1.4 <u>Tissue Distribution</u>   Methylene chloride is probably distributed throughout the body fluid by virtue of its water solubility, and its lipid solubility allows it to diffuse through lipid-containing membranes into various tissues. Methylene chloride readily crosses the blood-brain barrier (Winneke and Fodor 1976, Winneke 1981) and the placental barrier (Schwetz et al. 1975, Anders and Sunram 1982).

Concentrations of CH_2Cl_2 in various tissues depend upon exposure concentrations and durations and on tissue partition coefficients (EPA 1985a). Savolainen et al. (1977) found similar levels of CH_2Cl_2 in the brain, blood, and liver of rats exposed to 200 ppm CH_2Cl_2 for 5 days for 6 hours per day, but found a cumulative increase in CH_2Cl_2 concentrations in perirenal fat after the fifth day of exposure. At termination of the last 6-hour exposure, the ratios of CH_2Cl_2 concentrations in tissues versus blood were approximately 0.83 to 0.90 for brain and liver, and 6.6 for perirenal fat.

Studies of CH_2Cl_2 distribution in rats 48 hours after a 6-hour inhalation exposure to 50, 500, or 1,500 ppm or after a single oral dose of 1 or 50 mg/kg

have been conducted by McKenna et al. (1982) and McKenna and Zempel (1981) using ^{14}C-labelled CH_2Cl_2. Both routes of exposure resulted in the liver, kidney, and lung having the highest levels of ^{14}C-activity. Methylene chloride was not detected in any of the tissues assayed; it was assumed that the radioactivity found was due to the presence of nonvolatile metabolites.

2.1.5 <u>Elimination</u>   Virtually all unchanged CH_2Cl_2 is exhaled from the body (EPA 1985a). Less than 2% of absorbed CH_2Cl_2 has been found unchanged in the urine of human subjects exposed to 100 or 200 ppm for two hours (DiVincenzo et al. 1972), or in the urine of dogs exposed to 5,000 ppm (MacEwen et al. 1972).

After termination of exposure, CH_2Cl_2 is initially eliminated from the body via the lungs. Alveolar air rapidly equilibrates with pulmonary venous blood in which the CH_2Cl_2 concentration is a function of the first-order diffusion of CH_2Cl_2 from tissues, the arterial blood flow/tissue mass, and the relative solubility of CH_2Cl_2 in tissues (EPA 1985a).

Estimates of the half-times of pulmonary elimination of CH_2Cl_2 are given in Table 2-1. The alveolar air concentration of CH_2Cl_2 showed evidence of a three-phase decline with an initial rapid fall in alveolar concentration, a less rapid drop, and a longer-term slow decrease. The apparent three-component elimination for humans is relevant to the earlier discussion of tissues with high vascularity and blood flow (alpha), those with less flow (beta), and those with high amounts of adipose tissue (gamma). The values reported for the gamma components may not be indicative of actual diffusion

TABLE 2-1

COMPARISON OF HALF-TIMES OF PULMONARY ELIMINATION
OF METHYLENE CHLORIDE FOR HUMANS AND RATS

Subject	Exposure Method	Methylene Chloride Concentration	$t_{1/2}$ First-Order Components[a] (minutes)		
			Alpha[b]	Beta[c]	Gamma[d]
Human	Inhalation	50-500 ppm (1-7.5 hr)	8-23	40-80	360-390
Rat	Inhalation	500 ppm (6 hr)	2.4	14.8	--
		1,500 ppm(6 hr)	1.06	15.5	--
Rat	Gavage	1 mg/kg	--	12.6	46.5
		50 mg/kg	--	12.6	46.6

SOURCE: After EPA, 1985a

[a]
 Exponential elimination curve consistent with three distinct first-order rate
 constants.
[b]
 Attributed to elimination blood vessel-rich tissues with high blood flow,
 e.g., brain, heart, liver.
[c]
 Attributed to elimination from lean body mass, e.g., muscle and skin.
[d]
 Attributed to elimination from adipose tissues.

rates from fat tissues following repeated exposures since the gamma value reported in Table 2-1 was from a single exposure, and it has been previously shown that CH_2Cl_2 levels in fat tissues do not equilibrate after a single exposure. Fat biopsies of subcutaneous adipose tissue of obese subjects contained about 10 mg and 8 mg of CH_2Cl_2 at 1 and 4 hours repectively and between 1 and 2 mg/kg at 22 hours after exposure (EPA 1985a).

2.2 Metabolism

Methylene chloride is a single-carbon halogenated hydrocarbon for which few enzymatic pathways of metabolism are available. The primary known pathways for metabolizing CH_2Cl_2 are the mixed function oxidase system (i.e., microsomal oxidation) and a cytosolic pathway consisting of a glutathione transferase system which dehalogenates CH_2Cl_2. However, the specific mechanisms of metabolism and the formation of potentially reactive intermediates have not been directly demonstrated.

2.2.1 Enzymatic Metabolism

2.2.1.1 Microsomal oxidation Kubic and Anders (1975) first described the oxidative dehalogenation that appears to be a primary route of enzymatic metabolism of CH_2Cl_2 and other dihalomethanes. Working with an in vitro rat liver microsomal system, it was determined that the reaction required nicotinamide adenine dinucleotide phosphate (NADP), reduced NADP (NADPH), and molecular oxygen for maximal activity. Under optimal conditions, carbon monoxide (CO) was produced; in the absence of NADPH, CH_2Cl_2 and other dihalomethanes were dehalogenated without formation of carbon monoxide. When

Microsomal Pathway

$$X_2CH_2 \xrightarrow[\text{NADPH}]{\text{MFO}} X_2C\overset{H}{\underset{OH}{}} \xrightarrow{?} \longrightarrow \longrightarrow \longrightarrow CO_2$$

$$\downarrow \begin{array}{l} -H \\ -X \end{array} \text{Nonenzymatic rearrangement}$$

Covalent
Binding
lipid
protein $\longleftarrow$ $X-C\overset{H}{\underset{O}{}}$ $\xrightarrow[\substack{\text{Decomposition} \\ -H, -X}]{\text{Spontaneous}}$ CO $\xrightarrow{?}$ CO_2

formyl halide

Cytosolic Pathway

$$X - CH_2{-}^X \;+\; GSH \xrightarrow[\text{TRANSFERASE}]{-\,X} GS - CH_2X$$
S-halomethylglutathione

$$HOH \downarrow \text{Nonenzymatic hydrolysis}$$

$$GS - CH_2OH$$
S-hydroxymethyl glutathione

$$GS - C\overset{H}{\underset{O}{}} \xleftarrow{NAD}$$

S-formyl glutathione

$$HOH \downarrow \text{ S-formyl glutathione hydrolase}$$

$$HC\overset{O}{\underset{OH}{}} + GSH$$

formic acid

formaldehyde
dehydrogenase

$$H_2C{=}O + GSH$$
formaldehyde

one
carbon
pool

metabolic
incorporation

CO_2

Figure 1. Proposed reaction mechanisms for the metabolism of dihalomethanes to carbon monoxide, carbon dioxide, formaldehyde, formic acid, and inorganic halide. (EPA 1985b; Gargas et al. 1986)

the reaction was carried out under anaerobic conditions, the yield of the reaction was reduced by approximately 80% (20% of the maximal carbon monoxide was produced). Tissue activity studies showed that liver microsomes were about 5 times more active than lung microsomes and 30 times more active than kidney microsomes. Hogan et al. (1976) confirmed that CH_2Cl_2 was converted to carbon monoxide by the rat liver microsomal system, and noted a high correlation between carbon monoxide production in vitro and microsomal cytochrome P450 activity. Further studies by Kubic and Anders (1978) with $^{18}O_2$ and CD_2Cl_2 led to the current suggested scheme for P450 metabolism of CH_2Cl_2 to CO as indicated in Figure 1. In a recent study, Takano and Miyazaki (1988) used a perfused rat liver system and reported that the CO produced by P450 during metabolism could bind to the P450 cytochrome. However, in another study (Halpert et al. 1986) CH_2Cl_2 did not inactivate the phenobarbital-inducible isozyme of rat liver cytochrome P-450 in an in vitro assay system.

Studies by Anders and coworkers (Kubic and Anders 1978; Ahmed and Anders 1978; Stevens and Anders 1978, 1979) have shown that molecular oxygen, not water, is the source of the oxygen in carbon monoxide. They postulated that the cytochrome P450 mixed-function oxidase system hydroxylates dihalomethanes to yield an intermediate compound, a hydroxydihalomethane, which spontaneously breaks down to a formylhalide with the loss of one halogen atom. The formylhalide then decomposes to carbon monoxide by elimination of a halide anion.

A recent study by Reitz et al. (1988) provided some in vitro data on the kinetics of MFO enzymes in several species. The CH_2Cl_2 concentrations used varied from 1 to 10 mM. Reaction rates from liver microsomes (nmoles/min/mg

protein) were measured for hamster, mouse, rat and human tissue. The reaction rates were greatest in the hamster, followed by the mouse; and the rates of the human and rat tissue were equivalent and lower (per mg protein). The smallest difference between the mouse and human reaction rates occurred at the 5 mM level where the mean reaction rate for the mouse tissue (11.4) was 2.6 times the mean reaction rate for the human tissue (4.5). Although some mean values were presented, standard deviations were not, so it is difficult to ascertain the closeness of the responses. Reaction rates from lung microsomes were also measured in the mouse, rat and hamster, but no activity was measured in the single pooled human sample. At the 5 mM level, the reaction rate from mouse liver microsomes (11.4) was 2.5 times the reaction rate for the mouse lung microsomes (4.6). However, the lung data appears to represent information from a single experiment; means and standard deviations were not presented. Kinetic constants were calculated for the male liver tissue in the study and the KM for the mouse data (1.8 mM) was within the human range of data (0.9 - 2.6 mM). The VMAX for the mouse data (15.9 mmoles product/min/mg protein) was close to the upper range reported for humans (1.53 - 13.0 mmoles product/min/mg protein). However, the four human samples exhibited a 8.5-fold range indicating considerable human variability.

2.2.1.2 <u>Carboxyhemoglobin Formation</u> Blood COHb accumulates when the concentration of carbon monoxide exceeds that which can be eliminated through the lungs (EPA 1985a). Since carbon monoxide is a metabolite of CH_2Cl_2, there was concern for potential carbon monoxide poisoning from occupational exposures to CH_2Cl_2 (Stewart et al. 1972a,b). Pulmonary elimination of carbon monoxide from carboxyhemoglobin is a first-order process that involves the exchange of hemoglobin-bound carbon monoxide with oxygen and the diffusion of

carbon monoxide into the alveolar spaces. The half-time for elimination of carbon monoxide is 4 to 5 hours in humans (NIOSH 1972, Lambertsen 1974) and is 1.8 to 2.5 hours in rats (McKenna et al. 1982, McKenna and Zempel 1981). The rate of pulmonary elimination is independent of the concentration of carboxyhemoglobin in the blood, but is shortened by increased alveolar ventilation or the inspired partial pressure of oxygen (Lambertsen 1974).

EPA (1985a) concluded that the kinetics of CH_2Cl_2 metabolism to carbon monoxide and carboxyhemoglobin are strikingly similar for humans and rats. Both species demonstrate Michaelis-Menten dose-dependent kinetics with similar estimates for VMAX (15 and 12.5% carboxyhemoglobin per time unit, respectively) and KM (200 and 170 ppm CH_2Cl_2, respectively). Comparison of the kinetic parameters estimated for the carbon monoxide pathway in the rat with those estimated by McKenna et al. (1982) for the total metabolism of CH_2Cl_2 by both pathways suggests that the overall KM is approximately equal to 400 ppm and is approximately twice that of the carbon monoxide pathway alone.

Methylene chloride-induced carboxyhemoglobin depends on the CH_2Cl_2 dose and on the duration of exposure, but usually does not exceed 10% to 12% in either humans or animals (EPA 1985a). This limit may be determined by the resultant first-order pulmonary elimination of carbon monoxide and zero-order hepatic CH_2Cl_2 metabolism to carbon monoxide. This limit may also be the result of direct effects by CH_2Cl_2 on hemoglobin. An early study indicated that CH_2Cl_2 can deform binding sites of myoglobin (Nunes and Schoenborn 1973). Settle (1975) observed that carbon monoxide binding was affected by the presence of CH_2Cl_2. Spectrophotometric studies have shown that CH_2Cl_2 decreases the affinity of heme for CO and oxygen, and the cooperativity of oxygen binding of

hemoglobin in solution (Harkey et al. 1979). Consequently, the kinetics of CH_2Cl_2 metabolism and direct effects by CH_2Cl_2 on hemoglobin may be responsible for the levels of carboxyhemoglobin attained in experimental studies.

2.2.1.3   Cytosolic pathway   Methylene chloride is also metabolized to formaldehyde, formic acid, inorganic halide, and carbon dioxide (See Figure 1). The cytosolic enzyme system is a glutathione-S-transferase (GST) system that requires no cofactors other than glutathione. Ahmed and Anders (1976) proposed that the rate-limiting step of the reactions of CH_2Cl_2 with glutathione is displacement of the halogen to form a S-halomethylglutathione conjugate. This conjugate is postulated to undergo rapid nonenzymatic hydrolysis to S-hydroxymethylglutathione, which may subsequently break down to yield formaldehyde and regenerate glutathione. Alternatively, the S-hydroxymethylglutathione intermediate can be metabolized by formaldehyde dehydrogenase, a cytosolic liver enzyme, to S-formylglutathione, which can then be converted to formic acid and glutathione by the action of S-formylglutathione hydrolase.

Reitz et al. (1988) provided some in vitro data on the kinetics of GST in cytosol prepared from livers of several species. The CH_2Cl_2 concentrations used for all species varied from 12.5 to 100 mM, that is they were much higher than the concentrations used in the MFO studies (1 to 10 mM, see Section 2.2.1.1). Reaction rates from liver cytosol (nmoles/min/mg protein) were measured for hamster, mouse, rat and human tissue. The reaction rates were greatest in the mouse and least in the hamster. The relative rates of the human and rat tissue were dependant on the concentration chosen. The smallest

difference between the mouse and human reaction rates occurred at the lowest concentration tested, 12.5 mM, where the reaction rate for the mouse tissue (9.9) was 6.5 times the mean reaction rate for the human tissue (1.5). Reaction rates from lung cytosol were also measured in the mouse, rat, hamster, and human at the 40 mM CH_2Cl_2 exposure concentration. Although the data are extremely limited (apparently representing single experiments), the reaction rate from the mouse lung microsomes (7.3) was 20 times the reaction rate for the human lung microsomes (0.4); means and standard deviations were not presented. However, the report that human lung data exhibited GST activity while not exhibiting any P450 activity may be important in interpreting toxic effects that may result from GST metabolites, since this pathway may substantially predominate in human lung tissue. The GST pathway may predominate in the lung of mice as well. Kinetic constants were calculated for the male liver tissue of only mice and humans. The KM for the mouse data (137 mM) was 3-fold greater than the human value reported (44 mM). The VMAX for the mouse data (118 mmoles product/min/mg protein) was 17-fold greater than the value reported for humans (7 mmoles product/min/mg protein).

2.2.1.4 <u>Carbon dioxide formation</u> Oxidation of CH_2Cl_2 to CO_2 <u>in vivo</u> has been shown by several investigators (Angelo et al 1986a,b, Rodkey and Collison 1977a, DiVincenzo and Hamilton 1975, McKenna and Zemple 1981, McKenna et al 1982, and Yesair et al. 1977). Rodkey and Collison (1977a) studied the oxidation of CH_2Cl_2 to CO_2 in the rat. When exposed to 0.2 mmol $^{14}CH_2Cl_2$/kg animal (approximately 610 ppm for the 8 liter chamber), the evolution of $^{14}CO_2$ was measured to be 29% of the administered dose. Since the metabolic CO_2 produced in respiration greatly diluted (near 1000-fold) the CO_2 produced from CH_2Cl_2, proper measurement of CO_2 requires ^{14}C labeling.

There are two likely mechanisms of carbon dioxide formation from the metabolism of CH_2Cl_2. Studies in rats have shown that formaldehyde and formic acid produced by the cytosolic glutathione transferase system can be a source of carbon dioxide (McKenna et al. 1982). Gargas et al. (1986) hypothesize the possible production of CO_2 by the P450 pathway, but the study did not measure the metabolism of CH_2Cl_2 to CO_2 and data are not presented to substantiate the claim. An unpublished study (Green et al. 1987c) submitted to the Department implies that for mice exposed to low concentrations of CH_2Cl_2, CO_2 may be produced predominantly via the P450 pathway and not the GST pathway. Their conclusion is based on the assumption that GST "metabolism involves the breaking of C-Cl bond in the initial reaction with glutathione and hence the use of CD_2CCl_2 will not affect the rate of formation of carbon dioxide from this pathway." This is contrary to the results reported by Ahmed and Anders (1978) indicating that the use of a deuterated compound would decrease CO_2 production via the GST pathway. Specifically, Ahmed and Anders (1978) reported a primary isotope effect in the formation of formic acid that was dependant on NAD. As seen in Figure 1, the primary isotope effect would be expected to occur due to the oxidation of a C-H bond of S-hydroxymethyl glutathione by formaldehyde dehydrogenase to S-formyl glutathione. The results and conclusions of the Green et al. (1987c) study are discussed further in Section 2.2.2. Carbon monoxide may be metabolized to carbon dioxide by various animal tissues (Fenn 1970) by combining with the reduced form of tissue cytochrome oxidase at low oxygen levels (Coburn 1970. See Figure 1). Although the direct metabolism of carbon monoxide to carbon dioxide is thought to be a minor pathway, the metabolic conversion of CO to CO_2 appears to be a function of the body burden of CO in dogs (Luomanmaki and

Cobun, 1969), and thus the activity of the system may be inducible by increased body levels of carbon monoxide.

2.2.2 Dose-Dependent Metabolism: Saturation

There is evidence to suggest that the metabolism of CH_2Cl_2 is saturable under certain conditions. This evidence is based on increased expiration of unchanged CH_2Cl_2 at high dose levels, decreased expiration rates of CO and CO_2 at high dose levels, and the plateauing of carboxyhemoglobin production.

An early study on the extent of CH_2Cl_2 metabolism in the rat was conducted by DiVincenzo and Hamilton (1975). After injecting rats intraperitoneally with $^{14}CH_2Cl_2$ in corn oil, they determined the fate and disposition of radioactivity in exhaled air, urine, feces, and carcasses at 1, 8, and 24 hours after single doses (412 to 930 mg/kg). Approximately 90% of the administered CH_2Cl_2 was eliminated unchanged in the exhaled air, and 85% within two hours of administration. Two percent of the administered dose was metabolized to carbon monoxide, and approximately 3% was metabolized to a volatile compound that was not identified. Overall, less than 7% of the administered dose was metabolized. When rats were intravenously given a lower dose, 200 mg/kg CH_2Cl_2, they metabolized 6% of the dose to CO and 9% of the dose to CO_2 (Angelo et al. 1986b). At even lower doses of 50 and 10 mg/kg, slightly higher rates of CO and CO_2 production were reported (Angelo et al. 1986b). Rodkey and Collison (1977a) administered approximately 17 mg/kg $^{14}CH_2Cl_2$ by inhalation in an enclosed chamber. Approximately 47% of the administered ^{14}C was recovered as ^{14}CO and about 29% as $^{14}CO_2$. Thus, in this inhalation experiment a greater precentage of CH_2Cl_2 was metabolized. Only 2% of the $^{14}CH_2Cl_2$ remained unmetabolized in the chamber after 90 minutes.

TABLE 2-2

COMPARISON OF SATURATION DATA BASED ON THE PRODUCTION OF

CARBON MONOXIDE AND CARBON DIOXIDE

Species	Exposure Method	Dose or Concentration	Expired CO Level	Expired CO_2 Level	Study
Mouse	gavage	1 mg/kg	45%	> 50%	Yesair et al 1977
Mouse	i.v.[a]	10 mg/kg	15%	20%	Angelo et al 1986a
Mouse	i.v.	50 mg/kg	8%	14%	Angelo et al 1986a
Mouse	gavage	50 mg/kg	11%	18%	Angelo et al 1986a
Mouse	gavage	100 mg/kg	20%	25%	Yesair et al 1977
Mouse	gavage	500 mg/kg	9%	18%	Angelo et al 1986a
Mouse	gavage	1000 mg/kg	7%	19%	Angelo et al 1986a
Rat	i.p.[b]	412-881 mg/kg	2%	3%	DiVincenzo et al 1975
Rat	gavage	1 mg/kg	31%	35%	McKenna et al 1981
Rat	i.v.	10 mg/kg	12%	15%	Angelo et al 1986b
Rat	inhalation	17 mg/kg	47%	29%	Rodkey et al 1977a
Rat	i.v.	50 mg/kg	9%	10%	Angelo et al 1986b
Rat	gavage	50 mg/kg	6%	11%	McKenna et al 1981
Rat	gavage	50 mg/kg	16%	17%	Angelo et al 1986b
Rat	gavage	200 mg/kg	6%	9%	Angelo et al 1986b
Rat	inhalation	50 ppm	27%	26%	McKenna et al 1982
Rat	inhalation	500 ppm	18%	23%	McKenna et al 1982
Rat	inhalation	610 ppm	47%	29%	Rodkey et al 1977a
Rat	inhalation	1500 ppm	13%	10%	McKenna et al 1982

[a]The abbreviation i.v. refers to intravenous administration.
[b]The abbreviation i.p. refers to intraperitoneal administration.

McKenna and Zempel (1981) reported that rats dosed at 1 mg/kg metabolized 88% of the administered dose, whereas those given 50 mg/kg only metabolized 28%. In the same study, rats given 50 mg/kg CH_2Cl_2 exhibited a substantial decrease in the rate of CO and CO_2 production compared to the 1 mg/kg dose. The kinetics of pulmonary elimination of unmetabolized CH_2Cl_2 and of the metabolites carbon monoxide and carbon dioxide were reportedly first-order (essentially unaffected by the dose) despite large differences in the amounts of CH_2Cl_2 and its metabolites excreted. As summarized in Table 2-2, doses of 50 mg/kg or greater, administered to the rat, appear to be metabolized to a lesser extent than lower doses based on the decreased relative percentage of CO and CO_2 eliminated. This decreased elimination of CO and CO_2 implies saturation. Some indication of saturation of the P450 pathway was provided in studies of carboxyhemoglobin formation in rats following intraperitoneal injection (Kubic et al. 1974). Administration of 125, 250 and 500 mg/kg CH_2Cl_2 produced peak carboxyhemoglobin levels of 5, 8 and 9%, respectively. At the higher doses the peak levels came later, and the area under the dose-response curve indicates a greater response at the 250 and 500 mg/kg levels than the simple peak percent carboxyhemoglobin. Based on this study saturation would be expected at levels near 250 mg/kg for the rat.

As indicated in Table 2-2, the data for the mouse is similar to that produced for the rat, and similar trends are seen across routes. There is considerable variability from experiments on the concentration or dose saturating CO production. Furthermore, P450 saturation estimates based soley on CO production, instead of direct measurements of P450 activity, ignore the possibility of P450 inactivation by increasing production of reactive metabolites as the concentration increases. In a preliminary report, Yesair

et al. (1977) stated that the 100 mg/kg dose was metabolized by mice at 20 μmole/hr/kg, but the information in the abstract was never published as a complete report.

Evidence suggests that the CH_2Cl_2 metabolism may become saturated at inhalation exposure concentrations of 500 ppm and above (some have suggested levels as low as 200 ppm). When rats were exposed for 6 hours to 50, 500, and 1500 ppm CH_2Cl_2 there was a substantial decrease in CO and CO_2 production at the 1500 ppm level (McKenna et al. 1982). When rats were exposed for 6 hours to 500 or 1500 ppm CH_2Cl_2 the carboxyhemoglobin levels did not increase beyond the 500 ppm exposure level (McKenna et al. 1982). Rabbits do not exhibit signs of metabolic saturation of P450 at CH_2Cl_2 exposure levels up 11,000 ppm. A linear relationship between the change in % carboxyhemoglobin in blood resulting from increased CH_2Cl_2 inhalation was shown to occur in rabbits over the range tested of 1,270 to 11,520 ppm (Roth et al. 1975). Inhalation data for the mouse indicating percent CO and CO_2 production for the mouse has not been reported. However, an unpublished study by Green et al. (1987c), submitted to DHS, suggests saturation may occur near 500 ppm for the mouse.

Rodkey and Collison (1977b) investigated the relationship between administered dose and the extent of metabolism by the rat. Animals were exposed by inhalation to doses ranging from 80 to 800 μmol/kg (approximately 244 to 2440 ppm for the 8 liter chamber). They reported that the rate of CO production was virtually independent of CH_2Cl_2 dose, and that it suggested the metabolism of CH_2Cl_2 to carbon monoxide in rats was rate-limited by enzyme saturation The rate was approximated at the highest dose level to be 23 μmol/hour/kg body weight.

In another study, rats were exposed for 4 hours to concentrations up to 2000 ppm CH_2Cl_2 and some of the animals were pretreated with 2,3-EP, an hepatic glutathione depleting agent (Gargas et al. 1986). When naive animals were exposed to CH_2Cl_2, the carboxyhemoglobin level peaked with the 500 ppm exposure level. For rats pretreated with 2,3-EP, the carboxyhemoglobin levels did not peak until the 1000 ppm CH_2Cl_2 exposure level and the carbon monoxide yield attained was 43% greater than the level attained in the naive animals. That is, when the P450 pathway was supposedly saturated, addition of a cytosolic pathway inhibitor (2,3-EP), increased metabolism by the P450 pathway. One possible explanation for this result is that complete saturation of the MFO pathway had not occurred, but had simply reached some steady state.

Thus, although saturation of the P450 pathway is likely to occur at high doses, the precise exposure concentration has not been adequately defined. Information of this saturation concentration only exists for rats, and the saturation response based on carboxyhemoglobin formation may not be reliable based on the complicated mechanisms involved in carboxyhemoglobin formation and on the conflicting results produced in induction and inhibition studies discussed in Section 2.2.3.

DiVincenzo and Kaplan (1981a) evaluated the conversion of CH_2Cl_2 to carboxyhemoglobin (COHb) and CO in man for single exposure levels of 50, 100, 150, and 200 ppm for 7.5 hours and of 100, 150, and 200 ppm for 7.5 hours daily for 5 consecutive days. Exposure to 50, 100, 150, or 200 ppm of CH_2Cl_2 produced peak blood carboxyhemoglobin saturations of 1.9, 3.4, 5.3, and 6.8%, respectively, and produced net excretion levels of CO of 1.32, 2.66, 4.95, and 6.12 (mmol), respectively. The study concluded that CO concentrations were

directly proportional to the magnitude of CH_2Cl_2 exposure. Thus, the uptake of CH_2Cl_2, the exhalation of CO, the carboxyhemoglobin levels all increased proportionally with the dose, and saturation of the P450 pathway did not occur in concentrations up to 200 ppm in humans.

In an ealier human study volunteers were exposed to 213 or 514 ppm CH_2Cl_2 for 1.0 hour (Stewart et al., 1972b). The carboxyhemoglobin levels peaked at 2.4 in the one volunteer exposed to 213 ppm, while the levels ranged from 1.8 to 4.6 in the eight subjects exposed to 514 ppm. Based on this study it is difficult to determine whether saturation of the P450 metabolism occurred at 514 ppm, particularly since the carboxyhemoglobin levels are below those achieved by DiVincenzo and Kaplan (1981a) and humans exhibit considerable variability in this response.

In another study of healthy human male volunteers the kinetics of CH_2Cl_2 at exposures of 200 and 350 ppm during two 6-hour periods were compared (McKenna et al. 1980). No data were presented in the abstract but, the authors concluded that the metabolism of CH_2Cl_2 was dose-dependent, and that the data were consistent with metabolism by a Michaelis-Menten mechanism. Since the data were never reported in an abstract or in a subsequent publication, staff believe the conclusions made by the authors should not be considered in the evaluation of CH_2Cl_2 kinetics.

Some measurements of expired $^{14}CO_2$ have implied saturation of the cytosolic pathway at high doses in rats (McKenna and Zempel 1981, McKenna et al. 1982, Angelo et al. 1986b) and in mice (Angelo et al. 1986a, Yesair et al. 1977) following inhalation, oral and intravenous dosing. Evidence for the potential

saturation of CO_2 pathways is presented in Table 2-2. The expired level of CO_2 generally follows that of CO. Thus, based on production of CO_2, saturation of metabolic pathways producing CO_2 appears to follow the same trend as CO. Gargas et al. (1986), using male Fisher-344 rats in closed atmosphere gas uptake studies, measured formation of carboxyhemoglobin in the presence and absence of pyrazole to inhibit the microsomal pathway. Using the brominated methanes CH_2Br_2 and CH_2BrCl, they observed that the glutathione S-transferase pathway showed no indication of saturation at inhaled concentrations of up to 2,000 ppm based on plasma bromide concentrations. Since some carboxyhemoglobin was produced in the pyrazole-inhibited rats, another implication of the study is that CO may be produced to a small extent by the cytosolic or other non P450 pathway, since the study reported that after pyrazole was used, bromide ion was only released from the GSH-dependent pathway. However, since halide measurements were not made for CH_2Cl_2 and CO_2 was not measured in the study, conclusions regarding CO_2 production and cytosolic pathway activity are only hypothetical at this time. Furthermore, pyrazole not only inhibits MFO, but it also inhibits a variety of other metabolic systems including thyroid function and alcohol dehydrogenase (Szabo et al. 1978, Cornell et al. 1983). Thus, the decrease in production may have resulted from inhibition of numerous metabolic processes and not strictly MFO.

An unpublished study (Green et al. 1987c) submitted to DHS implies that for mice exposed to low concentrations of CH_2Cl_2, CO_2 may be produced predominantly via the P450 pathway and not the GST pathway. Deuterium isotope studies using CD_2Cl_2 reportedly produced CO_2 at a slower rate than CH_2Cl_2 at 100 and 500 ppm exposure concentrations. Green et al. assume that this slower rate is completely due to CO_2 metabolism via the P450 pathway, but literature

citations are not provided to substantiate this assumption. Since the rate of CO_2 production was not slowed as greatly as CO production, Green et al. calculated that 62% of the CO_2 production was via the P450 pathway at 100 ppm, 36% at 500 ppm and 10% at 4000 ppm. These calculations require that metabolism via the GST pathway would not be affected in any way by the use of CD_2Cl_2. This unpublished work is the only study that suggests the P450 pathway produced substantial quantities of CO_2 as well as CO. The results would be more convincing if glutathione depletion studies had been conducted which showed a substantial decrease in CO_2 production. The Green et al. (1987c) study disagrees with the published studies by Kubic and Anders (1978) which concluded that CO_2 production by P450 was minor, near 1%, when compared to the CO production. Furthermore, the basic assumption that the GST pathway would be unaffected by the use of a deuterated compound ignores the results reported by Ahmed and Anders (1978) which showed a primary isotope effect in the formation of formic acid that was dependant on NAD. This isotope efffect would be expected from the oxidation of a C-H bond of S-hydroxymethyl glutathione by formaldehyde dehydrogenase to S-formyl glutathione. Green et al. (1987c) conclude that the GST pathway is becoming saturated for rats in the 500 to 4000 ppm range because the 8-fold increase in concentration is only accompanied by a 2-fold increase in CO_2 production rate. However, the authors reject the possibility of GST pathway becoming saturated for mice even though an 8-fold increase in exposure concentration (500 to 4000 ppm) resulted in less than a 3-fold increase in CO_2 production rate. According to the authors, the less than 3-fold increase for mice shows "little or no evidence of saturation" while the 2-fold increase for rats indicates "it is becoming saturated." This distinction appears unwarranted. Although the studies are of great interest with regard to the underlying mechanism of CH_2Cl_2

carcinogenicity, their preliminary nature, their disagreement with other published results and the absence of impartial review of the methods, results and discussion make it difficult to place great weight on the study.

In summary, the metabolism of CH_2Cl_2 appears to be dose-dependent in the rat and mouse (EPA 1985a). In these species, saturation of metabolic capacity with zero-order kinetics appears to occur above 50 mg/kg orally and above 500 ppm from prolonged inhalation of CH_2Cl_2. At doses of 1 mg/kg orally (Yesair et al. 1977) or 50 ppm by inhalation (McKenna et al. 1982), 95% was metabolized. For humans, exposure to concentrations up to 200 ppm have not produced saturation, and at 200 ppm up to 95% of the absorbed dose may be metabolized (DiVincenzo and Kaplan 1981a).

2.2.3 Induction and Inhibition of Metabolism

Methylene chloride was shown to induce its own metabolism (based on the formation of carboxyhemoglobin) following repeated administration in rats (Kubic et al. 1974, Anders et al. 1977). An earlier study showed that repeated exposure of rats to CH_2BrCl had increased the rate of dehalogenation (Heppel and Porterfield 1948). Angelo et al. (1986a, b) noted an apparent increase from day 1 to day 14 in CH_2Cl_2 content in various tissues of rats and mice following repeated oral dosing. The authors concluded that neither pharmacokinetic nor metabolic induction occurred but a statistical analysis of the excretion data was not reported (Angelo et al. 1986a, b). Examination of the data by DHS staff indicate that for both rats and mice CO and CO_2 excretion rates were elevated at the doses up to 500 mg/kg. At the 1000 mg/kg

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level for mice the CO and CO_2 excretion rates appeared to be substantially decreased, which may be indicative of metabolic inactivation.

The nature of the induction is unclear since short-term exposure of exposed rats to CH_2Cl_2 at 500 and 1,000 pm for 6 hours per day did not produce any "consistent changes" in P450 content of liver microsomes at day 5 and 10 of exposure (Kurppa and Vainio 1981). However, at day 5 the P450 content was elevated for the three exposure groups and the elevation of the 500 ppm group was statiscally significant compared to the control group. At day 10 the P450 content was elevated in all groups compared to the 5-day control group, but exposure groups were not different from the 10-day control group. The P450 content of kidney microsomes was elevated at day 5 and 10 of exposure and in two groups the elevation was statistically significant. In the same study, glutathione levels at day 5 or 10 of exposure were elevated in renal tissue but not in hepatic tissue.

Daily exposure to CH_2Cl_2 for short periods may influence other enzyme systems (EPA 1985a). When Kurppa and Vainio (1981) exposed rats to CH_2Cl_2 at 500 and 1,000 ppm for 6 hours per day for 5 and 10 days, they reported a 35% drop in NADPH-cytochrome C and a two-fold increase in UDP-glucuronosyltransferase. Heppel and Porterfield (1948) exposed adult male rats to an atmosphere of 1000 ppm CH_2BrCl for 6 to 7 hours per day, 5 days per week for 4 to 6 weeks. They reported an approximate 1.6-fold increase in the rate of enzymatic hydrolysis of the bromide ion from CH_2BrCl in a crude liver homogenate of exposed rats as compared to unexposed control rats. However, under their assay conditions, it is not possible to determine if enzymes of the MFO, glutathione, or both pathways were induced. Pritchard et al. (1982) administered CH_2Cl_2 to male

mice for 3 days by gavage (doses of 5, 50, 100, 250, 500, and 1,000 mg/kg in corn oil) and found no significant changes in hepatic weight, microsomal protein, P450, cytochrome b_5 content, or activities of aminopyrene N-demethylase or biphenyl 4-hydroxylase.

Pretreatment with 3-methylcholanthrene or phenobarbital, did not increase the formation of carboxyhemoglobin following CH_2Cl_2 dosing (Miller et al. 1973, Kubic et al. 1974, Hogan et al. 1976, Anders et al. 1977). Although Hogan et al. (1976) state that phenobarbital pretreatment induced CO production, and colbaltous chloride reduced CO production, the data were not provided in the abstract and a complete report has not been published. Roth et al. (1975) examined the metabolism of CH_2Cl_2 to carboxyhemoglobin in rabbits. Pretreatment with phenobarbital inhibited the formation of carboxyhemoglobin from CH_2Cl_2 exposure (1400 to 1500 ppm for 20 minutes); however, P450 activity itself was shown to be induced by the pretreatment. A study by Miller et al. (1973) also reported (as an abstract) no effect on carbon monoxide production from CH_2Cl_2 following phenobarbital pretreatment (Miller et al. 1973).

The administration of a known inhibitor of P450 metabolism, SKF 525-A, did not decrease carboxyhemoglobin formation significantly (Miller et al. 1973, Kubic et al. 1974). As noted above, phenobarbital inhibited carboxyhemoglobin formation in rabbits (Roth et al. 1975). One study, using a pyrazole pretreatment decreased carboxyhemoglobin formation in rats exposed to CH_2Cl_2 (Gargas et al. 1986). Pyrazole has been shown to inhibit thyroid function and alcohol dehydrogenase (Szabo et al. 1978, Cornell et al. 1983). Numerous experiments have shown that administration of ethanol blocks the formation of carboxyhemoglobin from CH_2Cl_2 exposure in guinea pigs, monkeys, and rats

(Balmer et al. 1976, Ciuchta et al. 1979, Glatzel et al. 1987, Pankow et al. 1985), but the currently suggested scheme for CH_2Cl_2 metabolism does not account for this interaction. However, blockage of the P450 pathway is likely to result in increased metabolism by the conjugation pathway. Thus, persons consuming alcohol may be at greater risk to the chronic effects of CH_2Cl_2 induced by metabolites of the conjugation pathway.

Human exposure studies (Divincenzo and Kaplan, 1981b) indicate that exercise increased carboxyhemoglobin levels and the amount of CO exhaled. In the one volunteer where CO and carboxyhemoglobin levels were reported, the extent of CO production increased from moderate to heavy exercise but the carboxyhemoglobin levels did not increase. This result indicates that although CH_2Cl_2 P450 metabolism increased carboxyhemoglobin levels did not reflect the increase in metabolism and therefore may not be a sensitive indicator of either CH_2Cl_2 metabolism or CO production in humans. Consequently, although CO production is consistent with P450 metabolism experiments, measuring only the formation of carboxyhemoglobin may not accurately reflect P450 metabolism. The results are consistent with CH_2Cl_2 acting directly on hemoglobin at high concentrations. Possibly at high concentrations CH_2Cl_2 decreases the affinity of heme for CO, and reduces the cooperativity of binding of hemoglobin subunits (Nunes and Schoenborn 1973, Settle 1975, Harkey et al. 1979); thus preventing greater formation of carboxyhemoglobin. Such a situation would explain the inability of inducers to increase carboxyhemoglobin levels in experimental animals exposed to CH_2Cl_2.

In contrast, for the related compound CH_2Br_2, the formation of microsomal cytochrome P450, and the _in vitro_ production of carbon monoxide was found to be increased by _in vivo_ pretreatment with pentobarbital or 3-methylcholanthrene (Kubic and Anders 1975). Another set of experiments where CH_2Br_2 was injected indicated that pretreatment of animals with phenobarbital or 3-methylcholanthrene resulted in significant increases in carboxyhemoglobin levels (Stevens et al. 1980). Pretreatment with SKF 525-A resulted in a significant decrease in carboxyhemoglobin levels (Stevens et al. 1980). And pretreatment with diethyl maleate decreased blood CO levels from CH_2Br_2 exposure. Using an isolated rat hepatocyte system, diethyl maleate, SKF 525-A and ethanol inhibited the production of CO from CH_2Br_2 (Stevens et al. 1980). Stevens et al. (1980) pointed out that their results with CH_2Cl_2 were in contrast to those obtained for CH_2Cl_2 in the literature. Cobaltous chloride has also been found to reduce microsomal carbon monoxide production from CH_2Br_2 exposure (Kubic and Anders 1975).

The cytosolic enzyme system is a glutathione transferase system that requires no cofactors other than glutathione. Metabolism of CH_2Cl_2 by this pathway was not induced by phenobarbital or by a 4-day repeated exposure to CH_2Cl_2 (Ahmed and Anders 1976). In contrast, for a related compound CH_2BrCl the cytosolic system appears to have been induced following a 4 to 6-week inhalation exposure (Heppel and Porterfield 1948).

In conclusion, the metabolic induction and inhibition studies of CH_2Cl_2 to CO by P450 do not fit the simplistic scheme presented in Figure 1. Standard inhibitors and inducers of P450 do not affect the production of CO from CH_2Cl_2 as one would expect. Metabolism of a compound similar to CH_2Cl_2, CH_2Br_2, does

respond to metabolic inhibitors and inducers as one would expect, that is, in a manner different from CH_2Cl_2. Thus, results for studies with CH_2Br_2 or other dihalomethanes may not reflect CH_2Cl_2 metabolism. The inability to account for the metabolic results reported for CH_2Cl_2 makes it difficult to conclude that the metabolic scheme has been completely elucidated.

2.3 Physiologically based pharmacokinetic model

Andersen et al. (1987) have proposed a physiologically-based pharmacokinetic model (PBPK) to describe the metabolism of methylene chloride. Using the information on the relative metabolic contributions of the two metabolic pathways, the PBPK model is supposed to offer an estimate of the potential target tissue dose in comparison to the conventional risk assessment methods based on total dose to the subject. Since conventional risk assessments often involve linear extrapolations of external doses (combined with inter-species factors based on body surface areas), Andersen et al. (1987) proposed that their PBPK model might permit the calculation of realistic internal doses for various chemicals through integration of known information on the administered doses, the physiological structure of the mammalian species, and the biochemical properties of the specific chemicals.

Andersen et al. (1987) recommend that their model be used to estimate "internal doses" of CH_2Cl_2 before calculating the possible risks of CH_2Cl_2 to humans. The general approach appears reasonable, although DHS staff express concern over the increasing use of extremely complex mathematical models in risk assessment. Such models are often surrounded by a sophisticated mathematical or computerized approach that may tend to hide key assumptions

made in the model. Furthermore, since data to satisfy all the needs of the model are usually lacking, numerous assumptions need to be made about the data, data representing a single experimental result are often incorported in the models, and unpublished results are often used without external laboratory validation. And finally, data is continually being sought to "update" and "improve" such models. As a result, extensive review and reevaluations are required to assess the usefulness of the most current model. An intensive analysis of the model has been conducted by the U. S. Interagency Hazard/Risk Assessment Committee (HRAC) of the Integrated Chlorinated Solvents Project and a review draft report has been circulated by EPA (1987a). In addition to the comments made in the DHS health assessment the HRAC report could be consulted for further analysis. The Andersen et al. (1987) physiologically based-pharmacokinetic model is more fully described and discussed in Section 8 and Appendix E.

2.4 Covalent Binding to Cellular Macromolecules

The ability of a chemical to bind covalently to cellular macromolecules provides an indication of its potential genotoxicity and carcinogenicity. The likelihood of significant covalent binding of reactive metabolites of CH_2Cl_2 to cellular macromolecules depends on the postulated reactive intermediates (EPA 1985a): formyl chloride from microsomal oxidative metabolism and S-chloromethyl glutathione from cytosolic metabolism. These compounds would be expected to be capable of acylating cellular nucleophiles, and S-chloromethyl glutathione is structurally similar to the very reactive bis-halomethyl ethers, which are known carcinogens.

The extent and pattern of binding to microsomal lipid and protein after aerobic incubation of rat hepatic microsomes with $^{14}CH_2Cl_2$ were studied by Anders et al. (1977). They found that metabolites of CH_2Cl_2 bound covalently to microsomal protein and lipids under conditions optimized for metabolism of CH_2Cl_2 to carbon monoxide. Pretreatment of rats with phenobarbital to induce the mixed-function oxidase system resulted in increased binding by microsomes, suggesting that the formyl chloride intermediate may acylate macromolecules.

Cunningham et al. (1981) studied the comparative covalent binding of metabolites of CH_2Cl_2, carbon tetrachloride, and trichloroethylene to lipids and proteins in rat hepatocytes. They found that binding of CH_2Cl_2 metabolites was enhanced in the presence of oxygen, which is consistent with formation of formyl chloride, and that glutathione-depleted hepatocytes showed a marked drop in binding of labelled CH_2Cl_2 metabolites to both lipids and protein, suggesting inhibition of cytosolic glutathione conjugation with decreased production of S-chloromethyl glutathione and formaldehyde. Although they observed that in vitro administration of $^{14}CH_2Cl_2$ did not result in alkylation of RNA and DNA under the conditions of the experiment, they did not know the limits of detection of this assay system (Sipes 1987).

This work demonstrates that highly reactive intermediates are formed in the metabolism of CH_2Cl_2, from both the microsomal and cystolic pathways and is consistent with the proposed reaction pathways.

2.5 Summary and Conclusions

Limited data are available on the metabolism and pharmacokinetics of CH_2Cl_2 in humans, but there is extensive kinetic information in rats. Absorption from the gastrointestinal tract is rapid and complete, and occurs by first-order processes in the rat. Dermal absorption is very slow for CH_2Cl_2. Pulmonary absorption also occurs by first-order processes in humans and rats. CH_2Cl_2 is distributed throughout the body by virtue of its water solubility; it readily gains access to cells and accumulates in adipose tissues by virtue of its lipid solubility; and it is known to cross the blood-brain barrier and the placenta. The major processes of elimination of CH_2Cl_2 from the body are pulmonary exhalation of unchanged CH_2Cl_2, and pulmonary exhalation of metabolized CH_2Cl_2. Pulmonary elimination follows first-order kinetics, which suggests that there are at least three body-storage compartments.

Hepatic metabolism of CH_2Cl_2 occurs via two major enzymatic pathways. One is a cytochrome P450-mediated microsomal pathway where CH_2Cl_2 undergoes oxidative dehalogenation to carbon monoxide which can produce carboxyhemoglobin. Some evidence suggests possible production of CO_2 by this pathway as well. The other is a glutathione-S-transferase mediated dehalogenation cytosolic pathway, yielding formaldehyde and formic acid. These reaction products may be further metabolized to carbon dioxide. The relative utilization of the two pathways at low body burdens of CH_2Cl_2 is unclear, although some evidence indicates the P450-mediated pathway may be favored at low doses. Evidence indicates that CH_2Cl_2 metabolism is saturable at high concentrations. Both pathways appear saturable based on CO and CO_2 levels produced.

Carboxyhemoglobin levels in the blood of humans and animals increase as a consequence of metabolism of CH_2Cl_2 to carbon monoxide. The level of carboxyhemoglobin in the blood may be affected dose of CH_2Cl_2, first-order kinetics of pulmonary elimination of carbon monoxide, and direct effects of CH_2Cl_2 on hemoglobin. Experimental results indicate that the carbon monoxide pathway saturates above 500 ppm in rats and possibly in humans; comparable evidence in mice is not available. The glutathione pathway shows comparable evidence of saturation in rats based on CO_2 production. Some indirect evidence indicates an absence of saturation of the pathway.

Some studies indicate the CH_2Cl_2 may induce its own metabolism. However, traditional inducers such as phenobarbital and 3-methylcholanthrene do not induce any CH_2Cl_2 metabolism. Glutathione depleters decrease cytosolic metabolism, but tradition P450 inhibitors, such as SKF 525-A, do not inhibit P450 metabolism of CH_2Cl_2. Ethanol inhibits formation of carboxyhemoglobin from CH_2Cl_2. Consequently, the basic metabolic pathways have been established, but complex interactions within the pathways of metabolism of CH_2Cl_2 remain unclear due to unexpected (yet consistent) results obtained in induction and inhibition experiments.

3.0 ACUTE TOXICITY

3.1 Human Health Effects

Limited human data are available on the effects of acute exposures to CH_2Cl_2 vapors. A number of case reports have implicated exposures to exceedingly high levels of CH_2Cl_2 as one factor in human fatalities (Moskowitz and Shapiro 1952, Stewart and Hake 1976). The concentrations and durations of such exposures are not known and thus one cannot derive a dose-response relationship. Miller et al. (1985) reported a case of acute tubular necrosis in a 19-year-old male exposed to methylene chloride for two days in poorly ventilated rooms. Exposure estimates were not made. Acute renal failure, myoglobinuria, hypocomplementemia and elevations of liver enzymes were observed. The patient reportedly recovered after two weeks of hospitalization. Buie et al. (1986) described a case of diffuse pulmonary edema and pleural effusions in a 34-year-old male using furniture stripper containing methylene chloride. Horowitz (1986) reported a case of carboxyhemoglobinemia in a 35-year-old male who was a recurrent paint sniffer.

Experimental studies on human exposure to high concentrations of CH_2Cl_2 suggest that it depresses the central nervous system and alters behavior. Stewart et al. (1972b) reported that humans developed symptoms of "lightheadedness" and had difficulty with speech articulation following exposure to CH_2Cl_2 concentrations greater than 868 ppm for more than 1 hour. Subsequent experiments measured a decreased ability of human subjects exposed to concentrations greater than 300 ppm to perform a series of tasks, including

hand-eye coordination and auditory signal detection (Winneke 1974, Winneke and Fodor 1976, Fodor and Winneke 1971, Putz et al. 1976). Experimental and occupational exposure studies do not provide evidence that CH_2Cl_2 affects other organ systems following inhalation (Stewart et al. 1972 a,b, Gamberale et al. 1975, Ott et al. 1983 a,c,d). In comparison to other species, humans appear to be at least as sensitive, if not more sensitive, to the acutely toxic effects of CH_2Cl_2.

3.2 Animal Studies

Median lethal concentrations (LC_{50} values) for rats and guinea pigs exposed for 6 hours to CH_2Cl_2 vapors are approximately 16,000-18,000 ppm (Berger and Fodor 1968) and 11,500 ppm (Balmer et al. 1976), respectively. The LC_{50} values for mice exposed for 6 and 7 hours were 14,100 ppm (Gradiski et al. 1974) and 16,100 ppm (Svirbely et al. 1974), respectively. An LC_{50} value of 17,703 ppm was estimated for male B6C3F1 mice exposed for 4 hours (NTP 1986). A 30-minute LC_{50} for rats was reported to be approximately 25,400 ppm (Bakhishiev 1975).

Studies involving short-term exposures of experimental animals to CH_2Cl_2 vapors indicate that the central nervous system is the primary target organ. The nervous system appears to be affected at dose levels as low as 500 ppm.

Fodor and Winneke (1971) investigated the effects of CH_2Cl_2 inhalation on rapid eye movement (REM) sleep, sleeping time, and EEG patterns in rats during continuous sleep period exposure (approximately 13 hours). A concentration-dependent continuum of effects was observed. The duration of REM sleep was

reduced beginning at approximately 500 ppm. At 3,000 and 9,000 ppm, sleeping time increased, with a further decrease in REM sleep (Berger and Fodor 1968). Higher concentrations (27,000 ppm for 1.5 hours or 17,000 ppm for 6 hours) resulted in coma and subsequent brain death (Berger and Fodor 1968). Other investigators have reported that high airborne concentrations of CH_2Cl_2 (>1000 ppm) depress the nervous system and alter the behavior of laboratory animals (NTP 1986, Heppel and Neal 1944, Thomas et al. 1971, Weinstein et al. 1972).

Exposures to high levels of CH_2Cl_2 vapor also produce adverse effects on the liver, eyes, respiratory system, and cardiovascular system. Changes in cardiovascular function in rabbits and dogs (e.g., cardiac output and stroke volume) have been reported following short-term exposures ($\geq$ 5 minutes) to concentrations of CH_2Cl_2 exceeding 5,000 ppm (Taylor et al. 1976, Aviado et al. 1977). Short-term exposure (for 5 minutes or less) of animals to more than 5,000 ppm CH_2Cl_2 caused cardiac arrhythmias (Aviado 1975, Aviado et al. 1977, Zakhari 1977).

Hepatic effects (e.g., increased liver triglycerides, changes in liver weight, fatty infiltration) have been reported in guinea pigs and mice following continuous inhalation exposure to approximately 5,000 ppm for 24 hours (Morris et al. 1979, Weinstein et al. 1972).

The reaction product of methylene chloride with GSH seems to be implicated as the causative factor for acute toxicity in mice. Chellman et al. (1986) showed that depletion of glutathione with GSH inhibitors diminished acute liver toxicity as well as kidney and central nervous system toxicity that would have been expected from a single 6-hour exposure to 1500 ppm methylene

chloride. Pretreatment with GSH inhibitors also increased the LC_{50} from 2200 to 3200 ppm in male mice. Berger and Sozeri (1987) examined the effects of methylene chloride and other halogenated hydrocarbons on isolated rat hepatocytes. Methylene chloride (20 mM 1-hour incubation) caused only a very modest increase in the release of an asparate aminotransferase from the hepatocytes, and a reversible depression in cellular oxygen utilization. The effects caused by methylene chloride were much less than those of the more hepatotoxic solvents such as chloroform and carbon tetrachloride (Berger and Sozeri 1987).

Aranyi et al. (1986) examined the effects of inhaled methylene chloride or murine lung host susceptibility to laboratory-induced respiratory infections. A single three-hour exposure to 100 ppm CH_2Cl_2 significantly (p<0.001) reduced pulmonary bacteriocidal activity. It was hypothesized that inhaled methylene chloride can prolong bacterial viability in the lung and enhance severeness of disease, in part because of reduced macrophage function (Aranyi et al., 1986).

3.3 Summary and Conclusions

Methylene chloride has a relatively low acute toxicity. The LC_{50} for mice, rats, and guinea pigs is greater than 10,000 ppm for 7-hour exposures. Animal and human studies suggest that the CNS is the principal target organ resulting in CNS depression.

Human studies have shown a decrease in hand-eye coordination from exposures to 300 ppm for up to 3 hours, while animal studies show a decrease in sleep as measured by EEG and rapid eye movement from a continuous 500 ppm exposure of

CH_2Cl_2 beginning at approximately 50 to 100 minutes in the sleep cycle. Because of these diverse endpoints, it is not possible to precisely define a toxicological threshold. However, based on the observed concentrations for these effects, no adverse effects are expected from ambient CH_2Cl_2 concentrations.

4.0 SUBCHRONIC AND CHRONIC TOXICITY

4.1 Effects on Humans

There have been relatively few reports of subchronic or chronic toxic effects resulting from exposure to CH_2Cl_2. It is often unclear whether the reported toxicities were due to the direct action of CH_2Cl_2, to the action of one or more of its major metabolites or to a combination of these.

In a series of studies designed to evaluate the effects of inhaling CH_2Cl_2 on carboxyhemoglobin levels in the blood of normal volunteers, Stewart and colleagues (Hake et al. 1974, Forster et al. 1974) and Peterson (1978) found carboxyhemoglobin levels ranging from control levels of 0.9% (males) and 1.4% (females) to 9.6% (males) and 10.1% (females) in subjects exposed to 250 ppm (869 mg/m³) for 5 successive days. No other adverse effects were noted in a summary of the studies (EPA 1985a).

Several case reports of toxicity resulting from occupational exposure to CH_2Cl_2 exist. However, concurrent exposure to other chemicals make interpretation difficult. Reported effects included encephalopathy, eclampsia, and bilateral temporal lobe degeneration (Weiss 1967, Collier 1936, Barrowcliff 1978, Barrowcliff and Knell 1979).

Ott et al. (1983a,c) studied the mortality experience and did clinical laboratory evaluations of employees occupationally exposed to CH_2Cl_2. No mortality effects were attributed to time-weighted exposures of 60-475 ppm

(208-1650 mg/m^3). However, these studies had a low statistical power and suffered from several significant confounding factors. The mortality data are discussed in Section 8.2.2.

Ott et al. (1983a,c) studied six serum constituents that had the potential to detect possible liver injury in relation to CH_2Cl_2 exposures. The exposed group had 313 individuals; the reference group had 321. A dose-related rise in serum bilirubin was observed for both men and women. A consistent positive association between total bilirubin and CH_2Cl_2 exposure was found in three of four subgroups by exposure; however, the relationship of this endpoint to liver damage is unclear. Women in the subgroup exposed to 475 ppm (1650 mg/m^3) CH_2Cl_2 showed an increase in red cell counts, hemoglobin, and hematocrit, but men did not. These findings are suggestive of a hematopoietic effect. The carboxyhemoglobin concentration showed an increase and a possible association with CH_2Cl_2 exposure for all four subgroup exposures. Two standard liver function tests were not performed (SGOT, SGPT), and these might have proved to be more sensitive indicators of liver function.

4.2 Effects on Experimental Animals

Subchronic and chronic toxicity reported in animals have primarily involved weight loss and effects on the central nervous system, lungs, liver and kidneys. Many reports are only briefly summarized in this section because of the poor quality of the studies in general; more complete descriptions of chronic toxic effects reported in well-controlled, long-term studies are included in Section 8.3 and Appendix B, which report the results of rodent carcinogenicity studies.

4.2.1 Subchronic Exposures

Most of the data are qualitative and deal with neurobehavioral responses of various species to CH_2Cl_2. Based upon the observations reported, it is reasonable to assume that CH_2Cl_2 behaves similarly to other anesthetics (causing an initial excitatory phase followed by a progressive depression) (EPA 1985a, Heppel et al. 1944, Heppel and Neal 1944, Weinstein et al. 1972, Thomas et al. 1972, Savolainen et al. 1977). The lower threshold for excitatory central nervous system responses for subchronic exposures may be around 1,000 ppm (3,474 mg/m³).

Kim and Carlson (1986) exposed both rats and mice to 200, 500 or 1000 ppm CH_2Cl_2 for either eight hours/day for five days or ten hours per day for four days. Carboxyhemoglobin levels were not significantly higher for the animals exposed for ten hours daily than for those exposed eight hours daily, although for both groups the levels were significantly greater than those of controls. The peak blood levels of CH_2Cl_2 were dependent on the duration of exposure, but the half-life was independent of both exposure duration and concentration. In extrapolating their findings to the human work place, the authors concluded "that the exposure limit for a chemical with a short biological half-life and readily reversible toxic effect may not need to be adjusted for a longer workshift" (Kim and Carlson 1986).

A three-month continuous exposure to 210 ppm CH_2Cl_2 was found to reduce DNA concentrations in the hippocampus of gerbil brains (Karlsson et al. 1987). No changes in protein concentrations in different brain regions were noted. The significance of these effects are not known. An earlier study had shown an

increase in concentrations of the astroglial proteins S-100 and GFA in the frontal and sensory motor cerebral cortex of gerbils following a ten-week continuous exposure to 350 ppm of CH_2Cl_2 (Rosengren et al. 1986).

The NTP (1986) 13-week study exposed groups of 10 rats and 10 mice of each sex to five concentrations of CH_2Cl_2 (525, 1050, 2100, 4200 and 8400 ppm) for 6 hours per day, 5 days per week. This study found that rats developed foreign body pneumonia (foci of mononuclear and multinucleated giant cells), although lung lesions were not reported in mice. Mortality of 10% in male and female rats (1/10) and depression of final mean body weight were also noted at the highest exposure level (8,400 ppm); mice exposed to the same concentration had 40% (4/10) mortality in males and 20% (2/10) in females. Final mean body weight of female mice at this exposure level was depressed compared to controls.

4.2.2 Chronic Exposures

The liver is a primary target organ of animals chronically exposed to high levels of CH_2Cl_2. Histopathological changes have been reported in the liver of animals chronically exposed to high levels of CH_2Cl_2. These changes may be related to deaths observed in various studies. For example, Heppel et al. (1944) reported moderate centrilobular congestion and fatty degeneration of the liver in dogs and guinea pigs exposed to 10,000 ppm (34,740 mg/m^3) for 4 hours per day, 5 days per week, for 8 weeks. Identical findings were reported by Weinstein et al. (1972) after mice were exposed to 5,000 ppm (17,370 mg/m^3) continuously for 7 days. At lower exposure levels, Weinstein and Diamond (1972) found elevated triglycerides, centrilobular fat accumulation, and

decreased liver glycogen in ICR mice exposed continuously from 3 days to 10 weeks to 100 ppm (347 mg/m³) CH_2Cl_2.

Haun et al. (1972) continuously exposed mice, rats, dogs, and monkeys to 25 or 100 ppm (87 or 347 mg/m³) CH_2Cl_2 for up to 100 days. Exposure to 100 ppm resulted in fatty change in the liver and, in rats, nonspecific tubular degeneration in the kidney with evidence of regeneration. No adverse effects were observed at 25 ppm. Other hepatic effects were noted in exposed rats and mice by Norpoth et al. (1974) and the NTP (1986).

Serota et al. (1986a) exposed male and female Fisher 344 rats for 104 weeks to CH_2Cl_2 in their drinking water at concentrations of 5, 50, and 125 mg/kg/day. They observed a positive dose-related trend in both sexes for liver histomorphological liver alterations (foci and area cellular alterations) and fatty changes in both sexes at levels of CH_2Cl_2 as low as 50 mg/kg/day. The authors suggested a no-observable-effect level (NOEL) for these effects at an oral dose level of 5 mg/kg/day.

4.2.3 Summary and Conclusions

Chronic and subchronic effects observed in humans and animals occurred at very high exposure levels. On the basis of the Ott et al. (1983a-e) epidemiological data, the EPA (1983) estimated that chronic inhalation exposures of up to about 100 ppm (347 mg/m³) may represent a no-observed-effect level (NOEL) for humans. However, this estimate was made in the absence of a frank-effect adverse-effect level for mortality. The most

sensitive animal studies (Serota et al. 1986a) suggest an oral NOEL of 5 mg/kg/day. Assuming that the toxic effect is independent of exposure route, a NOEL for humans is 5 ppm. Since these concentrations are 3 to 4 orders of magnitude greater than those present in ambient air, the adverse health effects discussed in this chapter are not expected from current exposure levels.

5.0 REPRODUCTIVE EFFECTS

The potential reproductive toxicity of CH_2Cl_2 has not been extensively evaluated. In this section, developmental toxicity will be considered along with adverse effects on embryo development as a result of exposure before conception. Reproductive toxicity has not been reported for adult males or females.

Schwetz et al. (1975) exposed pregnant Swiss-Webster mice and Sprague-Dawley rats to 1,250 ppm CH_2Cl_2 (97.9% pure) by inhalation for 7 hours per day on days 6 through 15 of gestation. In both species there was a significant increase in liver weight and in carboxyhemoglobin content in exposed dams. Exposed mice exhibited a significant increase in body weight. The only statistically significant adverse effect on the mouse fetuses was on the number of litters in which fetuses exhibited an extra center of ossification in the sternum. This common anomaly may reflect the degree of embryonic development. Rat fetuses had significantly increased incidences of delayed ossification of lumbar ribs or spurs, of delayed ossification of sternebra, and of dilated renal pelvis, which may represent a slight, reversible delay in development. Due to the presence of maternal toxicity and the use of only one dose level of CH_2Cl_2, firm conclusions regarding the adverse effects of CH_2Cl_2 on fetal development cannot be made.

Hardin and Manson (1980) exposed female Long-Evans rats to 4,500 ppm CH_2Cl_2 (97% pure) by inhalation for 6 hours per day, 7 days per week, both before and during gestation. Increased liver weights and carboxyhemoglobin levels were

observed in the mothers, while fetuses exhibited lower body weights relative to controls. No other significant effects were observed. One or two individual animals from 10 of the 20 litters used in this study were evaluated for behavioral effects (Bornschein et al. 1980). Treatment-related effects were reported for the general activity tests. However, because of the small number of animals used, the presence of maternal toxicity, and the developing nature of the behavioral toxicology field, these results are inconclusive.

Nitschke et al. (1985) exposed male and female Fischer 344 rats to 0, 100, 500, or 1500 ppm CH_2Cl_2 for 6 hours/day, 5 days/week for 14 weeks. Male and female rats (F_0) from the same exposure group were mated, and they produced F_1 offspring. Selected F_1 rats were exposed to the same concentrations of CH_2Cl_2 for 17 weeks, at which point they were mated, and they gave birth to the F_2 offspring. No adverse effects on reproductive parameters, neonatal survival, or neonatal growth were noted in animals exposed to CH_2Cl_2 in either the F_0 or F_1 generations. Similarly, there were no treatment-related gross pathologic observations in F_0 and F_1 adults or in F_1 and F_2 weanlings at necropsy. Histopathologic examination of tissues from F_1 and F_2 weanlings did not reveal any lesions attributed to methylene chloride. These findings are consistent with those of Bornmann and Loeser (1967), who reported that ingestion of CH_2Cl_2 in the drinking water did not affect the reproductive performance of female rats. Thirty female Wistar rats were given 125 ppm CH_2Cl_2 in their drinking water for 3 months. An equal number of control rats were given drinking water alone. The estrous cycles of the females, evaluated by vaginal smear tests, were unaffected by treatment.

Fertility, litter size, neonatal growth and survival were evaluated in a two-generational study of F344 rats exposed to methylene chloride during the gestation period (Nitschke et al. 1988b). Maternal and paternal exposure to 0, 100, 500, or 1500 ppm CH_2Cl_2 (six hours/day, five days/week for fourteen weeks) caused no adverse reproductive or neonatal effects in either the F_1 or F_2 generation; histopathologic examination did not reveal any lesions attributable to CH_2Cl_2. The exposure to levels as high as 1500 ppm did not appear to significantly effect reproductive parameters in mice (Nitschke et al. 1988b).

Based on the available information, inhalation of high, maternally toxic concentrations (1250-4500 ppm) by pregnant rodents throughout pregnancy can result in fetotoxicity as evidenced by reduced fetal body weight and changes in ossification of the fetal skeleton. The results to date indicate that CH_2Cl_2 is of low teratogenic potential in rodents, but no firm conclusions as to the potential human health impact of CH_2Cl_2 exposure can be drawn. Experiments performed to date have been limited in that, except for rat studies, either they employed only one dose level, utilized relatively few numbers of test animals, or demonstrated signs of maternal toxicity possibly as a result of carboxyhemoglobin formation.

An epidemiological study conducted among women in the pharmaceutical industry found that with increasing frequency of exposure to CH_2Cl_2, the odds of having a spontaneous abortion were increased, although the statistical significance was borderline (Taskinen et al. 1987). No estimation of the range of exposure concentrations was given.

6.0 GENETIC TOXICITY

6.1 Introduction

CH_2Cl_2 has been tested in a variety of in vitro and in vivo systems designed to detect gene mutations, chromosomal aberrations, and other DNA binding or damage (see Table 6-1). This section summarizes the existing data and discusses what

conclusions may be drawn. Individual studies, with references, are summarized in Appendix A.

6.2 Mutagenicity

CH_2Cl_2 has been tested in bacteria, yeast, Drosophila, Panagrellus, and cultured mammalian cells. CH_2Cl_2 showed a positive, dose-related response in Salmonella strains TA1535, TA98, and TA100, both in the presence and absence of a metabolic activating system, when tested as a vapor in a sealed chamber in several laboratories (see Table A-1 for references). Cytotoxicity data (survival of bacteria) and the purity of the CH_2Cl_2 were not given in most of the published reports. (However, the most common contaminants in the ppm range are phosgene and cyclohexene, neither of which is genotoxic in the Ames test.) The addition of rat liver cytosol to the assay system slightly increased the mutagenicity of CH_2Cl_2 in Salmonella, which is consistent with formation of the electrophilic intermediate S-chloromethylglutathione following enzyme-mediated conjugation by the glutathione-S-transferases (see Section 3) (Green 1983, Jongen et al. 1982). The EPA (1985a) stated that on the basis of data obtained from Dr. Eugene Barber of the Eastman Kodak Company as a personal

communication, the mutagenic responses at the highest dose (115 μmoles/plate) for strains TA1535, TA98, and TA100 were 0.0006, 0.006, and 0.003 revertants/μmole, respectively. These determinations were made on the basis of GC/MS measurements of CH_2Cl_2 concentrations in the bacteria-containing aqueous phase of the petri dish and the vapor-phase head space of the closed incubation chamber, and as such, reflect the actual concentrations to which the bacteria were exposed (Barber et al. 1980). These results indicate that CH_2Cl_2 is a much weaker mutagen in Salmonella than benzo(a)pyrene or 3-(2-chloroethoxy)-1,2-dichloropropene. Benzo(a)pyrene induces 121,000 revertants/μmole in Salmonella (McCann and Ames 1977), and 3-(2-chloroethoxy)-1,2-dichloropropene, a volatile chloroallyl ether, induces 74,400 revertants/μmole, based on the amount incorporated into the assay and not accounting for volatilization (Distlerath et al. 1984).

Methylene chloride has exhibited mutagenic activity in two strains of E. coli, WU361089 (tyr⁻) (tested for mutation to tyrosine prototrophy) and K49, a lamda lysogenic strain (tested for prophage induction). (However, as indicated by the authors, difference in solubility and volality made it difficult to estimate the doses and thus to compare these results to the mutagenicity of other substances [Osterman-Golkar et al. 1983]). CH_2Cl_2 was not mutagenic in the B. subtilis rec assay, with or without the addition of a microsomal enzyme-activating system (Kanada and Uyeta 1978).

CH_2Cl_2 was tested for gene conversion, mitotic recombination, and reverse mutation in two strains of the yeast Saccharomyces cerevisiae without the addition of exogenous enzymes (Callen et al. 1980, Simmon et al. 1977).

TABLE 6-1

SUMMARY OF TESTS OF THE GENETIC TOXICITY OF METHYLENE CHLORIDE

System	Results		
	Positive	Negative	Equivocal
Salmonella typhimurium (vapor exposure)	X		
E. coli tyrosine prototrophy (in desiccator)	X		
B. subtilis rec assay		X	
Saccharomyces cerevisiae			X
Drosophila sex-linked recessive lethal test			X
Mammalian cell mutation (V79, CHO, L5178Y cells)		X	
Mammalian cell transformation (C3H-10T-1/2, BALB/C-3T3, F1706 cells)			X
Syrian hamster embryo (adenovirus-transformed foci (vapor exposure))	X		
Unscheduled DNA synthesis		X	
Mammalian cell cytogenetics Chinese hamster ovary cell (chromatid gaps, chromatid breaks)	X		
Sister chromatid exchange	X		

CH_2Cl_2 had weak effects on mutation and recombination in one strain and no effects in the other. Callen et al. (1980) observed a greater than two-fold dose-related response in Saccharomyces cerevisiae for each of three endpoints, gene conversion, mitotic recombination, and reverse mutation. These yeast strains contain endogenous cytochrome P450 and hence are capable of metabolic activation. The differences in response between the two strains may be attributable to different levels of P450 or different experimental conditions. Extending the incubation period from 1 to 4 hours eliminated the genotoxic activity of CH_2Cl_2 in the one positive strain. Since an extended incubation time usually increases mutagenicity as a result of increased metabolic activation, the positive result may have been artifactual. Alternatively, the longer incubation time may have permitted detoxication and DNA repair.

Two feeding studies and an injection study were conducted with CH_2Cl_2 to detect sex-linked recessive lethal mutations in Drosophila (Abrahamson and Valencia 1980, Gocke et al. 1981). CH_2Cl_2 was negative in the injection study and in one of the feeding studies, but it was weakly mutagenic at very high doses in one of the three broods tested in the other feeding study. Several mammalian cell culture systems have been used to test the mutagenic potential of CH_2Cl_2. Jongen et al. (1981) exposed V79 and Chinese hamster ovary (CHO) cells to both liquid- and vapor-phase CH_2Cl_2 in a sealed container and obtained negative results for the induction of 6-thioguanine resistance over the dose range tested. Thilagar et al. (1984a) reported that CH_2Cl_2 was negative as a mutagen at the thymidine kinase locus of mouse lymphoma L5178Y cells.

6.3 Chromosomal Aberrations

Several studies to evaluate the ability of CH_2Cl_2 to cause chromosomal aberrations have been performed. Sprague-Dawley rats were exposed to CH_2Cl_2 via inhalation (Burek et al. 1984) and NMRI mice were exposed intraperitoneally (Gocke et al. 1981). Chromosomal preparations of bone marrow cells from the rats showed no increase in specific aberrations or in the frequency of abnormal cells. Bone marrow smears from the mice were examined for micronuclei, and at the two highest dose levels an increase in polychromatic erythrocytes was observed. Since the response was not dose-related and did not double the control value, the results are inconclusive although suggestive of a positive response.

Sheldon et al. (1987) also evaluated the ability of CH_2Cl_2 to induce micronuclei in mice. Bone marrow samples were taken 24, 36, 48 and 72 hours after oral dosing with 1250, 2500 or 4000 mg/kg of CH_2Cl_2. No significant increase in the incidence of micronuclei over control values were observed for any of the test groups (Sheldon et al. 1987).

Thilagar and Kumaroo (1983) exposed CHO cells in vitro to CH_2Cl_2 and found a replicable, dose-related positive response for specific aberrations, number of aberrations per cell, and total cells with chromosomal aberrations. A positive response was also reported for human peripheral lymphocytes and mouse lymphoma L5178Y cells in culture (Thilagar et al. 1984b).

6.3.1 Sister Chromatid Exchange

CH_2Cl_2 has been tested at concentrations of 0.5%, 1.0%, 2.0%, 3.0% and 4% with and without S9 for its ability to induce sister chromatid exchange in vitro, and it exhibited a weak positive response in Chinese hamster V79 cells (Jongen et al. 1981) and CHO cells (McCarroll et al. 1983). At lower doses in CHO cells (up to approximately 1.5%), the response was not significant (Thilagar and Kumaroo 1983).

6.3.2 Unscheduled DNA Synthesis

CH_2Cl_2 has been reported to be negative as an inducer of unscheduled DNA synthesis for V79 cells and primary human fibroblasts or lymphocytes with and without metabolic activation (Jongen et al. 1981, Perocco and Prodi 1981); it induced a "marginal positive response" in primary rat hepatocytes (Thilagar et al. 1984a). Oral exposure of rats or inhalational exposure of mice and rats to methylene chloride did not cause any unscheduled DNA synthesis in hepatocytes isolated from exposed animals (Trueman and Ashby 1987). Additional unpublished studies on unscheduled DNA synthesis have been reviewed in the recent draft EPA documents (EPA 1987a,b).

6.3.3 DNA Alkylation

The potential of CH_2Cl_2 to alkylate DNA in vivo has also been evaluated. DNA was isolated from the liver and salivary tissue of rats and hamsters that had been exposed to $^{14}CH_2Cl_2$ via inhalation (Schumann et al. 1983). Some labeling of DNA was detected, but it appeared to be associated only with normal bases

and nucleosides; no alkylated bases or nucleosides were detected above a detection level of <12 and 1 alkylation per 10^6 nucleotides for nucleosides and bases, respectively. Incorporation of ^{14}C may have occurred through normal biosynthetic pathways (one-carbon pool) or by unstable adduct formation. Another study examined the labeling of DNA in mouse and rat liver and lung tissue and allowed for a distinction between alkylation and labeling via the one-carbon pool; no evidence for DNA adduct formation was found (Green et al. 1988). The sensitivity of the assay was reported in terms of a covalent binding index (CBI), which is used as a measure of DNA alkylation in relation to dose (Lutz 1979). The minimum detectable CBI was 0.002 for the liver and 0.01 for the lung for mice. By comparison, the strong aklylating agent dimethylnitrosamine has CBIs of 5500 and 500 for mouse liver and lung DNA, respectively (Lutz 1979). CH_2Cl_2 was reported not to bind covalently to DNA following incubation with isolated rat hepatocytes (Cunningham et al. 1981); however, the limit of detection was not known (Sipes 1987).

Green et al. (1986a, 1988) studied the in vivo interaction of methylene chloride and its metabolites with F344 rats and B6C3F1 mouse lung and liver DNA following a three-hour exposure to 4000 ppm ^{14}C-methylene chloride. The DNA was isolated from the exposed animals either 6, 12 or 24 hours after the start of exposure, and was found to contain radioactivity at all time points. Higher radioactivity levels were obtained in the lung than in the liver, and levels in the mouse were higher than levels in the rat. The radioactivity associated with DNA in lung and liver tissue following methylene chloride exposure was compared to that produced by an intravenous dose of ^{14}C[formate]. Chromatographic comparison of hydrolyzed DNA indicated that the major nucleotide peaks were likely to be the result of radioactive incorporation via

the C-1 pool instead of direct alkylation. However, chromatographic analysis of hydrolyzed DNA from lungs and livers of mice exposed to CH_2Cl_2 exhibited a different radioactivity fingerprint and considerably more radioactive "noise" than that in animals given [14]C[formate]. This study is consistent with other genotoxicity studies indicating the mutagenic potential of CH_2Cl_2 is relatively weak.

6.4 Neoplastic Transformation

Purified, food-grade CH_2Cl_2 did not induce neoplastic transformation in BALB/c-3T3 or C3H-10T-1/2 cells (Sivak 1978, Thilagar et al. 1984a). Reagent-grade CH_2Cl_2, without further purification, was reported to elicit a positive response for transformation using an F1706 cell line (Price et al. 1978). The authors asserted that the positive response for reagent-grade CH_2Cl_2 was presumably due to a contaminant because no positive response was elicited when the purified sample that had been negative in the BALB/c-3T3 system was tested in the F1706 cell line. To be responsible for transformation of the F1706 cells, a contaminant of CH_2Cl_2 would have to be present in large quantities or be a very potent mutagen (i.e., similar to benzo(a)pyrene). DHS staff members have been told in telephone conversations with chemical supply companies that reagent-grade methylene chloride is 99% to 99.9% pure; the two primary contaminants are phosgene and cyclohexene neither of which is a potent mutagen. It should be noted that since reagent grade CH_2Cl_2 is as pure or purer than the high volume chemical used primarily in industrial operations, human exposures are likely to include exposure to any contaminants as well as CH_2Cl_2.

Roghani et al. (1987) demonstrated that CH_2Cl_2 as well as chloroform and carbon tetrachloride are potent activators of protein kinase C. This phospholipid-dependent kinase has a pivotal role in the transduction of various effectors triggering cell activation by polyphosphoinositide turnover and has been implicated in the tumor-promoting activity of phorbol esters (Sukla and Albra 1987, Castagna et al. 1982).

6.5 Conclusions

CH_2Cl_2 is weakly mutagenic in Salmonella, Saccharomyces, and E. coli using a variety of endpoints. In contrast, mutagenic activity has not been definitively demonstrated in mammalian systems. There is evidence that exposure of mammalian cells to CH_2Cl_2 in vitro can result in chromosomal aberrations and DNA damage. However, the evidence for DNA adduct formation in mammalian tissues is scant. In summary, methylene chloride is a mutagen in prokaryotes (Salmonella, E. coli) and lower eukaryotes (yeast). In mammalian cells, CH_2Cl_2 exhibits clastogenic (chromosome damaging) activity. Thus, methylene chloride should be classified as genotoxic.

7.0 CARCINOGENICITY

7.1 Introduction

This chapter reviews the epidemiological and experimental evaluations of the carcinogenicity of CH_2Cl_2. Two cohorts of workers exposed to CH_2Cl_2 have been studied, and several studies of rodents exposed orally or via inhalation have been reported. The animal studies are summarized in this section and discussed in greater detail in Appendices B and C.

7.2 Epidemiological Studies

7.2.1 Kodak Employees

Friedlander and colleagues studied male workers employed by the Eastman Kodak Company at its film-making operation in Rochester, New York, where CH_2Cl_2 was used as the primary solvent (Friedlander et al. 1978, Hearne and Friedlander 1981, Friedlander et al. 1985 [a report issued by the Eastman Kodak Company], Friedlander et al. 1986 [a presentation at a toxicology forum], Hearne et al. 1987). Industrial hygiene surveys conducted there had found airborne levels of methylene chloride in the workroom ranging, in general, from 30 to 100 ppm. As control groups, the researchers considered (1) males in New York State not including New York City (this area was called "upstate New York" although it includes "downstate" areas such as Nassau and Suffolk Counties on Long Island) and (2) other males employed by Kodak in Rochester. Questionnaire data indicated that the prevalence tobacco use in the exposed cohort was about as extensive as in the control groups; however, the investigators did not note whether other relevant

variables, such as average duration of cigarette smoking and the number of cigarettes smoked per day, were similar (Hearne et al. 1987).

The major findings are contained in the papers of Friedlander et al. (1985) and Hearne et al. (1987), both of which are described below. In both reports, the investigators employed two tests to determine whether observed-expected differences were statistically significant: (1) a one-tailed test of significance at p<0.05 for outcomes that the investigators hypothesized might be associated with methylene chloride (such as lung and liver cancer and ischemic heart disease) and (2) a two-tailed test of significance at p≤0.01 for nonhypothesized outcomes (including pancreatic cancer).

The 1985 report from Friedlander and colleagues noted that overall, significantly fewer deaths occurred during the follow-up period (January 1964 thru December 1984) than expected based on the New York State data (165 vs. 231.1) (Friedlander et al. 1985). However, the number of deaths among exposed workers was similar to that of the other Kodak employees (165 vs. 167.0). Thirty-nine deaths from malignant neoplasms were observed in the exposed cohort compared to 54.7 expected based on the New York State data or 43.2 expected on the basis of the data from other Kodak employees. The follow-up rate for this report was 94% for the exposed cohort (N=751). The above findings are consistent with the "healthy worker effect," in which working populations tend to experience lower mortality than the general population.

Using the statistical tests employed by the investigators, no cancer site displayed a significantly elevated death rate. Nevertheless, eight deaths from pancreatic cancer were observed compared to 3.0 expected based on the New

York data or 2.6 based on the other employees' data. Although this finding was not considered significant based on the two-tailed test described above, an exact one-sided Poisson test using the other Kodak employees as the control group yields a p-value of 0.0053 and suggests a possible relationship between exposure to CH_2Cl_2 and pancreatic cancer mortality. Somewhat smaller-than-expected death rates were observed for certain tumor sites, including the colon, and the genital and urinary organs; these observations did not reach statistical significance.

In the subset of the cohort which had been exposed for a minimum of 20 years by 1964 (N=252), fewer cancer deaths were observed than expected when compared to the New York State data (23 vs. 33.8) or to the data from other Kodak employees (23 vs. 27.0). A slight excess of deaths from pancreatic neoplasms was observed here (4 compared to 1.9 or 1.6 expected). None of these differences were statistically significant.

This report noted that the study had insufficient power to address mortality from rare disorders such as liver or male breast cancer. Assuming comparable smoking characteristics, the study had 97% power to detect a relative risk of 2.0 for lung cancer in the entire exposed cohort and 85% power in the 252-man subset with the upstate New York controls. With the Kodak employee controls, the corresponding power figures were 91% and 74%. To detect a relative risk of 1.5, the power figures were 62% or 41% with the upstate New York controls and 50% or 33% with the Kodak employee controls).

In the most recent report (Hearne et al. 1987), the investigators presented estimates of exposure and expanded the exposed cohort to 1,013 men who had at

least one year of experience in the methylene chloride operation between January 1964 and December 1970. The 1985 report analysed approximately 14,000 person-years of follow-up in the exposed cohort; this 1987 report presented data from 19,465 person-years, with a follow-up rate of 99% for the exposed cohort. Again, compared to either control group, no statistically significant difference was found between observed and expected deaths for respiratory or hepatic cancer mortality, based on a one-sided test, $p<0.05$. Among the nonhypothesized outcomes, workers exposed to CH_2Cl_2 still experienced more than a two-fold greater rate of mortality from pancreatic cancer (8 observed vs. 3.2 or 3.1 expected [New York State or Kodak controls]). The SMR for this site was 2.5 with 95% confidence limits of 1.1 to 4.9. DHS staff calculations indicate an exact one-sided Poisson test using the New York State data as a control yields a p-value of 0.017. Thus, the results still suggest a possible relationship between exposure to CH_2Cl_2 and pancreatic cancer mortality. The number of deaths remained somewhat smaller than expected for certain malignancies, including those of the large intestine (2 observed vs. 8.0 or 6.9 expected) and the genitourinary organs (3 observed vs. 8.0 or 7.6 expected). Overall cancer mortality in the exposed cohort was still low (41 observed vs. 59.3 or 52.7 expected). None of these differences was statistically significant at the 1% level ($p>0.01$) based on a two-tailed test.

Exposure estimates were based on over 1200 area and task-specific air samples collected between 1945 and 1986 and more than 900 full-shift personal samples collected between 1980 and 1986. Longstanding job progression patterns facilitated estimation of career methylene chloride exposure. The bulk of the cohort was exposed to eight-hour time-weighted average (TWA) CH_2Cl_2 concentrations averaging 10-100 ppm. Over 150 members of the cohort were

exposed to peak concentrations of 10,000 ppm an average of three times per day, 190 days per year. The majority of the cohort was exposed to peak concentrations of 500 ppm, an average of three times per day, 10 or 40 days per year (depending on occupational classification). Other solvents, including 1,2-dichloropropane and 1,2-dichloroethane were present at lower levels in the workroom. The sampling results, in combination with information on the amount of time each employee spent in each job, were used to develop a career exposure index in ppm-years. For the analysis, nine subgroups were created corresponding to three categories each of exposure (<350, 350-749 and >750 ppm-years) and latency, the time between initial exposure and the end of follow-up (<20, 20-29 and >30 years). Each person-year of observation for each worker was assigned to one of the nine cells (subgroups). The researchers found no evidence of a dose-response relationship according to career exposure, latency or both indexes for any of the hypothesized outcomes or total cancer mortality.

Hearne and colleagues also compared their observations of lung and liver cancer mortality with risk estimates based on the NTP (1986) animal bioassay. They estimated excess risk for the three career exposure categories using a unit risk estimate (0.014 for lifetime exposure to 1 ppm CH_2Cl_2) derived from the EPA (1985b) risk assessment. They then presented an animal-based prediction of cancer mortality for lung and liver (36.3, an excess of 14.5 over the New York State control rate) and noted that the observed 14 cases (all lung cancer) were significantly lower than the animal-based predictions. The investigators concluded that the projections based on animal data were "clearly inconsistent" with their observations (Hearne et al. 1987). Using data provided by Friedlander (personal communication), DHS staff members

concur with other researchers in finding such a conclusion is not warranted (Mirer et al., 1988, Tollefson et al., 1988). (See Appendix D).

Dr. Hearne has reported (November 1988) to DHS staff that preliminary findings of an update of mortality results in the Kodak cohort for an additional three years (through 1987) are consistent with the earlier data indicating fewer total cancer deaths than expected among exposed workers. Furthermore, there was no increase in pancreatic cancer deaths in the update, however, in these three years, 0.6 pancreatic cancer deaths were expected.

7.2.2 <u>Employees at a Fiber Production Plant</u>. In a series of reports, Ott and colleagues (Ott et al. 1983a-e) evaluated the health of employees working at a fiber production plant in Rock Hill, South Carolina, where CH_2Cl_2 was used as part of a solvent system. Workers in this plant were exposed to a mixture of methylene chloride and methanol from one process and to acetone from a second process. Median 8-hr TWA CH_2Cl_2 concentrations in the plant's two main work areas were 140 and 475 ppm. Health evaluations of these employees were compared to those of employees at a similar fiber production plant in Narrows, Virginia, where only acetone was used. One of the reports (Ott et al. 1983b) evaluated whether exposure to CH_2Cl_2 was associated with cancer deaths. Smoking habits were not considered in the analyses, although age was controlled for.

The target population was production and service employees who had worked for at least three months in the preparation or extrusion areas of either plant between January 1, 1954, and December 31, 1976. Mortality experience within the two cohorts was followed through June 1977. Few nonwhites were in either

cohort, so many of the analyses were restricted to the whites. The cohorts were characterized as follows:

COHORT	YEAR OF FIRST EXPOSURE[1]			YEARS OF EXPOSURE[1]			
	1954-59	1960-69	1970-76	<1	1-4	5-9	≥10
Exposed (N=1271)	45%	31%	24%	17%	39%	16%	28%
Unexposed (N=948)	36%	38%	26%	15%	32%	14%	39%

[1]For the unexposed cohort, "exposure" refers to work in the reference plant.

Compared with United States death rates, no excess mortality from any cause in either cohort was evident. A slight deficit in the observed deaths from cancer was noted among exposed white workers (7 observed vs. 12.4 expected). The cancer mortality experience of the methylene chloride-exposed workers was not significantly different from that of the reference cohort. One death from pancreatic cancer was reported in the exposed cohort. Of the seven cancer deaths in the reference cohort, one was from pancreatic cancer (Bond 1988).

Statistically significant risk ratios (RRs) for mortality from all causes (RR=2.2), circulatory diseases (RR=2.2), and external causes (including accidents and suicides, RR=2.5) were found among white men when comparing the two cohorts.

This study is of somewhat limited usefulness for evaluating the association between exposure to methylene chloride and cancer mortality. It had limited power to detect increases in malignancy rates in the exposed cohort. In that cohort, only 54 deaths, of which 7 were from cancer, were reported. There were no deaths observed among the 108 nonwhite women in the study. In addition, the follow-up period was probably insufficient for any carcinogenic effects from CH_2Cl_2 exposures to be manifested.

7.2.3 Conclusions Based on Epidemiological Studies

International Agency for Research on Cancer (1986) has concluded that no excess risk of death from malignancies was observed in these epidemiological studies, but noted that the studies had a limited power to detect excess risk. To date, epidemiological studies on methylene chloride do not provide sufficient evidence either to prove or disprove human carcinogencity.

7.3 Summary of Chronic Toxicity/Oncogenicity Studies in Rodents

The effects of long-term (2-year) exposure to methylene chloride by inhalation have been studied in rats, mice, and Syrian hamsters, and by ingestion of drinking water in rats and mice. These studies are evaluated in detail in Appendix B, and the results are summarized in this section. In Appendix C, the biological relevance of the exposure-related neoplasms is discussed.

There have been five long-term rodent bioassays examining the effects of inhaling CH_2Cl_2: two using an outbred stock of Spartan/Sprague-Dawley (SD) rats (Burek et al. 1984, EPA 1985a, Nitschke et al. 1988a), one with inbred Fischer 344 (F344) rats (NTP 1986), one using hybrid B6C3Fl mice (NTP 1986), and one with Ela:Eng (Syr) Syrian hamsters (Burek et al. 1984). The NTP (1986) studies have also been published by Mennear et al. 1988. Bioassays exposing animals orally to CH_2Cl_2 in drinking water were conducted in F344 rats and B6C3Fl mice (Serota et al. 1986a, 1986b). The experimental designs of these bioassays and the results obtained are summarized in Table 7-1. The NTP also conducted a bioassay in which CH_2Cl_2 was administered by gavage in

TABLE 7·1

SUMMARY OF CHRONIC TOXICITY/ONCOGENICITY STUDIES OF METHYLENE CHLORIDE IN RODENTS

Species/Strain	Sex	No. in Group	Route of Exposure	Duration of Exposure	Exposure Levels	Results	Reference
Rat/Spartan Sprague Dawley	M,F	129 (95 chronic; 34 interim sac. & ancillary studies)	Inhalation	6 hr/day, 5 day/wk, for 2 yr	0, 500, 1,500, 3,500 ppm	**Neoplasms**: Sarcomas of the salivary gland region in males. Increase in benign mammary tumors per tumor-bearing rat (both sexes). **Nonneoplastic lesions**: Hepatocellular vacuolization in males and females. Multinucleated hepatocytes in females; increased number of foci and areas of hepatocellular alteration in high dose females.	Burek et al. 1984, EPA 1985a (Dow 1980)
Hamster/ Ela:Eng (Syr)	M,F	107-109 (95 chronic; rest for interim sac. & ancillary studies)	Inhalation	6 hr/day, 5 day/wk, for 2 yr	0, 500, 1,500, 3,500 ppm	No exposure-related neoplasms or nonneoplastic lesions.	Burek et al. 1984, EPA 1985a (Dow 1980)
Rat/Spartan Sprague Dawley	M,F	90 (70 chronic) 500/0[c]:25F 0/500[c]:25F	Inhalation	6 hr/day, 5 day/wk, for 20 (males) or 24 (females) months	0, 50, 200, 500 ppm	**Neoplasms**: Some increase in benign mammary tumors in tumor-bearing females. **Nonneoplastic lesions**: Hepatocellular vacuolization and multinucleated hepatocytes in females.	EPA 1985a (Dow 1982) (Nitschke et al. 1988a)
Rat/F344	M,F	85 C1[a] and treated; 50 C2[a] and 25 high dose recovery group	Oral/ drinking water	7 day/wk, for 2 yr; recovery group-- 78-wk treatment & 2-wk recovery	0, 5, 50, 125, 250 mkd[b]	**Neoplasms**: The EPA's Carcinogen Assessment Group reported borderline increase in liver tumors (combined neoplastic nodule and hepatocellular carcinomas) in females. Serota et al. considered this response not significant because incidence was within the laboratory's historical control range. **Nonneoplastic lesions**: Hepatocellular fatty change and foci of cellular alteration in males and females.	Serota (et al. 1986a)

TABLE 7·1 (continued)

Species/Strain	Sex	No. in Group	Route of Exposure	Duration of Exposure	Exposure Levels	Results	Reference
Mouse/B6C3F1	M	C1:60 C2:65 60 mkd :200 125 " :100 185 " :100 250 " :125	Oral/ drinking water	7 day/wk for 2 yr	0,60,125, 185,250 mkd	Neoplasms: Increased incidence of liver tumors (combined hepatocellular adenoma and carcinoma) in males at 125 and 185 mkd and borderline increase at 250 mkd compared to combined control groups. Nonneoplastic lesions: Hepatocellular fatty change in high dose males and females.	Serota et al. (1986b)
	F	50/group					
Rat/F344	M,F	50	Inhala-tion	6 hr/day, 5 day/wk, for 102 wk	0, 1,000, 2,000, 4,000 ppm	Neoplasms: Treatment-related increase in benign mammary tumors (fibroadenoma, adenoma, or fibroma in males; fibroadenoma or adenoma in females). Nonneoplastic lesions: Treatment - but not dose - related increase in hepatocytomegaly, cytoplasmic vacuolization, hemosiderosis,and bile duct fibrosis in both sexes.	NTP 1986 (Mennear et al. 1988)
Mouse/B6C3F1	M,F	50	Inhala-tion	6 hr/day, 5 day/wk, for 102 wk	0, 2,000, 4,000 ppm	Neoplasms: Dose-related increase in incidence and multiplicity of alveolar-bronchiolar lung tumors (adenoma and carcinoma) in both sexes. Elevated incidence and multiplicity of liver tumors (hepatocellular carcinoma or combined hepatocellular adenoma and carcinoma) in high dose males. Dose-related increase in incidence of hepatocellular adenoma and carcinoma and in multiplicity of liver tumors in females. Nonneoplastic lesions: Liver cytologic degeneration in both sexes. Atrophic and degenerative lesions in reproductive organs considered secondary to severe lung and liver cancer.	NTP 1986 (Mennear et al. 1988)

[a] C1 = control 1 group; C2 = control 2 group.
[b] mkd = mg/kg/day.
[c] Additional groups: 500/0 = 500 ppm for 12 months, air for 12 months; 0/500 = no exposure to methylene chloride for 12 months, then 500 ppm for 12 months.

corn oil to F344 rats and B6C3F1 mice. On July 23, 1983, the NTP announced that the results of these bioassays would not be published in final form. The NTP's decision was "based on some recently discovered significant discrepancies in experimental data that compromise a clear interpretation" (Fed. Reg. 1983). The NTP did not cite this study in its technical report (NTP 1986) when summarizing the results of two-year studies on CH_2Cl_2, however, it is expected that NTP will release the final results without a statistical evaluation.

7.3.1 Rats In the Dow (1980) study, male and female Sprague-Dawley rats were exposed to CH_2Cl_2 by inhalation for two years (EPA 1985a, Burek et al. 1984). (For concentrations of CH_2Cl_2 used, exposure durations, and other details of this and the other studies discussed, see Table 7-1.) The most notable increase in tumor incidence was in sarcomas in the salivary gland region of male rats. These tumors did not appear to arise from the salivary gland itself but were thought to involve the salivary gland.

Control and exposed female rats all had a high incidence (above 80%) of benign mammary tumors. However, female rats exhibited a dose-related increase in the number of benign mammary tumors per tumor-bearing animal. The proportion of male rats with mammary tumors increased somewhat at the highest exposure level, but the increase was not statistically significant. (For a more detailed description of the numbers and types of tumors found at various doses for this and the other studies discussed, see Appendix B).

In another 2-year inhalation study of CH_2Cl_2 (Dow 1982, Nitschke et al. 1988a) using male and female Sprague-Dawley rats, a slight increase in the number of

tumor-bearing female rats was observed (EPA 1985a). No other significant effect on tumor incidence was noted. However, the doses were considerably lower than in the first study.

In an NTP inhalation study (NTP 1986, Mennear et al. 1988), Fischer 344 rats of both sexes were exposed to CH_2Cl_2 for 2 years and exhibited a dose-related increase in the number of animals with benign mammary tumors (both sexes), which was statistically significant by life table tests ($p<0.001$) and also by incidental tumor tests in females ($p<0.001$).

A study in which CH_2Cl_2 was administered to F344 rats in drinking water for 2 years was conducted by the National Coffee Association (Serota et al. 1986a). Male rats showed no treatment-related tumor response. There was an increase in liver tumors in female rats compared to concurrent controls ($p<0.05$), particularly for the 50 and 250 mg/kg groups. However, the increase was not significant when compared to historical controls.

7.3.2 <u>Mice</u> Male and female B6C3F1 mice were exposed to CH_2Cl_2 by inhalation for 2 years (NTP 1986, Mennear et al. 1988). Both sexes of mice had statistically significant, dose-related increases in the incidence and multiplicity of adenomas ($p<0.001$) and carcinomas ($p<0.001$) of the lung. However, concurrent controls had lower incidences of such tumors than historical controls. Male mice had an elevated incidence of hepatocellular adenomas ($p<0.05$) and carcinomas of the liver at the high dose ($p=0.02$), while females had dose-related increases of both hepatocellular adenomas ($p<0.001$) and carcinomas ($p<0.001$) of the liver.

B6C3F1 mice were also exposed to CH_2Cl_2 in their drinking water for 2 years in the National Coffee Association study (Serota et al. 1986b). No treatment-related effect on tumor incidence was seen in female mice. Male mice had a slight increase in hepatic tumors of borderline statistical significance. However, the incidences fell within the range of historical controls. Compared to the rats exposed in a similar study (Serota et al. 1986a), mice appear to be as sensitive or less sensitive to the effects of CH_2Cl_2.

7.3.3   Hamsters   An inhalation study in which Syrian golden hamsters were exposed to CH_2Cl_2 for 2 years was conducted by Dow (EPA 1985a, Burek et al. 1984). No exposure-related tumors were reported.

7.3.4   Conclusions   The strongest evidence for the carcinogenicity of CH_2Cl_2 to rodents was provided by the NTP inhalation bioassays. Under the conditions of the study, benign mammary tumors were induced in F344 rats, and the female rats exhibited a dose-related response ($p<0.001$). Further evidence of exposure-related mammary tumorigenesis in female rats was provided by a dose-related increase in the number of benign mammary tumors per tumor-bearing rat in the Dow (1980) inhalation study, although the high spontaneous incidence of mammary tumors in control SD females obscured any dose-related response in the incidence, i.e., in the number of tumor-bearing animals.

Subcutaneous sarcomas of the ventral cervical-salivary gland region occurred in male SD rats exposed by inhalation to 1,500 and 3,500 ppm of CH_2Cl_2 but not in F344 rats exposed to 1,000, 2,000, and 4,000 ppm. In the Dow and the NTP studies, there was evidence that the rats had been infected by sialodacryoadenitis virus and rat-coronavirus based on clinical signs in SD

rats (Burek et al. 1984) and serological results in F344 rats (NTP 1986, Mennear et al. 1988). Burek et al. (1984) suggested that the combination of this viral infection and exposure to high concentrations of CH_2Cl_2 may have been associated with the tumor response in SD rats in the Dow study. However, F344 rats exposed to higher CH_2Cl_2 concentrations and the same viral agent did not develop similar tumors in the salivary gland region.

In B6C3F1 mice, exposure to CH_2Cl_2 by inhalation (NTP 1986, Mennear et al. 1988) was associated with an increased incidence and multiplicity of alveolar and bronchiolar tumors (adenoma and carcinoma) in the lungs of both sexes. The incidence and multiplicity of liver tumors were also increased in both sexes. Male mice had an increased incidence of hepatocellular carcinomas and of adenomas or carcinomas (combined) at the high exposure level, while female mice had dose-related increases in hepatocellular adenoma and hepatocellular carcinoma. The survival in both male and female high dose groups was significantly (p<0.001, trend test) decreased as compared to controls.

Treatment-related increases in the combined incidence of liver tumors, hepatocellular adenomas, and carcinomas occurred in B6C3F1 mice exposed orally (via drinking water) to lower doses (125 and 185 mg/kg/day) of CH_2Cl_2 but were not significant in the highest (250 mg/kg/day) exposure group.

8.0 RISK ASSESSMENT

8.1 Introduction

Qualitative evaluation of the carcinogenicity of an agent is an essential part of the assessment of the hazard posed by that agent. However, to quantify human risk, the observed animal cancer response must be extrapolated from high to low doses and, in some cases, from rodents to humans. Such extrapolations commonly involve the use of mathematical dose-response models.

Several different cancer dose-response models are discussed in this section. To demonstrate how different extrapolation models will affect low-dose risk, the risks from ambient exposures are calculated using both mechanistic and distribution models. Additionally, the Andersen et al. (1987) physiologically based pharmacokinetic (PBPK) model is used to estimate the delivered dose of CH_2Cl_2 to the mouse lung. The cancer risk is then estimated for humans using this calculated delivered dosage.

8.2 Mathematical Models

This section will briefly discuss some of the dose-response models used for low-dose extrapolation. A more detailed mathematical description of the different forms of the multistage models can be found in Appendix C.

8.2.1 Multistage Models
Most mathematical models of multistage carcinogenesis are simplifications of the original time-dependent model

developed by Armitage and Doll (1954; See Appendix C). More recently, Moolgavkar and Venzon (1979) suggested a generalized two-stage form of this model, which Thorslund et al. (1987) subsequently simplified to a time-independent form and modified to a dose-dependent form by incorporating the effect of environmental agents on cell transition rates and first-stage proliferation rates. These two-stage models are discussed in Appendix C.

8.2.1.1 Linearized multistage model (Global 82)

Guess and Crump (1977) generalized the multistage model as a time-independent form with a less restrictive polynomial form. This form of the multistage model is described in Appendix C.

8.2.1.2 Time-to-tumor model (WEIBULL 82)

This model was originally intended to estimate the cancer risk when the death rate due to the agent is dependent on the exposure level and independent of the tumor of concern. Under this model the estimation of the tumor incidence requires the specification of whether the tumor caused death or was only found incidentally at necropsy. Few veterinary pathologists have reported this distinction. However, the model can be used for a modified lifetable analysis of the tumor rate when the agent of concern does not induce lethal tumors with very short latency periods. The Weibull 82 model is used to correct for mortality which does not have as its underlying cause the tumor(s) of concern. A mathematical description of the model is provided in Appendix C.

8.2.2 Other Mathematical Models

A number of alternatives to the multistage model have been suggested as being potentially useful for estimating low-dose

cancer risk. Among them are the probit, logit, Weibull, and gamma multihit models. The computer program RISK 81 was used to estimate the parameters in these models (Kovar and Krewski 1981). Whittemore (1978) and Whittemore and Keller (1978) note that the underlying biological basis of each of these models is inconsistent with our present understanding of carcinogenesis. Even so, these models are useful in representing the potential range of estimates that might result due to the selection of a particular parametric form. It has been shown (Crump et al. 1976) that if background tumor rates are caused by factors that are dose-additive with the carcinogenic agent, low-level linearity is rapidly achieved as exposures are decreased from the high levels used in animal bioassays. In this case, the parametric form of the model has only a limited effect on low-dose risk estimates.

8.3 Estimates of Cancer Risk Based Upon Animal Bioassay Data

The tumor types used to obtain risk estimates were judged to be both biologically relevant and significantly different statistically from controls. Biological relevance was defined as a malignant tumor or a benign tumor with a reasonable chance of progressing to a malignant form. Statistical significance was measured by a Cochran-Armitage trend test that had a "p" value of less than 0.05 associated with it. The following tumor types met both of these criteria:

o Sarcomas of the cervical-salivary gland region in Sprague-Dawley male rats (Table B-1, Dow, 1980 inhalation study, reported by Burek et al. 1984),

o Alveolar and/or bronchiolar neoplasms of the lung in B6C3F1 male and
female mice (Table B-9, NTP, 1986 inhalation study, Mennear et al.
1988), and

o Hepatocellular adenomas or carcinomas in female B6C3F1 mice (Table B-
10, NTP, 1986 inhalation study, Mennear et al. 1988).

The tumor dose-response data that are used to estimate risk are shown in
Tables B-1, B-9, B-10, and B-12 and are summarized in Table 8-1.

8.3.1 Linearized-Multistage (L-M), 95% Upper-Bound Estimates for All Significant Data Sets

Standard unit risk estimates (q_1^* in units ppb^{-1}) were obtained for these
endpoints using the (L-M) 95% upper-bound approach as discussed in Appendix C.
These results are shown in Table 8-1.

The lung tumors in mice are used as a basis for a more detailed, quantitative,
risk-modeling effort. The rationale for this is:

o The lung is the organ of direct contact for inhalation exposures.

o The tumor data indicate a strong increasing response with dose ($p \leq$
0.001 by Cochran-Armitage trend test) and are consistent with the
multistage models.

TABLE 8-1

TUMOR DOSE-RESPONSE DATA USED TO ESTIMATE RISK TO RODENTS

Exposure Level[a] (ppm CH_2Cl_2)	Sarcomas of the Cervical/Salivary Gland Region in SD Male Rats[b]	Alveolar and/or Bronchiolar Adenomas or Carcinomas in B6C3F1 Mice[c]		Hepatocellular Adenomas or Carcinomas in B6C3F1 Female Mice[c]
		Male	Female	
0	1/93	5/50[d]	3/50	3/50
500	0/94	--	--	--
1,500	5/91	--	--	--
2,000	--	27/50	30/48	16/48
3,500	11/88	--	--	--
4,000	--	40/50	41/48	40/48
95% upper bound[e] linear term q_1^* in ppb^{-1}	2×10^{-7}	3×10^{-6}	3×10^{-6}	9×10^{-7}

[a] Animals were exposed 6 hours per day, 5 days per week.
[b] Dow 1980 inhalation study.
[c] NTP 1986 inhalation study, Mennear et al. 1988
[d] No exposure group included at this level.
[e] Lifetime excess risk for continous exposure. Adjusted for experimental conditions.

NOTE: All Cochran-Armitage trend tests are significant at p<0.001 level. All Fisher exact tests are significant at p<0.001 level except control versus 500 and 1,500 ppm in Dow study, which were not significant, i.e., p>0.05.

o The response is consistent between sexes.

o The largest 95% upper-bound linear coefficient (q_1^*) for the four data

   sets considered is associated with the lung tumor response in female

   mice.

8.3.2 Risk Estimates Based on Lung Tumors and Various Forms of the Multistage
Model

A statistically significant, monotonically increasing dose-response

relationship was obtained for both sexes of B6C3F1 mice in the NTP (1986)

study (see Tables B-9 and B-10). The lung tumor response to be used in this

analysis is alveolar and/or bronchiolar adenomas and carcinomas, since these

stages of lung tumors are thought to be part of the same etiologic entity.

Stimulation of cell proliferation is believed to be a possible mechanism of

tumor induction.   However, a model based on this mechanism was rejected for

both sexes in this case since the observed data did not exhibit sufficient

curvature to be consistent with the hypothesis of preneoplastic cell

proliferation.

Other two-stage models based on mechanistic descriptions of tumorigenesis

adequately fit the data for both sexes. An exact fit is always obtained under

certain conditions described in Appendix C. An exact fit was obtained for

the lung tumor data sets from both male and female mice. As a result, the

two-stage, different transition rate model (Equation 3, Appendix C) and the

standard multistage model (Equation 7, Appendix C) give identical results.

However, a goodness-of-fit test is meaningless since the number of parameters estimated is equal to the number of exposure groups. The more restricted model, with identical transition rates, is also consistent with the observed data based on the chi-square goodness-of-fit test (p>0.1). The risks obtained from the various forms of the models are summarized in Table 8-2. The estimates obtained from the two-stage models are relatively consistent. The largest risk is associated with female mice using the upper-bound time-dependent multistage model with all tumors regarded as "incidental." This analysis uses the term "incidental" tumors for all tumors found at necropsy, including those discovered at terminal sacrifice. This terminology is used to distinguish between the "fatal" tumor analysis which excludes tumors found at terminal sacrifice. Using the female mice tumor data, the maximum likelihood estimates of risk and the 95% upper bounds are compared for the various models in the next section.

8.3.3 <u>Comparison of Various Risk Models Using Female Mouse Lung Tumors</u>.

The multistage time-dependent and time-independent models can be compared to a number of alternative models that have been used in risk assessment (Table 8-3). The risk values in Table 8-3 assume that human and animals exposed to equal doses of a carcinogen on a mg per surface area basis, for an equivalent proportion of a lifetime will encounter the same degree of risk. This is the surface area conversion for virtually completely absorbed gases. The basis for this conversion and methodology are shown in Appendix F. Since the observed background rates are not equal to zero, the various models give very comparable results as shown in Table 8-3. This is indicated by the ratio of

TABLE 8-2

MAXIMUM LIKELIHOOD ESTIMATES OF RISK FOR MICE BASED ON VARIOUS FORMS
OF THE MULTISTAGE MODEL AND LUNG ALVEOLAR AND/OR BRONCHIOLAR
ADENOMAS OR CARCINOMAS IN B6C3F1 MICE

Mathematical Model	Biological Hypothesis	Lifetime Excess Risk from Exposure Under Experimental Conditions to 1 ppb CH_2Cl_2[a]	
		Male	Female
Two-stage, dose-dependent, preneoplastic cell proliferation	Mitogenic stimulation of preneoplastic cells	Model rejected based on chi-square goodness-of-fit test, p<.01	
Two-stage, dose-dependent, different transition rates	Genotoxic at two different loci	2×10^{-6}	3×10^{-6}
Two-stage, dose-dependent, identical transition rates	Genotoxic at same locus on homologous chromosomes	9×10^{-7}	9×10^{-7}
(L-M) GLOBAL 82	Exposure additive with other factors causing background rates	2×10^{-6}	3×10^{-6}
Multistage time-to-tumor WEIBULL82 (incidental tumors)	Multiple linear unspecified events	2×10^{-3}	1×10^{-6}

[a]Linear term multiplied by age function evaluated at 104 weeks and the values were
adjusted for continuous exposure.

TABLE 8-3

COMPARISON OF HUMAN RISKS OBTAINED USING DIFFERENT MODELS
ASSUMING CONSTANT LIFETIME INHALATION EXPOSURE
TO 1 ppb CH_2Cl_2[a,b]
(Risk from CH_2Cl_2 Minus Background Tumor Rate)

Model Used	Excess Risk from Continuous Exposure to 1 ppb in Air	
	Maximum Likelihood Estimate	95% Upper Confidence Limit
Multistage (Global 82)	7×10^{-6}	9×10^{-6}
Time-dependent multistage assuming all tumors are incidental (Weibull 82)	4×10^{-6}	10×10^{-6}
Probit	4×10^{-6}	8×10^{-6}
Logit	3×10^{-6}	7×10^{-6}
Weibull	7×10^{-7}	2×10^{-5}
Gamma multihit	4×10^{-6}	7×10^{-6}

[a] Crump multistage Global 82, Weibull 82, and Risk 81 (with additive background), surface area corrected for conversion from animals to humans.

[b] Based on female B6C3F1 mice with alveolar and/or bronchiolar adenomas or carcinomas of the lung observed in the NTP (NTP 1986, Mennear et al. 1988) inhalation bioassay.

the highest to the lowest value is only about a factor of two for the 95% upper-bound estimates.

Dose-additive forms for logit, probit, Weibull and gamma multihit models were used for the comparison. If a major portion of the background tumor rates were induced by a mechanism different from that of CH_2Cl_2, the dose-additive assumption would not be valid. Under the alternative assumption of independence, much greater variability in risk estimates would be expected.

8.4 Postulated Mechanisms of Action for Observed Tumor Responses Induced by Methylene Chloride

Several of the carcinogenicity studies of CH_2Cl_2 using both rats and mice yielded significantly increased incidences of tumors in organs of species and strains that have been reported to have relatively high spontaneous tumor incidences (Tarone et al. 1981). These included mammary tumors in F344 and Sprague-Dawley rats following inhalation exposure, liver tumors in B6C3F1 male mice exposed via ingestion and inhalation, and liver tumors in B6C3F1 female mice exposed via inhalation (see Table 7-1 in Chapter 7).

The mechanism of formation for tumors with high spontaneous incidence is controversial. Some authors have associated the increased incidence rates for these tumors with exposure to chemicals that have nongenotoxic mechanisms, i.e., do not interact directly with DNA. However, this does not imply that these tumors cannot also be caused by known genotoxic substances. Several potential nongenotoxic and genotoxic mechanisms might account for an increased rate of tumor formation specifically for the B6C3F1 mouse liver tumor.

The role of cell proliferation in tumorigenesis is not fully understood. It has been suggested that since the total number of spontaneous mutations in a tissue is proportional to the total number of mitotic divisions (Knudson 1985), cell proliferation results in more mutations, and in turn may yield more tumors.

The NTP (NTP 1986, Mennear et al. 1988) study in which B6C3F1 mice were exposed chronically to CH_2Cl_2 via inhalation revealed that hepatic "cytological degeneration" occurred as a result of treatment in both male and female mice. This lesion was not described further in the report and the NTP provided no dose-response information. Hepatocellular toxicity can be followed by regenerative hyperplasia, a common response when a tissue attempts to replace damaged cells by increasing the cell proliferation rates of the undamaged cells. Thus, CH_2Cl_2 could induce mouse liver tumor formation simply by stimulating cell proliferation in that organ in response to cytotoxicity. However, there are no experimental studies using CH_2Cl_2 that can be used to specifically document regenerative hyperplasia resulting in tumor formation.

Chemicals that are cytotoxic, i.e., that cause cell death and stimulate cellular regeneration, may damage DNA by virtue of cell lysis (Sina et al. 1983), perhaps through the release of lysosomal contents containing DNA hydrolases (Bradley 1985). Indirect cytotoxicity and genotoxicity may result from the stimulation of peroxisomal proliferation and concomitant overabundance of reactive oxygen species (Reddy et al. 1980). There is no direct evidence, however, that the carcinogenic effects of CH_2Cl_2 are due to any of these mechanisms.

CH_2Cl_2 may produce tumors via direct genotoxic action. The mutagenic activity of CH_2Cl_2 has been demonstrated in the <u>Salmonella</u> mutagenicity assay. DNA adducts have not been observed, although alkylation of DNA may have occurred below the detection limit in the experiments designed to detect adduct formation. The possibility of DNA binding is also suggested by covalent binding of CH_2Cl_2 metabolites to microsomal lipids and proteins, which demonstrates the formation of highly reactive intermediates. DNA alkylation may be significant for carcinogenesis at levels below the limit of detection for an experimental assay. Such alkylation might lead to mutation, so the possibility that CH_2Cl_2 interacts with DNA directly cannot be ruled out.

8.5 Estimation of Risk Adjusted for Pharmacokinetic Information

In addition to estimates derived under the standard "applied dose" approach, risk estimates for CH_2Cl_2 have been derived using variations of the physiologically-based pharmacokinetic model [PBPK] described by Andersen et al. (1987). The purpose of such modeling is to identify the dose of actual carcinogenic agent that will be present at the target tissue as accurately as possible. This type of approach requires the extensive use of data on the physiology of the species under study and on the pharmacokinetics of the substance being considered. The approach uses data available on the chemical in a quantitative manner whereas previously such data was only considered qualitatively. Thus, by using more information, it is the intention of the modelers to develop a more accurate picture of the potential for the chemical to induce cancer in humans. The limitations of the method basicly reflect the limitations of our knowledge of the chemical. Although DHS staff have not independently derived PBPK values for this risk assessment, staff of the EPA

(1987a,b) and the U.S. Consumer Product Safety Commission (Cohn 1987) have done so through the U.S. Interagency Hazard/Risk Assessment Committee of the Integrated Chlorinated Solvents Project (HRAC). The purpose of this subsection is to discuss application of the PBPK model to risk assessment and to indicate which values should be used in reporting the range of risks from CH_2Cl_2 exposure.

8.5.1 Identification of Carcinogenically Active Metabolites

Use of the PBPK model requires determination of the active species involved in CH_2Cl_2 carcinogenesis. Several hypotheses have been generated concerning the biologically active form of CH_2Cl_2 responsible for tumor induction in mice. One hypothesis is that the observed effects are related to the parent chemical. Due to the low reactivity of CH_2Cl_2 and evidence suggesting that it behaves like a weak alkylating agent, it is unlikely that CH_2Cl_2 functions as a direct-acting carcinogen. However, none of the available evidence precludes the possibility that the parent compound is carcinogenically active (EPA 1987a). As indicated in Section 6 (page 6-1) positive dose-related responses in Salmonella strains TA1535, TA98 and TA100 have been reported both in the presence and in the absence of a metabolic activating system.

As indicated in Section 2, metabolism of CH_2Cl_2 has been demonstrated to occur by two pathways (Ahmed and Anders 1978). One is catalyzed by mixed-function oxidase (MFO) cytochrome P450, while the other is catalyzed by glutathione S-transferase (GST). Each metabolic pathway involves formation of an active intermediate that is theoretically capable of irreversibly binding to cellular

macromolecules such as DNA (Ahmed and Anders 1976, Ahmed et al. 1980, Kubic and Anders 1978, EPA 1985b).

A second hypothesis is that the tumorigenicity of CH_2Cl_2 is related to the formation of metabolites derived from oxidative metabolism via the MFO pathway. Several lines of evidence suggest the production of genotoxic intermediates by this pathway. Jongen et al. (1982) reported that the addition of either microsomes (i.e., MFO) or cytosol (i.e., GST) increased the mutagenic response of CH_2Cl_2 in TA100 using a 6-hour incubation period. Snow et al. (1979) reported that with the addition of S9 (an MFO-containing fraction) from Syrian Golden Hamsters there was an increase in mutagenic response at all dose levels. These results are in contrast to Green T. (1983) who reported that the addition of cytosol increased the mutagenic response of CH_2Cl_2 in TA100, but the addition of an S9 fraction or of microsomes did not. However, Green T. (1983) incubated the assay for 3 days which is much longer than most other investigators. Consequently, the MFO pathway appears to exhibit mutagenic activity.

Compounds closely related to CH_2Cl_2 exhibit the production of mutagenic metabolites via the MFO pathway. The mutagenicity of chlorofluoromethane was increased in TA100 by the addition of S9 (Green T. 1983). There was a marked increase in mutagenicity of dibromoethane and diiodomethane when microsomes or cytosol were added to the medium and incubated 15 minutes (Van Bladeren et al. 1980). Thus, for three closely related compounds the MFO metabolism produced mutagenic metabolite(s).

As demonstrated in unpublished data of Green et al. (1987b) submitted to DHS, the nonciliated Clara cell of the bronchiolar epithelium of the mouse lung exhibited toxic effects and MFO damage following CH_2Cl_2 exposure. It appears that in this cell type the oxidative route produced metabolites which destroyed the enzymes from which they were formed, and caused cytotoxicity. That is, the MFO pathway produced reactive intermediates, which reacted with proteins and consequently may react with and damage DNA. Therefore there is a direct and indirect evidence that the metabolic intermediate(s) produced by the MFO pathway are mutagenic and possibly genotoxic.

Other data suggests that the MFO pathway is not important with regard to the formation carcinogenic metabolites. Cytochrome P450 appeared to be saturated at 500 ppm in rats (McKenna et al. 1982), while a dose-related increase in tumor incidence is observed in mice at higher concentrations. Indirect evidence has been presented (Andersen et al. 1987) which indicates that the MFO pathway may not be the primary carcinogenic pathway. This evidence consists of comparing the results of the NTP (1986) inhalation study, where tumors were clearly observed, with those of the National Coffee Association (NCA) drinking water study (Serota et al. 1986a,b) where there was a borderline increase in tumors (see Appendix B, page B-11). The PBPK target dose by the MFO pathway was higher in the NCA study, but the GST target dose was lower. Cohn (1987) of the CPSC concluded that the MFO pathway was saturated in both studies, and indicated that if the MFO pathway were of primary importance, similar carcinogenic responses would have occurred in the two studies. However, the basis for the conclusion is limited. First, the difference in carcinogenic response in the two studies can also be explained in terms of the applied dose since the dose in the NTP study was, on a mg/kg

basis, more than 10 times greater than the dose in the Serota et al. study. The EPA (1985b) stated that the response in the Serota et al. study was consistent with the calculated carcinogenic risk based on the potency estimated using data from the NTP study, without adjustments for pharmacokinetics. Second, this comparison does not imply that the MFO pathway is unimportant in terms of the carcinogenic response since it may still produce a significant amount of carcinogenic intermediates. Third, comparison of the two studies would be consistent with production of carcinogenic intermediates via both metabolic pathways. Fourth, Cohn's conclusion assumes that the calculation of the MFO target dose by the PBPK method is correct. This target dose has not been directly measured experimentally.

Cohn (1987) indicated that the "dose-response relationship for lung and liver would not be expected to be as pronounced as in the NTP bioassay if the MFO pathway was of primary importance," since this pathway is expected to be saturated based on the PBPK model. However, he states further that "this argument does not eliminate the MFO pathway from consideration with regard to a role in the carcinogenic response of methylene chloride, but it does indicate that some other pathway or chemical species is likely to be of greater importance." Although saturation of the total MFO pathway, as measured by CO production, and the PBPK analysis indicate overall MFO and GST activity they do not indicate the number of reactive intermediates produced by each pathway. Thus it is the opinion of DHS staff that the carcinogenicity data in animals do not indicate which pathway(s) produce carcinogenic metabolites. However, PBPK models have focused on the GST pathway as the primary producer of the carcinogenic metabolite, and have placed less weight on the metabolites produced via the MFO pathway, as is discussed below.

A third hypothesis suggests that CH_2Cl_2 metabolites formed by the GST system are responsible for tumorigenicity. This hypothesis is supported by several lines of evidence: One is that the increased production of GST pathway metabolites is consistent with the treatment-related tumor incidence, since the GST pathway was assumed to be not saturated at the exposure concentrations used in the inhalation bioassay. Second, the addition of a cytosolic fraction increased the yield of bacterial mutagens in a _Salmonella_ assay system (Jongen et al. 1982, Green 1983). The latter observation also finds support from studies of the activation of two other halogenated hydrocarbons by glutathione (GSH), 1,2-dichloroethane (Guengerich et al. 1980) and 1,2-dibromoethane (Rannug et al. 1978). The third type of evidence implying that the GST pathway is important in carcinogenesis is the indication that the MFO pathway is unlikely to be fully responsible for the carcinogenic activity of CH_2Cl_2.

The observed tumorigenicity of CH_2Cl_2 appears to be related to the formation of GSH conjugates via the GST pathway. However, it is unclear as to how significant a role the GST pathway plays and whether the contribution of the MFO pathway is significant. That is, there is considerable uncertainty with regard to the identification of the methylene chloride-glutathione (CH_2Cl_2-GSH) conjugate as the sole metabolite responsible for carcinogenicity in laboratory animals and with regard to the exclusion MFO activity from the production of carcinogenic metabolites. The HRAC (EPA 1987a) indicated that "the mechanism of carcinogenic action of [methylene chloride] remains problematical..." and "...there remains some uncertainty as to whether or not the GST pathway is the sole path to carcinogenicity." The CPSC document (Cohn 1987) stated "again, of course, some or all of the intermediates of the various pathways, or the parent compound, may contribute to the carcinogenic

process." Although there is uncertainty in positively identifying the carcinogenic species, EPA and CPSC staff concluded that the MFO pathway only plays a minor role in the carcinogenic process in animals, and risk estimates were made using the PBPK model assssuming that the carcinogenic activity of CH_2Cl_2 is not dependant on the MFO pathway.

An extensive analysis of the PBPK model was presented in the federal interagency health/risk assessment committee (HRAC) draft report (EPA 1987a). The HRAC suggested that pharmacokinetic model adjustments consisted of two separate analyses (EPA 1987a). The first analysis adjusts for saturated metabolic pathways during the experimental exposure (referred to as a high-to-low-dose adjustment). The second adjusts for interspecies differences in the handling of the substance (referred to as the species-to-species adjustment). The HRAC analysis concluded that the PBPK model could be used to adjust for saturation of the MFO pathway in calculating the risk estimate for CH_2Cl_2. The EPA analysis (EPA 1987b) went further and suggested that an adjustment for interspecies differences could also be made using the PBPK model. The analyses discussed below will be based on the HRAC report (EPA 1987a), the CPSC report (Cohn 1987), and the EPA report (EPA 1987b). The PBPK model is itself described in Appendix E.

8.5.2 Adjustment for MFO Saturation: High-to-Low Dose Adjustment

The CPSC analysis (Cohn 1987) concluded that "based on the weight of evidence, some adjustment utilizing pharmacokinetic data would appear to be appropriate when extrapolating from high to low dose in the case of [methylene chloride]." Based on data discussed in Section 2.2.2 and the PBPK model, the MFO pathway

is expected to be saturated at all dose levels in mice in the NTP (1986) inhalation bioassay. CPSC viewed the PBPK model as generally able to estimate levels of CH_2Cl_2 in blood, lung and liver. The HRAC report (EPA 1987a) indicated that the PBPK model appeared sensitive to changes in the metabolic constants KF (the first order rate constant for the GST pathway), KM (the Michaelis-Menten constant for the MFO pathway), and VMAX (the maximum velocity of metabolism by MFO). Based upon data the CPSC obtained from the authors of the PBPK model for estimates of the three constants in humans (KF=0.53; KM=0.58; VMAX=118.9), the CPSC reported that consideration of the pharmacokinetic data required an adjustment of 2.2 for the lung tumor data (Cohn 1987). A sensitivity analysis to determine how large changes in the above three variables (KF, KM, and VMAX) would influence the adjustment factor was conducted. The adjustment was shown to be relatively insensitive (varying from 1.6 to 2.4) even if the three metabolic variables were in error by an order of magnitude. Consequently, consideration of the pharmacokinetic adjustments from high-to-low dose would reduce the estimated lung cancer potency by approximately 2.2-fold.

It is not possible to validate the appropriateness of this adjustment. As indicated in Table 8-1 the upper bound estimate of risk was based on the incidence of alveolar and/or bronchiolar adenomas or carcinomas in mice in the NTP (1986) inhalation study. The dose-response data are presented in Table B-10 in Appendix B. If the cancer dose-response model using an adjusted dose fit the data better, that would support the high-to-low dose adjustment. However, when the several carcinoma and adenoma endpoints in Table B-10 of Appendix B are examined, there is considerable variability in fit with the applied doses. For alveolar/broncheolar adenomas the doubling of applied dose

does not correlate well with the increase in tumor response (56 to 75%), and may indicate a saturating metabolism. However, for alveolar/bronchiolar carcinomas, the doubling of the applied dose correlates very well with the tumor response (27 to 60%). As indicated above, the carcinogenic response would not be expected to increase with dose if the MFO pathway was the only pathway producing carcinogenic intermediates and if the MFO pathway was saturated in the NTP (1986) study. However, the response would be expected to increase with dose if the MFO and GST pathways both contributed appreciably to the production of carcinogenic intermediates. Thus, considering the wide variability in dose-response, support for or against the high-to-low dose adjustment does not appear to be available based on an improved model fit of the tumor data.

8.5.3 Species-to-Species Adjustment

Extrapolation between species involves numerous factors, including metabolism and pharmacokinetics. As Cohn (1987) indicated "the ability to elucidate one component of a species difference does necessarily indicate what, if any, adjustments should be made; it does not provide more certainty than the empirical process currently used; in fact, making the necessary assumptions may introduce even more uncertainty than the present empirical procedure." As a result, the CPSC did not make a species-to-species adjustment for CH_2Cl_2. However, in addition to the high-to-low dose adjustment, the EPA did calculate an adjustment factor for species-to-species extrapolation (EPA 1987b), and the results are described below.

Two PBPK models were studied by HRAC (EPA 1987a). One was the model developed by Andersen et al. (Andersen et al. 1984, Andersen et al. 1987) which is based on inhalation of CH_2Cl_2. The other model was developed by Angelo et al. (Angelo et al. 1984, Angelo and Pritchard 1984, Angelo et al. 1986a,b) which is based on intravenous and oral exposure to CH_2Cl_2. The two models differ significantly in structure. EPA (1987b) chose to develop risk estimates based on the Andersen et al. (1987) approach and to use the Angelo et al. (1986a,b) model to obtain greater insight into the Andersen et al. model. The Anderson et al. model is briefly described in Appendix E and references to the "PBPK" model refer to the Anderson et al. model unless otherwise stated.

The Andersen et al. model estimates tissue-level doses for CH_2Cl_2 and its metabolites. Andersen et al. (1987) concluded that the estimate of risk from CH_2Cl_2 exposure to the human lung was 144-fold lower using the PBPK approach compared to the applied-dose approach. EPA (1987b) indicated that this difference was composed of an 11.3-fold difference based on the estimated dose adjustment, and a 12.7-fold factor used for surface area correction for dose when extrapolating across species. EPA further indicated that the difference between the two approaches was overstated for several reasons. Andersen et al. (1987) used a lower human breathing rate that represented a resting state (12.5 m^3/day) instead of a 24-hour average (20 m^3/day). Andersen et al. (1987) used a higher mouse breathing rate (0.084 m^3/day) than the standard EPA estimate (0.043 m^3/day). Furthermore, Andersen et al. compared their estimates using their breathing rates to the EPA estimates using EPA breathing rates. When the same breathing estimates are used in the applied-dose and PBPK approaches, the estimated PBPK dose is not 11.3-fold lower but

only 3.6-fold (using Andersen et al. rates) to 9.4-fold (using EPA rates) lower.

Using the PBPK model developed by Andersen et al. (1987), the average daily concentration of CH_2Cl_2 metabolites in the lung via the GST pathway was calculated for mice exposed to 2,000 or 4,000 ppm. The HRAC calculated the internal mouse and human dose levels as indicated in Table 8-4 based on the female mouse lung tumor data of the NTP (1986) bioassay. The internal dose calculated for humans in the lung from a 1 ppm exposure was 0.008386 mg/L/day. The unit risk reported by HRAC (EPA 1987a) was $1 \times 10^{-6}(ppb)^{-1}$ (or 3×10^{-7} $(\mu g/m^3)^{-1}$). The risks from CH_2Cl_2 calculated from the female mouse lung tumor data using various assumptions are shown in Table 8-5. Approximately a 9-fold reduction in estimated risk is achieved by the combined high-to-low dose and species-to-species adjustments. This reduction is mostly due to the assumptions concerning the species-to-species adjustment. As indicated in Section 8.5.2, accounting for saturation of CH_2Cl_2 metabolism (high-to-low dose adjustment) would account for about 28% of the total adjustment.

8.5.3.1 Use of a Surface Area Corection Factor.

The HRAC (EPA 1987a) analysis differed with the approach suggested by Andersen et al. (1987) regarding the surface area correction on applied dose. Andersen et al. (1987) assumed that the adjustment for metabolic differences across species in the PBPK model should replace the surface area adjustment (EPA 1987a). However, in this case the HRAC concluded that the surface area conversion factor (12.7) is still needed to account for interspecies differences in sensitivity of the tissues to the internal dose. Currently,

TABLE 8-4

HUMAN AND MOUSE CH_2Cl_2 INTERNAL DOSE LEVELS BASED UPON THE RESULTS
OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL[a]

Exposure Level (ppm)	Internal Lung Dose of CH_2Cl_2 (mg/liter blood)	
	Mice	Humans
4,000	240	
2,000	110	
1		0.008

[a]Based on the model of Andersen et al. (1987) as reported by EPA (1987a,b). The values were adjusted for continuous exposure. That is, the mouse exposure in ppm was multiplied by (6 hours/day)/(24 hours/day)x(5 days/week)/(7 days/week). The concentrations are calculated from the female mouse lung tumor data (NTP 1986).

TABLE 8-5

COMPARISON OF HUMAN CANCER RISK ESTIMATES FROM A 1 ppb CH_2Cl_2

EXPOSURE, BASED ON LUNG TUMORS IN FEMALE MICE,

WITH AND WITHOUT ADJUSTING FOR METABOLISM[a]

Approach	Lifetime Risk	
	ppb^{-1}	$(\mu g/m^3)^{-1}$
Applied Dose	9×10^{-6}	3×10^{-6}
High-to-Low Dose Adjustment[b]	4×10^{-6}	1×10^{-6}
Total PBPK Adjustment[c]	1×10^{-6}	3×10^{-7}
Total PBPK Adjustment without Surface Area Correction[d]	9×10^{-8}	3×10^{-8}

[a]All values were adjusted for continuous exposure. That is, the mouse exposure in ppm was multiplied by (6 hours/day)/(24 hours/day) x (5 days/week)/(7 days/week). The concentrations are calculated from the female mouse lung tumor data (NTP 1986). The extrapolation was also corrected for surface area (a factor of 12.7) unless indicated otherwise. The risks are based on the linearized multistage model.

[b]High-to-low adjustment for saturation of MFO pathway based on the HRAC report (EPA 1987a).

[c]High-to-low adjustment for saturation of MFO pathway and the species-to-species adjustment based on the HRAC report which includes a 12.7 surface area correction (EPA 1987a).

[d]High-to-low adjustment for saturation of MFO pathway and the species adjustment based on the HRAC report (EPA 1987a), except without use of a surface area correction.

the PBPK model itself does not incorporate a susceptibility factor. The CPSC analysis (Cohn 1987) indicated that the surface area adjustment has been used to account for the many differences between species (e.g., metabolic activation rates, clearance, distribution, cell sensitivity, number of cells in exposed tissue, immunosurveillance, DNA longevity, efficiency of DNA repair, and cell proliferation rates). Knowledge of a single interspecies difference, such as the pharmacokinetics, does not shed light on the total interspecies correction since other factors may be compensating for the effect, such as the pharmacodynamics (i.e., the effect the chemical has on a living system).

There is likely to be difference in tissue susceptibility within species. Reitz et al. (1988) recently reported the PBPK internal dose estimates for lung and liver for the NTP (1986) mouse bioassay. The internal doses (in mg equivalent CH_2Cl_2 /liter volume of tissue) are reported in Table 8-6. As shown in the table, despite a higher PBPK dose delivered to the liver than to the lung, the tumor response is slightly lower in the liver than the lung. The potency of the PBPK dose was calculated for each tissue and the lung was found to be approximately 9-fold more sensitive to the PBPK dose than the liver. Thus, for this very homogeneous species, there was a substantial difference in tissue susceptibility to the PBPK dose. Consequently, the PBPK dose should not be considered the sole factor affecting intraspecies or interspecies differences.

There may be differences in susceptibility across species to the PBPK dose. At present an estimate of the difference cannot be made using published PBPK doses. One possible comparison would be production of lung tumors in female

mice to production of mammary tumors in female rats. This would compare the most sensitive sites of two species. However, the PBPK model is currently not able to calculate an internal dose for mammary tumors. A second possible comparison, would be of combined liver tumors in rats to that of mice based on the Serota et al. (1986a,b) studies. PBPK doses for all dose levels for these studies have not been published although they could be calculated. The difficulty in interpreting this comparison would be the borderline significant tumor response obtained in the studies.

In summary, the PBPK model assumes that sensivitity to the same target dose is identical in different species. However, based on the 9-fold difference between two tissues in the mouse NTP (1986) study, there is likely to be differences in susceptibility across species. Consequently, staff at DHS agree that the application of the surface area correction to account for sensitivity differences is justified, particularly in light of the numerous uncertainties involved in the PBPK procedure as discussed in Section 8.5.3.2, and questions regarding whether the carcinogenic pathway has been identified in humans. Furthermore, an uncertainty factor (such as the surface area correction) is needed to account for the potential variability in human response to CH_2Cl_2 exposure. Thus, DHS staff choose to use the uncertainty factor, (referred to as the surface area correction), adopted by EPA in the HRAC (EPA 1987a) document.

8.5.3.2 Concerns Regarding the Species-to-Species Adjustment.

There is a large number of variables and equations used in the PBPK models. As indicated above, a sensitivity analysis has been conducted on a few of the

parameters (Cohn 1987). A complete analysis of all the parameters would assist in identifying which parameters have the greatest impact on the final numerical outcome of the model. Once the most influential parameters have been identified, reporting that information would be very helpful in evaluating how such complex models operate.

The roles of either the CH_2Cl_2-GSH conjugates or MFO reactive metabolites have not been elucidated in human carcinogenesis. There is limited information on the *in vitro* GST activity with CH_2Cl_2 in human and rodent liver (Rietz et al. 1988). Based on a per mg protein basis, mice produce 6- to 10-fold more reactive metabolite *in vitro*. However, the human liver (2198g) is more than 1000-fold larger than the mouse liver (1.84g) and would still be expected to produce 100-fold more of the putative metabolite per liver. The information for GST activity in the lung is more limited. Comparison of a single *in vitro* mouse tissue experiment to a single *in vitro* human tissue experiment (Rietz et al. 1988) indicated a 20-fold difference in GST activity on a per mg protein basis. The human lung (1065g) is more than 5000-fold larger than the mouse lung (0.2g) (Crosfill and Widdicombe 1961), and would be expected to produce 250-fold more of the putative metabolite per lung. The importance of this information to risk assessment probably depends on the relative contribution of other differences between species as discussed in Section 8.5.3.1.

Currently the PBPK model is restricted to considerating only the liver and the lung metabolism of CH_2Cl_2. Tumors at several sites have been associated with CH_2Cl_2 exposure in rodents. These tumors include salivary gland sarcomas in rats (Burek et al. 1984), mammary gland tumors in rats (Burek et al. 1984, NTP

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1986), combined liver tumors in rats (Serota et al. 1986a), hepatocellular adenomas and carcinomas in mice (Serota et al. 1986b, NTP 1986), and alveolar/bronchiolar adenomas and carcinomas in mice (NTP 1986). Increases in tumors in a variety of organs indicates that the carcinogenic activity of CH_2Cl_2 is not tissue-specific. Humans may respond to an extent similar to other animals though at a different site. The relative distributions of GSH and GST throughout the body in laboratory animals versus humans have not been identified; thus, some human tissues may exhibit greater (or lesser) production of the CH_2Cl_2-GSH conjugate than their counterparts in rodents. Furthermore, more information needs to be developed regarding the relative activities of the MFO and GST pathways in various tissues across species.

PBPK models have to rely on information from the test species. Human estimates of enzymatic activity in vivo have been limited to methods relying on exhalation of CO or carboxyhemoglobin (COHb) levels. Measurements of enzyme activities and rates of formation of products in vitro are not available for all animal tissues and are only available for a few human liver and lung samples. Using 1,2-dibromoethane as a substrate to measure GST activity in the rat indicated that the highest activity occurred in the liver and kidneys and significant GST activity was reported in the lung, testis, spleen, and heart (Hill et al. 1978). Thus, based on GST activity levels, other tissues are possible sites of carcinogenicity for CH_2Cl_2. Studies by Black and Howerton (1984) and Mukhtar et al. (1981) suggest that both animal and human pancreatic tissue exhibit significant GST activity; as discussed in Sections 7.2.1 and 8.5, there is some suggestive evidence of increased pancreatic cancer in humans resulting from CH_2Cl_2 exposure (Hearne et al., 1987). However, it is not possible to estimate a PBPK dose for the pancreas

based on the data currently available. Although the primary site of GST and MFO activity is the liver, carcinogenicity is not restricted to that tissue. In extrahepatic organs, GST activity may predominate over MFO activity. Based on the limited data of Rietz et al. (1988), GST activity is more important in the human than the mouse relative to MFO activity (the ratio of GST/MFO activity is greater for humans). Another possibility is that simple formation of a toxic metabolite may not be the overriding factor in carcinogenesis. Instead, the relative toxifying and detoxifying activities in the specific organ may be more important.

In addition to the above general comments regarding the PBPK approach, DHS staff have some specific concerns regarding use of the Andersen et al. (1987) model in risk assessment. Several metabolic constants incorporated into the PBPK model (e.g., VMAX for MFO in the liver, KM for MFO, and KF for GST in the liver) were developed by an optimization procedure to obtain the best fit of the model to the data on the disappearance of the compound from the exposure chamber (Andersen et al. 1987). The model does not account for sequestering into lipid-rich compartments of some organs. In contrast to the sensitivity analysis for the high-to-low dose adjustment, the results for a species-to-species extrapolation is reportedly very sensitive to the value of KF (Cohn 1987, EPA 1987a). The human variability in KF has not been established.

There is uncertainty in the human variability of other physiological parameters, partition coefficients, and metabolic constants used in pharmacokinetic models. The catalytic affinity (KM) and maximum metabolic capability (VMAX) for a particular substrate may vary widely due to different MFO and GST isoenzymes predominanting in different individuals and tissues.

These isoenzymes may also differ in the responses of the genetic systems controlling their induction or repression to chronic exposure. GST is known to have at least 7 isoenzymes which differ in catalytic activity, in substrate specificity, and in both subcellular and tissue distribution. The specific activity of GSTs may vary by orders of magnitude for the same substrate when tested with different isoenzymes (Jakoby and Habig 1980). The isozymes of GST responsible for CH_2Cl_2 metabolism have not been identified. It is not clear if one or more isozymes is involved. The stability or variability of the isozymes in the human liver assay have not been verified. Up to 50% of the human population lack a specific GST isozyme present in the rest of the population (Seidergard and Pero 1985, Seidegard et al. 1985, 1986). Thus, if one or more of the pooled human livers lacked a potential CH_2Cl_2-specific isozyme, there would be a decreased GST activity in the sample. Since very few human livers have been assayed for GST activity, the inter-individual variability is not known. Furthermore, since the livers used may have been affected by the use of drugs or anesthetics in heroic measures to save trauma victims, it is unclear how representative the these measurements are of the general population.

The human internal dose estimates depend upon the parameter values selected for use in the PBPK model. Key parameters used in the PBPK model are the metabolic constants for the two biotransformation pathways proposed for the formation of specific metabolites in the liver and the lung. Due to the lack of quantitative data on the metabolism of CH_2Cl_2 itself, surrogate substrates were used to apportion the activity of the MFO and GST pathways of each tissue (Andersen et al. 1987, Gargas et al. 1986, Reitz et al. 1986) based on the study of Lorenz et al. (1984). To partition activities between the liver and

the lung, 2,4-dinitrochlorobenzene was used for the GST estimate, and 7-ethoxycoumarin was used for MFO estimate.

Partition coefficients incorporated into the model were obtained using homogenized tissues and a vial equilibration technique (Andersen et al. 1987). Homogenization alters the normal architecture of the tissue, destroys proteins that may interact with the compound, disrupts normal membranes, deteriorates the normal binding components and decreases the ability of the tissue to metabolize the compound. Applying such metabolic constants measured in acute experiments to chronic exposure may not take into account responses such as enzyme induction, tolerance, or other long-term adaptive or pathological tissue changes. Furthermore, due to the absence of available data, the PBPK model assumed that the tissue/air partition coefficients for liver, muscle and fat in humans was identical to that measured in the rat (Andersen et al. 1987).

As indicated in Andersen et al. (1987), there is no data to allow direct calculation of the first order rate constant KF for the GST pathway in humans. Based on an apparent allometric relationship of intrinsic clearance (range of 34 to 59 ml/hr/kg) between rodent species, the human value for the kinetic constant KF was derived by scaling from the rodent values based on body weight to the 0.7 power. However, Reitz et al. (1988) indicate that in vitro KF values are roughly equivalent to the scaled values.

Due to the use of test results from a variety of studies and procedures, there may be considerable variability in the parameters due to experimental error, systematic differences in assay methods, and tissue preparation which have not

been taken into account. For example, the KM values for the metabolism of CH_2Cl_2 to CO via the oxidative pathway reportedly vary by a factor of 58 (ECETOC, 1987: 0.86 mM; Ahmed and Anders 1976: 50.1 mM), and the VMAX values vary by a factor of 27 (ECETOC, 1987: 0.58 nmoles/min/mg protein; Ahmed and Anders, 1976: 15.5 nmoles/min/mg protein). The values for 2,4-nitrochlorobenzene metabolism (used to partition lung and liver activities) in rat liver cytosolic fractions for GST activity range over 8000-fold (Green et al. 1986c: 0.16 nmol/min/mg; Moron et al. (1979): 567 nmol/min/mg; Lorenz et al. (1984): 1380 nmol/min/mg). The values for 2,4-nitrochlorobenzene metabolism in rat lung cytosolic fractions for GST activity had over a 6000-fold range (Green et al. 1986c: 0.015 nmol/min/mg; Lorenz et al. (1984): 77 nmol/min/mg; Moron et al. (1979): 100 nmol/min/mg). Andersen et al. (1987) presented four estimates of the human VMAX for the MFO pathway that varied by a factor of 2 (82 to 159 mg/hr). Thus, there is considerable variation in the estimate of many of the parameters used in the PBPK model. Variation in the individual parameters may combine to increase the uncertainty of the overall outcome of the model.

References have been made to unpublished studies and draft reports throughout this section. Generally, DHS staff do not rely heavily on such information since unpublished data may be very preliminary in nature. Information that is unpublished or peer-reviewed may be useful for clarification of the issues discussed or to acknowledge those who have evaluated and analyzed the complex issues involved in using the PBPK model. In several instances in this document DHS staff have extensively evaluated the unpublished data. In some cases, the preliminary information has been found to be incorrect. For example, the initial unpublished report submitted by the Halogenated Solvents

Industry Alliance (HSIA) to DHS indicated that the activity of the human liver GST was zero (ECETOC 1987). This result, obtained from a small sample size, and an inadequate assay method, was reported to regulatory agencies and widely to the scientific community. However, the more recent submissions indicate some activity (Green et al. 1987a; Reitz et al. 1987, submission by DOW Chemical Co. to EPA; ECETOC 1988). A recent publication (Rietz et al. 1988) also reports GST activity in the liver.

In other instances, unpublished data may indicated results contrary to information already published. For example, Green et al. (1986b, 1987c) reported that mice exhaled more CO_2 than rats and that there was a resulting species difference. However the work of McKenna et al. (1982) and Angelo et al. (1986a,b) indicate the absence of a species difference. In such instances, the published information would be relied upon to draw conclusions. Another example is that Ahmed and Anders (1976) reported that the metabolism of CH_2Cl_2 to formaldehyde by rat liver cytosol was significantly greater than that reported in Green et al. (1986c). In another example, the values of 2,4-nitrochlorobenzene metabolism in liver and lung cytosolic fractions for GST activity were significantly lower in Green et al. (1986c) than in either Lorenz et al. (1984) or Moron et al (1979).

Other data in unpublished reports that appear contradictory often cannot be explained. For example, Green et al. (1986) describes exposure of mice for 6 hours to CH_2Cl_2. In this study blood levels of CH_2Cl_2 for the 2000 ppm exposure were lower at 6 hours (20 μg/ml) than at 1.5 hours (40 μg/ml). Such information may simply reflect the prelimary nature of the experiment and of scientific knowledge in this area.

However, the use of unpublished human data from studies conducted at DOW Chemical Co. (VMAX and KM for the MFO pathway in humans) in the PBPK models is of particular concern to DHS staff. Similar human data from other sources is not available for comparison. In Andersen et al. (1987) the source of the data is attributed to unpublished data by Nolan and McKenna at Dow Chemical Co. However, in a recent publication by Rietz et al. (1988) of Dow Chemical Co. the human data is cited by referencing Andersen et al. (1987) without mention of their unpublished nature. This circular referencing may lead to the misunderstanding that the data and study have been peer-reviewed and published.

In summary, while the general approach and structure of the PBPK model is attractive and seems reasonable, several issues regarding the ultimate accuracy of the PBPK model over the concentrations of interest remain unresolved. More information is needed with regard to validation of the predicted results of the PBPK model. The effects of some variations in model design on the final results needs to be quantified. Further research is needed to elucidate the relative importance of the methylene chloride-glutathione conjugates and the MFO pathway in human carcinogenesis. The accuracy of estimates of the individual parameters is highly variable. The sensitivity of the model to changes in parameter values should be further evaluated with regard to the uncertainty in the parameter values. In addition, sites other than the lung and liver should be assessed in terms of their contribution to metabolism and their susceptibility to carcinogenicity. On the other hand, the PBPK approach does allow for consideration of pertinent data that cannot be taken into account in the applied dose approach. Thus, despite the concerns enumerated above, cautious application of the PBPK model

to methylene chloride risk assessment is appropriate in conjunction with other more traditional approaches.

8.6 Conclusions

A variety of approaches were used to develop models to obtain estimates of cancer risk associated with exposure to CH_2Cl_2. The results obtained from those models, which DHS staff conclude are most relevant to the risk assessment of airborne CH_2Cl_2, are summarized in Table 8-7. The values shown represent the potential increase in cancer risk associated with continuous lifetime exposure to 1 ppm of CH_2Cl_2 in ambient air. The risks estimated for a 1 ppb lifetime exposure to CH_2Cl_2, based on induction of lung tumors, range from 1×10^{-6} to 10×10^{-6} (0.3 to 3×10^{-6} for 1 $\mu g/m^3$ for exposure). The State of New York reported a unit risk of 2.7×10^{-6} for a lifetime exposure to 1 $\mu g/m^3$ (Riano and Rest 1986). The PBPK-adjusted values required parameter estimates from a model as reported by HRAC (EPA 1987a), EPA (1987b) and the CPSC (Cohn 1987). The lower estimate (1×10^{-6}/ppb) incorporates a complete pharmacokinetic adjustment as calculated by EPA (1987b). DHS staff believe that the complete pharmacokinetic adjustment retains considerable uncertainty. In contrast, the applied dose value (10×10^{-6}/ppb) does not incorporate any of the available pharmacokinetic information, and thus ignores information regarding saturation of the MFO pathway in the NTP study; the likely result is that the applied dose value overestimates the risk. The high-to-low dose adjustment used by CPSC to generate a risk of 4×10^{-6}/ppb incorporates information regarding saturation of the MFO pathway in the NTP study. However, the high-to-low dose adjustment assumes a negligible contribution by the MFO pathway to the cancer risk of CH_2Cl_2, and thus may underestimate the

risk. EPA staff have indicated that incorporation of the MFO pathway would increase the value only slightly. DHS staff conclude that the full range of values presented above is scientifically plausible, however, after evaluation of the HRAC data (EPA 1987a) and other available evidence presented Sections 2 and 8, the CPSC adjusted value (Cohn 1987) of 4×10^{-6}/ppb appears to be the most likely estimate of the risk of CH_2Cl_2 exposure.

Air monitoring in several areas in California indicates that approximately 20.3 million people are exposed to a range of CH_2Cl_2 concentrations from 1.1 to 2.4 ppb weighted by population. This range of exposure would be associated with an upper 95% confidence interval of risk ranging from 1×10^{-6} (1.1 ppb $\times$ 1×10^{-6}/ppb) to 24×10^{-6} (2.4 ppb $\times$ 10×10^{-6}/ppb). With an estimated exposed population of 20.3×10^{6}, the 95% upper confidence limit range of estimates of excess cancer cases over a lifetime exposure to CH_2Cl_2 would be 20 to 500. This is the range of plausible upper bound estimates, and due to the uncertainties in the risk extrapolation process (over five orders of magnitude), the actual number of excess cancer cases may be as low as zero.

Air monitoring of CH_2Cl_2 in the South Coast basin has yielded an estimated range of ambient concentrations of 1.5 to 3.1 ppb weighted by population. This exposure would be associated with an upper 95% confidence interval of risk ranging from 2×10^{-6} (1.5 ppb $\times$ 1.2 $\times 10^{-6}$/ppb) to 3.0×10^{-5} (3.1 ppb $\times$ 10×10^{-6}/ppb). With an estimated population of 10.09×10^{6}, the 95% upper confidence limit range of estimates of excess cancer cases over a lifetime due to exposure to CH_2Cl_2 would not likely exceed 20 to 300. Due to the uncertainties in the extrapolation process (five orders of magnitude) the actual number of cancer cases may be as low as zero.

Table 8-6

DIFFERENCES IN TISSUE SUSCEPTIBILITY TO THE PBPK DOSE

Exposure[a] (ppm)	LUNG		LIVER	
	PBPK Dose[b]	Tumors	PBPK Dose	Tumors
0	0	3/50	0	3/50
2000	321	30/48	785	16/48
4000	482	41/48	1670	40/48
Potency of PBPK dose[c] (per PBPK unit)	3.9×10^{-3}		4.5×10^{-4}	

[a]Exposure of B6C3F1 mice for 6 hours/day, 5 days/week.

[b]Internal dose calculated by Rietz et al. (1988) in average mg equivalents of CH_2Cl_2 metabolized by GST pathway per day per liter volume of tissue. The values were corrected for fraction of a lifetime exposed.

[c]Potency estimate using Global 86 per mg equivalent of CH_2Cl_2 metabolized by the GST pathway per day per liter volume of tissue.

TABLE 8-7

SUMMARY OF HUMAN CANCER RISKS ASSOCIATED WITH CONTINUOUS
LIFETIME EXPOSURE TO 1 ppb CH_2Cl_2 BASED ON

LUNG TUMORS IN FEMALE MICE USING VARIOUS MODELS AND ASSUMPTIONS[a]

Exposure Model	Mathematical Model	Endpoint	Increased Lifetime Cancer Risk Due to Continuous Lifetime Exposure to 1 ppb CH_2Cl in Ambient Air
Applied Dose	Linearized Multistage	Lung Tumors	9×10^{-6}
Applied Dose	Time-Dependent Multistage	Lung Tumors	10×10^{-6}
High-to-Low Dose[b]	Linearized Multistage	Lung Tumors	4×10^{-6}
High-to-Low Dose	Time-Dependent Multistage	Lung Tumors	5×10^{-6}
Total PBPK[c]	Linearized Multistage	Lung Tumors	1×10^{-6}

[a]Surface area conversion for rodent to human risks. Risk estimates are reported at 95% upper bound values.

[b]High-to-Low dose adjustment for saturation of MFO pathway based on the HRAC report (EPA 1987a).

[c]High-to-Low dose adjustment for saturation of MFO pathway and species-to-species adjustment based on the HRAC report (EPA 1987a).

9.0 CONCLUSIONS

The evidence characterizing acute, subchronic, and chronic health effects of exposure to CH_2Cl_2 may be summarized as follows. Acute toxicity studies conducted in laboratory animals indicate that the central nervous system is the primary target organ, with nervous system depression appearing at dose levels as low as 500 ppm. The available experimental studies of acute human exposure to CH_2Cl_2 suggest that it caused central nervous system depression and altered behavior at similar concentrations. The levels of CH_2Cl_2 required to elicit such effects are well above those which can be expected to be encountered in ambient air.

Subchronic or chronic exposure of experimental animals to 500 to 1,000 ppm or more of CH_2Cl_2 has been reported to cause behavioral changes, hepatic dysfunction, and, less frequently, lung lesions. There have been relatively few reports of subchronic or chronic toxic effects resulting from exposure of humans to CH_2Cl_2. Subchronic and chronic effects reported include alterations in carboxyhemoglobin levels, encephalosis, toxemia, and bilateral temporal lobe degeneration. In many cases, it is unclear whether the reported toxicities were caused by the direct action of CH_2Cl_2 or by the indirect action of carbon monoxide, a major metabolite of CH_2Cl_2. These effects are unlikely to occur at ambient concentrations.

The reproductive and teratogenic potential of CH_2Cl_2 cannot be adequately characterized because the data to evaluate toxicity to germ cells and potential teratogenicity are limited. The minor effects that have been

observed may have resulted from maternal toxicity due to carboxyhemoglobinemia.

Methylene chloride is a weak mutagen as indicated by positive responses in the Salmonella and E. coli bacteria test systems and in the yeast Saccharomyces. Methylene chloride induces cell transformation and chromosomal aberrations in vivo. Other in vitro and in vivo test systems designed to detect gene mutations and DNA binding have resulted in negative or equivocal results, although tests of clastogenic potential were positive.

There is sufficient evidence to regard CH_2Cl_2 as a probable human carcinogen based on responses in the B6C3F1 mouse liver and lung and the F344 rat mammary gland. Although a positive response was not obtained in the B6C3F1 mice exposed to CH_2Cl_2 in drinking water, the dose levels per unit bodyweight were lower than the levels resulting in positive effects in B6C3F1 mice exposed via inhalation. The available evidence from human epidemiology studies should be regarded as inadequate. Applying the EPA's criteria for evaluating the overall weight of evidence of carcinogenicity to humans places CH_2Cl_2 in Group B2. The EPA suggests that agents classified in Group B2 are "probable" human carcinogens. This category includes agents for which there is inadequate evidence from human epidemiologic studies and sufficient evidence from animal studies. An International Agency for Research on Cancer working group has recently evaluated the potential carcinogenicity of CH_2Cl_2 and also found sufficient evidence for carcinogenicity in animals, inadequate evidence in humans, and thus it recommended that CH_2Cl_2 be classified as a Group 2B agent (IARC 1986). International Agency for Research on Cancer suggests that agents classified in Group 2B are "possibly carcinogenic to humans."

There is no evidence from the known metabolic pathways for CH_2Cl_2 to suggest that there may be a biochemical threshold for carcinogenic action. Additionally, because the metabolism of CH_2Cl_2 results in mutagenic metabolites, a carcinogenic threshold dose cannot be estimated.

Mathematical models for the prediction of cancer risk associated with CH_2Cl_2 consistent with genotoxic and cell proliferation mechanisms of tumor induction were discussed. Cancer risks were estimated using models based on standard assumptions regarding biological mechanisms of carcinogenesis that fit the experimental data. Low-dose linear terms for the models were estimated and used as measures of cancer potency. The Andersen et al. (1987) PBPK model was also used and adjusted to account for exposure to a delivered dose of the putative biologically active CH_2Cl_2 metabolite. Estimation of delivered dose was based on several additional assumptions, so the potency developed in this manner should be viewed with caution. The DHS staff have presented the potency estimates developed by the EPA and the U. S. Consumer Product Safety Commission using a PBPK model. Thus, the range of risks presented include standard applied dose calculations and internal dose calculations.

Assuming that the airborne concentrations of CH_2Cl_2 to which animals were exposed can be extrapolated between species, potency estimates for CH_2Cl_2 were calculated using dose-response models that are linear at low doses. The 95% upper bound for unit risk estimates span the range 1×10^{-6} to 10×10^{-6} per ppb (or 3×10^{-7} to 3×10^{-6} per $(\mu g/m^3)$).

REFERENCES

ABRAHAMSON, S. and VALENCIA, R. 1980. Evaluation of substances of interest for genetic damage using Drosophila melanogaster. Prepared for FDA Contract No. 233-77-2119

AHMED, A.E. and ANDERS, M.W. 1976. Metabolism of dihalomethane to formaldehyde and inorganic chloride. Drug Metab. Dispos. 4:357-361

AHMED, A.E. and ANDERS, M.W. 1978. Metabolism of dihalomethanes to formaldehyde and inorganic halides. II. Studies on the mechanism of the reaction. Biochem. Pharmacol. 27:2021-2025

AHMED, A.E., KUBIC, V.L., STEVENS, J.L. and ANDERS. M.W. 1980. Halogenated methanes: Metabolism and toxicity. Feder. Proceed. 39, 1:3150-3155

ALTMAN, N.H. and GOODMAN, P.G. 1979. Neoplastic diseases. Chpt. 13. In: The Laboratory Rat. Vol. I. Biology and Diseases. Baker, H.J., Lindsey, J.R., and Weisbroth, S.H., eds., Academic Press, New York. pp. 339-340, 361-362

ANDERS, M.W. and SUNRAM, J.M. 1982. Transplacental passage of dichloromethane and carbon monoxide. Toxicol. Lett. 12:231-234

ANDERS, M.W., KUBIC, V.L., and AHMED, A.E. 1977. Metabolism of halogenated methanes and macromolecular binding. J. Environ. Pathol. Toxicol. 1:117-124

ANDERSEN, M.E., GARGAS, M.L., and RAMSEY J.C. 1984. Inhalation pharmacokinetics: Evaluating systemic extraction, total in vivo metabolism, and the time course of enzyme induction of inhaled styrene in rats based on arterial blood:inhaled air concentration ratios. Toxicol. Appl. Pharmacol. 73:176-187

ANDERSEN, M.E., CLEWELL, M.J., III, GARGAS, M.L., SMITH, F.A., and REITZ, R.H. 1987. Physiologically-based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol. Appl. Pharmacol. 87:185-205

ANGELO, M.J., BISCHOFF, K.B., PRITCHARD, A.B. and PRESSER, M.A. 1984. A physiological model for the pharmacokinetics of methylene chloride in $B_6C_3F_1$ mice following i.v. administration. J. Pharmacokinet. Biopharm. 12, 4:413-436

ANGELO, M.J., PRITCHARD, A.B. 1984. Simulations of methylene chloride pharmacokinetics using a physiologically based model. Reg. Toxicol. Pharmacol. 4: 329-339.

ANGELO, M.J., PRITCHARD, A.B., HAWKINS, D.R., WALLER, A.R., and ROBERTS, A. 1986a. The pharmacokinetics of dichloromethane I. Disposition in $B6C3F_1$ mice following intravenous and oral administration. Food Chem. Toxicol. 24, 9:965-974

ANGELO, M.J., PRITCHARD, A.B., HAWKINS, D.R., WALLER, A.R., and ROBERTS, A. 1986b. The pharmacokinetics of dichloromethane II. Disposition in Fischer 344 rats following intravenous and oral administration. Food Chem. Toxicol. 24, 9:975-980

ARANYI, C., O'SHEA, W.J., GRAHAM, J.A. and MILLER, F.J. 1986. The effects of inhalation of organic chemical air contaminants on murine lung host defenses. Fundam. Appl. Toxicol. 6:713-720

ARMITAGE, P. and DOLL, R. 1957. A two-stage theory of carcinogenesis in relation to the age distribution of human cancer. Br. J. Cancer 11:161-169

ARMITAGE, P. and DOLL, R. 1954. The age distribution of human cancer and a multistage theory of carcinogenesis. Br. J. Cancer 8:1-12

AVIADO, D.M. 1975. Toxicity of aerosol propellants in the respiratory and circulatory systems: X. Proposed classification. Toxicol. 3:321-322

AVIADO, D.M, ZAKHARI, S., and WATANABE, T. 1977. Non-Fluorinated Propellants and Solvents for Aerosols. CRC Press, Cleveland

BAKHISHEV. G.N. 1975. Relationship between chemical structure and toxicity for some aliphatic halogenated series. Fiziologicheski Aktivnye Veshesta 7:35-36

BALMER, M.F., SMITH, F.A., LEACH, L.J., and YUILLE, C.L. 1976. Effects in the liver of methylene chloride inhaled alone and with ethyl alcohol. Am. Ind. Hyg. Assoc. J. 37:345-352

BARBER, E.D., DONISH, W.H., and MUELLER, K.R. 1980 (Abstract). Quantitative Measurement of the Mutagenicity of Volatile Liquids in the Ames Salmonella/Microsome Assay. P-39, Environmental Mutagen Society, 11th Annual Meeting (As cited in EPA 1985a)

BARROWCLIFF, D.F. 1978. Chronic carbon monoxide posioning caused by methylene chloride paint stripper. Med. Sci. Law 18:238

BARROWCLIFF, D.F. and KNELL, A.J. 1979. Cerebral damage due to endogenous chronic carbon monoxide poisoning caused by exposure to methylene chloride. J. Soc. Occup. Med. 29:12-14

BERGER, M., and FODOR, G.G. 1968. CNS disorders under the influence of air mixtures containing dichloromethane. Zentralbl. Bakteriol. (Ref.) 215:517

BERGER, M.L. and SOZERI, T. 1987. Rapid halogenated hydrocarbon toxicity in isolated hepatocytes is mediated by direct solvent effects. Toxicol. 45:319-330

BLACK, O. and HOWERTON, B.K. 1984. Glutathione S-transferase activity in rat pancreas. J. Natl. Cancer Inst. 72:121-123

BOND, G. 1988. Personal communication. Dow Chemical Company.

BORNMANN, G. and LOESER, A. 1967. Zur Frage einer cronish-toxischen Wirkung von Dischloromethan. Zeit. Lebenomit.-Untersuch. Forsch. 136:14-18

BORNSCHEIN, R.L., HASTINGS, L., and MANSON, L. 1980. Behavioral toxicology in the offspring of rats following maternal exposure to dichloromethane. Toxicol. Appl. Pharmacol. 52:29-37

BRADLEY, M.O. 1985. Cytotoxicity as a mechanism of carcinogenesis. In: Basic and Applied Mutagenesis. Muhammed, A. and von Borstel, R.C., eds., Plenum Press, New York, pp. 99-109

BUIE, S.E., PRATT, D.S. and MAY, J.J. 1986. Diffuse pulmonary injury following paint remover exposure. Am. J. Med 81:702-704

BUREK, J.D., NITSCHKE, K.D., BELL, T.J., WACKERLE, D.L., CHILDS, R.C., BEYER, J.E., DITTENBER, D.A., RAMPY, L.W., and McKENNA, M.J. 1984. Methylene chloride: A two year inhalation toxicity and oncogenicity study in rats and hamsters. Fundam. Appl. Toxicol. 4:30-47

CALLEN, D.F., WOLF, C.R., and PHILPOT, R.M. 1980. Cytochrome P450-mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae. Mutat. Res. 77:55-63

CARLSON G.P. and KIM, Y.C. 1986 (Abstract) Effect of forced exercise on dichloromethane blood levels and metabolism to carbon monoxide in rats. IRCS Journal of Medical Science 14:795

CASTAGNA, M., TAKAI, Y., KARBUCHI, K. et al. 1982. Direct activation of calcium-activated phoshalipid-dependent protein kinase by tumor-promoting pharbol esters. J. Biol. Chem. 257:7874-7851

CHELLMAN, et al. 1986. Inhibition of the acute toxicity of methylene chloride in male B6C3F1 mice by glutathione depletion. Toxicol. Appl. Pharmacol. 86:93-104

CIUCHTA, H.P., SAVELL G.M., SPIKER R.C. 1979. The effect of alcohols and toluene upon methylene chloride-induced carboxyhemoglobin in the rat and monkey. Toxicol. Appl. Pharmacol. 49:347-354

COBURN, R.E. 1970. The carbon monoxide body stores. In Biological effects of carbon monoxide. Proceedings of a Conference, New York Academy of Science, New York Academy of Science, New York, January 12-14, 1970 Ann. N.Y. Acad. Sci. 174:11-22

COHN, M.S. 1987. Updated risk assessment for methylene chloride (dichloromethane). In: Methylene chloride briefing package for U.S. Consumer Products Safety Commission, Washington, D.C.

COLLIER, H. 1936. Methylene dichloride intoxication in industry--a report of two cases. Lancet 1:594-595

CORNELL, N.W., HANSCH, C., KIM, K.H. and HENEGAR, K. 1983. The inhibition of alcohol dehydrogenase _in vitro_ and in isolated hepatocytes by 4-substituted pyrazoles. Arch Biochem. Biophys. 227:81-90

CRAMER, H. 1946. Mathematical Methods of Statistics. Princeton University Press

CROSFILL, M.L. AND WIDDICOMBE, J.G. 1961.£ Physical characteristics of the chest and lungs and the work of breathing in different mammalian species. J. Physiol. 158:1-14

CRUMP, K.S., HOEL, D., LANGLEY, C., and PETO, R. 1976. Fundamental Carcinogenic processes and their implications for low-dose risk assessment. Cancer Res. 36:2973-9

CUNNINGHAM, M.L., GANDOLFI, A.J., BRENDEL, K., and SIPES, I.G. 1981. Covalent binding of halogenated volatile solvents to subcellular macromolecules in hepatocytes. Life Sci. 29:1207-1212

DISTLERATH, L.M., LOPER, J.C., and DEY, C.R.. 1984. Aliphatic halogenated hydrocarbons produce volatile Salmonella mutogens. Mutat. Res. 136:55-64

DiVINCENZO, G.D., YANNO, F.J., and ASTILL, B.D. 1972. Human and canine exposures to methylene chloride vapor. Am. Ind. Hyg. Assoc. J. 33:125-135

DiVINCENZO, G.D. and HAMILTON, M.L. 1975. Fate and disposition of 14C-methylene chloride in the rat. Toxicol. Appl. Pharmacol. 32:385-393

DiVINCENZO, G.D. and KAPLAN, C.J. 1981a. Uptake, metabolism, and elimination of methylene chloride vapor by humans. Toxicol. Appl. Pharmacol. 59:130-140

DiVINCENZO, G.D. and KAPLAN, C.J. 1981b. The effect of exercise or smoking on the uptake, metabolism and excretion of methylene chloride vapor. Toxicol. Appl. Pharmacol. 59:141-148

DOW CHEMICAL COMPANY. 1980. Methylene chloride: A two-year inhalation toxicity and oncogenicity study in rats and hamsters. FYI-OTS-0281-0097. Follow-up response A. Washington, D.C.: U.S. Environmental Protection Agency, Office of Toxic Substances

DOW CHEMICAL COMPANY. 1982. Nitschke, K.D., Bell, T.J., Rampy, L.W., and McKenna, M.J. Methylene chloride: A two-year inhalation toxicity and oncogenicity study in rats. Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical Company, Midland, MI (October 11, 1983)

ECETOC Internal Document. 1987. The assessment of carcinogenic hazard for human beings exposed to methylene chloride. Technical Report No. 26. ISSN 07773-8072-26

ECETOC Internal Document. 1988. Methylene chloride (dichloromethane): Human risk assessment using experimental animal data. Technical Report No. 32. ISSN 07773-8072-32

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1983. Reportable quantity document for dichloromethane. ECAO-CIN-R104

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1985a. Health Assessment Document for Dichloromethane (Methylene Chloride). Final Report. EPA 600-8-82/004F

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1985b. Addendum to the Health Assessment Document for Dichloromethane (Methylene Chloride). Updated Carcinogenicity Assessment of Dichloromethane (Methylene Chloride). EPA 600-8-82/004F

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1987a. External Review Draft. Technical analysis of new methods and data regarding dichloromethane. EPA 600-8-8-7/029A.

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1987b. External Review Draft. Update to the Health Assessment Document and Addendum for Dichloromethane (Methylene Chloride): Pharmacokinetics, Mechanism of Action, and Epidemiology. EPA 600-8-87/030A.

FEDERAL REGISTER, August 4, 1983 48(151):35508

FENN, W.O. 1970. The burning of CO in tissues. Ann. N.Y. Acad. Sci. 174:64-71

FODOR, G.G. and WINNEKE, G. 1971. Nervous system disturbances in men and animals experimentally exposed to industrial solvent vapors in England. In Proceedings of the 2nd International Clean Air Congress. Academic Press, New York. pp. 238-243

FOOD SOLVENTS WORKSHOP. 1984. I: Methylene chloride. 1984. Proceedings of Workshop March 8-9, 1984, Bethesda, Maryland

FORSTER, H.V., GRAFF, S., HAKE, C.L., SOTO, R., and STEWART, R.D. 1974. Pulmonary-hematological studies on humans during exposure to methylne chloride. Report No. NIOSH-MCOW-ENVM-MC-74-4. The Medical College of Wisconsin. Department of Environmental Medicine, Milwaukee

FRIEDLANDER, B.R. 1987. Personal communications. Eastman Kodak Company

FRIEDLANDER, B.R., HEARNE, T., HALL, S. 1978. Epidemiologic investigation of employees chronically exposed to methylene chloride. Mortality analysis. J. Occup. Med. 20:657-666

FRIEDLANDER, B.R., HEARNE, F.T., PIFER, J.W., and GROSE, F.H. 1986. Epidemiologic evidence regarding methylene chloride. Presented at the Winter Toxicology Forum, Washington, D.C., February 18

FRIEDLANDER, B.R., PIFER, J.W., and HEARNE, F.T. 1985. 1964 methylene chloride cohort mortality study. Update through 1984. Eastman Kodak Company. Rochester, N.Y.

GAMBERALE, F., ANNWALL, B.A., and HULTENGREN, M. 1975. Exposure to methylene chloride: II. Psychological functions. Scand. J. Work. Environ. Health 1:95-103

GARGAS, M.L., CLEWELL, H.J., and ANDERSEN, M.E. 1986. Metabolism of inhaled dihalomethanes *in vivo*: Differentiation of kinetic constants for two independent pathways. Toxicol. Appl. Pharmacol. 82:211-223

GLATZEL, W., TIETZE, K., GUTEWORT, R. and PANKOW, D. 1987. Interaction of dichloromethane and ethanol in rats. Toxicokinetics and nerve conduction velocity. Alcohol: Clin. Exper. Res. 11:450-452

GOCKE, E., KING, M.T., ECKHARDT, K., and WILD, D. 1981. Mutagenicity of cosmetics ingredients licensed by the European communities. Mutat. Res. 90:91-109

GRADISKI, D., MEGADUR, J.L., BAILLOT, M., DANIERE, M.C., and SCHUH, M.B. 1974. Comparative toxicity of the principle chlorinated aliphatic solvents. J. Eur. Toxicol. 7:247

GREEN, T. 1983. The metabolic activation of dichloromethane and chlorofluoromethane in a bacterial mutation assay using *Salmonella typhimurium*. Mutat. Res. 118:227-288

GREEN, T., PROVAN, W.M., COLLINGE, D.C., and GUEST, A.E. Internal ECETOC Document. 1986a. Methylene Chloride (Dichloromethane): Interaction with Rat and Mouse Liver and Lung DNA *in vivo*. Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/851

GREEN, T., PROVAN, W.M., GOWANS, N. Internal ECETOC Document. 1986b. Methylene Chloride (Dichloromethane): *In vivo* inhalation pharmacokinetics and metabolism in F344 rats and B6C3F1 mice Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/880

GREEN, T., NASH, J.A., and MAINWARDING., G. 1986c. Methylene Chloride (Dichloromethane): *In vitro* metabolism in rat, mouse, hamster liver and lung fractions and in human liver fractions. Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/879

GREEN, T., NASH, J.A., and HILL, S.J. Internal ECETOC Document. 1987a. Methylene Chloride (Dichloromethane): Glutathione-S-transferase metabolism *in vitro* in rat, mouse, hamster and human liver cytosol fractions. Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/934

GREEN, T., NASH, J.A., HILL, S.J., and FOSTER, J.R. Internal ECETOC Document. 1987b. Methylene Chloride (Dichloromethane): The effects of exposure to 4000 ppm on mouse lung enzymes. Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/935

GREEN, T., PROVAN, W.M., GOWANS, N. Internal ECETOC Document. 1987c. Methylene Chloride (Dichloromethane): In vivo inhalation pharmacokinetics in B6C3F1 mice (using stable isotpes) and F344 Rats. Imperial Chemical Industries PLC Central Toxicology Laboratory Alderley Park Macclesfield Cheshire, United Kingdom. Report No. CTL/R/931

GREEN, T., PROVAN, W.M., COLLINGE, D.C. and GUEST, A.E. 1988. Macromolecular interactions of inhaled methylene chloride in rats and mice. Toxicol. Appl. Pharmacol. 93:1-10

GUENGERICH, F.P., CRAWFORD, W.M., Jr., DOMORADZKI, J.Y., MACDONALD, T.L., and WATANABE, P.G. 1980. In vitro activation of 1,2-dichloroethane by microsomal and cytosolic enzymes. Toxicol. Appl. Pharmacol. 55:303-317

GUESS, H.A., CRUMP, K.S., and PETO, R. 1977. Uncertainty estimates for low-dose extrapolations of animal carcinogenicity data. Cancer Res. 37:3475-3483

HAKE, C.L, STEWART, R.D., FORSTER, H.V., LEBRUN, A.J., PETERSON, J.E., and WU, A. 1974. Results of the controlled exposure of human females to the vapor of methylene chloride. Report No. NIOSH-MCOW-ENVM-MC-74-3. Milwaukee, Wis. The Medical College of Wisconsin, Department of Environmental Medicine, Milwaukee

HALPERT, J.R., BALFOUR C., MILLER, N.E. and KAMINSKY, L.S. 1986. Dichloromethyl compounds as mechanism-based inactivators of rat liver cytochromes P-450 in vitro. Molec. Pharmacol. 30:19-24

HARDIN, B.D. and MANSON, J.M. 1980. Absence of dichloromethane teratogenicity with inhalation exposure in rats. Toxicol. Appl. Pharmacol. 52:22-28

HARKEY, M.R., SETTLE, W.L., JAMES, T.L., and TREVOR, A.J. 1979. Spectral studies on the interaction of dichloromethane with hemoglobin. Molec. Pharmacol. 16:1065-1074

HAUN, C.C., VERNOT, E.H., DARMER, K.I., and DIAMOND, S.S. 1972. Continuous animal exposure to low levels of dichloroemethane. AMRL-TR-130, No. 2. In Proceedings of the 3rd Conference on Environmental Toxicology, Wright-Patterson Air Force Base, OH, Aerospace Medical Research Laboratory, pp. 199-208

HEARNE, F.T. and FRIEDLANDER, B.R. 1981. Follow-up of methylene chloride study. J. Occup. Med. 23:660

HEARNE, F.T., GROSE, R., PIFER, J.W., FRIEDLANDER, B.R., and RALEIGH, R.L. 1987. Methylene Chloride Mortality Study: Dose response to characterization and animal model comparison. J. Occup. Med. 29:217-228

HEPPEL, L.A. and NEAL, P.A. 1944. Toxicology of dichloromethane (methylene chloride): II. Its effects on running activity in the male rat. J. Ind. Hyg. Toxicol. 26:17-21

HEPPEL, L.A., NEAL, P.A., PERRIN, T.L., ORR, M.L., and PORTERFIELD, V.T. 1944. Toxicology of dichloromethane (methylene chloride): I. Studies on effects of daily inhalation. J. Ind. Hyg. Toxicol. 26:8-16

HEPPEL, L.A. and PORTERFIELD. 1948. Enzymatic dehalogenation of certain brominated and chlorinated compounds. J. Biol. Chem. 76:763-769

HILL, D.L., SHIH, T.W., JOHNSTON, T.P., and STRUCK, R.F. 1978. Macromolecular binding and metabolism of the carcinogen 1,2-dibromoethane. Cancer Res. 38:2438-2442

HOGAN, G.K., SMITH, R.G., and CORNISH, H.H. 1976 (Abstract). Studies on the microsomal conversion of CH2Cl2 to CO. Toxicol. Appl. Pharmacol. 37:112

HOROWITZ, B.Z. 1986. Carboxyhemoglobinemia caused by inhalation of methylene chloride. Am. J. Emer. Med. 4(1):48-51

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC). 1986. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. 41:43-85. IARC, Lyon, France.

JACOBY, R.O., BHATT, P.N., and JONAS, A.M. 1979. Viral diseases. Chpt. 11. In: The Laboratory Rat Vol. I. Biology and Diseases. Baker H.J., Lindsey, J.R., and Weisbroth, S.H., eds., Academic Press, New York. pp. 284-294

JAKOBY, W.B. and HABIG, W. 1980. Glutathione Transferases. Jakoby, W.B., ed. Enzymatic Basis of Detoxification, Vol. II. Academic Press, New York, pp. 63-64

JONGEN, W.M.F., ALINK, G.M., and KOEMAN, J.H. 1978. Mutagenic effect of dichloromethane on Salmonella typhimurium. Mutat. Res. 56:245-248

JONGEN, W.M.F., LOHMAN, P.H.M., KETTENHAGEN, M.J., ALINK, G.M., BERENDS, F., and KOEMAN, J.H. 1981. Mutagenicity testing of dichloromethane in short-term mammalian test systems. Mutat. Res. 81:203-213

JONGEN, W.M.F., HARMSON, E.G.M., ALINK, G.M., and KOEMAN, J.H. 1982. The effect of glutathione conjugation and microsomal oxidation on the mutagenicity of dichloromethane in S. typhimurium. Mutat. Res. 95:183-189

KALBFLEISCH, J.D., KREWSKI, D.R., and VAN RYZIN, J. 1983. Dose-response models for time-to-response toxicity data. Can. J. Stat. 11:25-49

KANADA, T. and UYETA, M. 1978 (Abstract). Mutagenic screening of organic solvents in microbial systems. Mutat. Res. 54:215

KARLSSON, J.E., ROSENGREN, L.E., KJELLSTRAND, P. and HAGLID, K. G. 1987. Effects of low-dose inhalation of three chlorinated aliphatic organic solvents of deoxyribonucleic acid in gerbil brain. Scand. J. Work Environ. Health 13:453-458

KIM, Y.C. and CARLSON, G.P. 1986. The effect on an unusual workshift on chemical toxicity. I. Studies on the exposure of rats and mice to dichloromethane. Fundam. Appl. Toxicol. 6:162-171

KLAASEN, C.D. 1986. Distribution, excretion and absorption of toxicants. In: Casarett and Doull's toxicology: the basic science of poisons. Klaassen, C.D., Amdur, M.O., and Doull, J. eds., Macmillan Publishing Co., NY pp. 56-57

KNUDSON, A.G. 1985. Hereditary cancer, oncogenes, and antioncogenes. Cancer Res. 45:1437-1443

KOVAR, J. and KREWSKI, D. 1981. Risk 81: a computer program for low-dose extrapolation of quantal-response toxicity data. Health & Welfare, Ottawa, Ontario

KUBIC, V.L., ANDERS, M.W., ENGEL, R.R., BARROW, C.H., and CAUGHEY, W.S. 1974. Metabolism of dihalomethanes to carbon monoxide. I. In vivo studies. Drug Metab. Dispos. 2:53-57

KUBIC, V.L. and ANDERS, M.W. 1975. Metabolism of dihalomethanes to carbon monoxide. II. In vitro studies. Drug Metab. Dispos. 3:104-112

KUBIC, V.L. and ANDERS, M.W. 1978. Metabolism in dihalomethanes to carbon monoxide. III. Studies on the mechanism of the reaction. Biochem. Pharmacol. 27:2349-2355

KURPPA, K. and VAINIO, H. 1981. Effects of intermittent dichloromethane inhalation on blod carboxyhemoglobin concentration and drug metabolizing enzymes in rat. Res. Comm. Chem. Path. Pharmacol. 32:535-544

LAMBERTSEN, C.J. 1974. Effects of excessive pressures of oxygen, nitrogen, carbon dioxide, and carbon monoxide: Implications in aerospace, undersea, and industrial environments. In Mountcastle, V.B., Vol. 2, 13th ed. C.V. Mosby Co., St. Louis, Missouri

LORENZ, J., GLATT, H.R., FLEISCHMANN, R., FERLINZ, R. and OESCH, F. 1984. Drug metabolism in man and its relationship to that in three rodent species: Mono-oxygena se, eposide hydrolase, and elutathione-s-transferase activities in subcellular fractions of lung and liver. Biochem. Med. 32:43-56

LUOMANMAKI, K., and COBURN, R.E. 1969. Effects of metabolism and distribution of carbon monoxide on blood and body stores. Am. J. Physiol. 271:354-363

LUTZ, W.K. 1979. In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. Mutat. Res. 65:289-356

MACEWEN, J.D., VERNOT, E.H., and HAUN, C.C. 1972. Continuous animal exposure to dichloromethane. Report AMRL-TR-72-28. Aerospace Medical Research Lab. Wright-Patterson Air Force Base, Ohio, P 33

McCANN, J. and AMES, B.N. 1977. The Salmonella/microsome mutagenicity test: Predictive value for animal carcinogenicity. In: Origins of Human Cancer: Book C. Human Risk Assessment. Hiatt, H.H., Watson, J.D., and Winsten, J.A., eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. pp. 1431-1450

McCARROLL, N.E., CORTINA, T.A., ZITO, M.J., and FARROW, M.G. 1983. Evaluation of methylene chloride and vinylidene chloride in mutational assays. Environ. Mutagen 5:426-427

McGREGOR, D.G. 1979. Practical experience in testing unknowns in vitro. Chapter 4. In Paget, G.E., ed. Topics in Toxicology. Mutagenesis in submammalian systems: Status and Significance. University Park Press, Baltimore, Maryland. pp. 53-71

MCKENNA, M.J. and ZEMPEL, J.A. 1981. The dose-dependent metabolism of 14C-methylene chloride following oral administration to rats. Food. Cosmetic. Toxicol. 19:73-78

MCKENNA, M.J., ZEMPEL, J.A., BRAUN, W.H. 1982. The pharmacokinetics of inhaled methylene chloride in rats. Toxicol. Appl. Pharmacol. 65:1-10

MCKENNA, M.J., SAUNDEERS, J.H., BOECKLER, W.R., KARBOWSKI, R.J., NITSCHKE, K.D., and CHENOWETH, M.B. 1980 (Abstract). The pharmacokinetics of inhaled methylene chloride in human volunteers. Paper No. 176, 19th Annual Meeting of the Society of Toxicology, Washington, D.C., March 9-13

MENNEAR, J.H., MCCONNELL, E.E., HUFF, J.E. RENNE, R.A., AND GIDDENS, E. 1988. Inhalation toxicology and carcinogenesis studies of methylene chloride (Dichloromethane) in F344/N rats and B6C3F1 mice. Ann. New York Acad. Sci. Vol., 534 pp.343-351

MILLER, V.L., ENGEL, R.R., and ANDERS, M.W. 1973 (Abstract). In vivo metabolism of dihalomethanes to carbon monoxide. Pharmacologist 15:190

MILLER L., PATERAS, V., FRIEDERICI H., and ENGEL, G. 1985. Acute tubular necrosis after inhalation exposure to methylene chloride. Arch. Intern. Med. 145:145-146

MIRER F.E., SILVERSTEIN M., PARK R. 1988. Methylene chloride and cancer of the pancreas. J. Occup. Med. 210 (6):475-476

MOOLGAVKAR, S.H. and VENZON, D.J. 1979. Two-event models for carcinogenesis: Incidence curves for childhood and adult tumors. Math. Biosci. 47:55

MOOLGAVKAR, S.H. and KNUDSON, A.G. 1981. Mutation and cancer: A model for human carcinogenesis. JNCI 66:1037

MOOLGAVKAR, S.H. 1986. Carcinogenesis modeling: From molecular biology to epidemiology. Ann. Rev. Public Health 7:151-169

MORON, M.S., DEPIERRE, J.W., MANNERVIK, B. 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochem. Biophys. Acta 582:67-78

MORRIS, J.B., SMITH, F.A., and GARMAN, R.H. 1979. Studies on methylene chloride induced fatty liver. Exp. Mol. Pathol. 30:386-393

MOSKOWITZ, S. and SHAPIRO, H. 1952. Fatal exposure to methylene chloride vapor. Arch. Ind. Hyg. Occp. Med. 6:116-123

MUKHTAR, H., ZOETEMELK, C.E.M., BAARS, A.J., WIJNEN, J.T., BLANKENSTEIN-WIJNEN, L.M.M., KHAN, P.M., and BREIMER, D.D. 1981. Glutathione S-transferase activity in human fetal and adult tissues. Pharmacol. 22:322-329

NATIONAL CANCER INSTITUTE (NCI). 1981. Surveillance Epidemiology End Results. Incidence and Mortality Data: 1973-77. Monograph 57. U.S. Dept. of Health and Human Services. NIH Publ. No. 81-2330

NATIONAL INSTITUTE FOR OCCUPATIONAL HEALTH (NIOSH). 1972. Criteria for a Recommended Standard for Occupational Exposure to Carbon Monoxide. HSM 73-11000

NATIONAL TOXICOlOGY PROGRAM (NTP). 1982. Draft technical report on the carcinogenesis bioassay of dichloromethane (methylene chloride), gavage study. Research Triangle Park, NC, and Bethesda, Maryland

NATIONAL TOXICOLOGY PROGRAM (NTP). 1986. Toxicology and Carcinogenesis Studies of Dichloromethane (Methylene Chloride) (CAS No. 75-09-2) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). TR No. 306, NIH (USDHHS, PHS, NIH) Publication No. 86-2562

NESTMANN, E.R., LEE, E.G.H., MATULA, T.I., DOUGLAS, G.R., and MUELLER, J.C. 1980. Mutagenicity of constituents identified in pulp and paper mill effluents using the Salmonella/mammalian microsome assay. Mutat. Res. 79:203-212

NESTMANN, E.R., OTSON, R., WILLIAMS, D.T., and KOWBEL, D.J. 1981. Mutagenicity of paint removers containing dichloromethane. Cancer Lett. 11:295-302

NITSCHKE, K.D., EISENBRANDT, D.L., and LOMAX, L.G. 1985 (Unpublished Study). Methylene chloride: Two-generation inhalation reproduction study in Fischer 344 rats. Dow Chemical Co., Midland, Michigan

NITSCHKE, K.D., BUREK, J.D., BELL, T.J., KOCIBA, R.J., RAMPY, L.W., and MCKENNA, M.J. 1988a. Methylene chloride A two-year inhalation toxicity and oncogenicity study in rats. Fundam. Appl. Toxicol. 11:48-59.

NITSCHKE, K.D., EISENBRANDT, D.L., and LOMAX, L.G. and RAO, K.S., 1988b. Methylene chloride. Two generation inhalation reproductive study in rats. Fundam. Appl. Toxicol. 11:60-67

NORPOTH, K., WITTING, U., SPRINGORUM, M., and WITTING, C. 1974. Induction of microsomal enzymes in the rat liver by inhalation of hydrocarbon solvents. Int. Arch. Arbeitsmed. 33:315-321

NUNES, A.C. AND SCHOENBORN, B.P. 1973. Dicholoromethane and myoglobin function. Molec. Pharmocol. 9:835-839

OSTERMAN-GOLKAR. S., HUSSAIN, S., WALLES, S., ANDRSTAM, B., and SIGVARDSSON, K. 1983 Chemical reactivity and mutagenicity of some dihalomethanes. Chem-Biol Interactions 46:121-130

OTT, M.G., SKORY, L.K., HOLDER, B.B, BRONSON, J.M., and WILLIAMS, P.R. 1983a. Health evaluation of employees occupationally exposed to methylene chloride: General study design and environmental considerations. Scand. J. Work.Environ. Health 9:1-7

OTT, M.G., SKORY, L.K., HOLDER, B.B., BRONSON, J.M., and WILLIAMS, P.R. 1983b. Health evaluation of employees occupationally exposed to Methylene chloride: Mortality. Scand. J. Work Environ. Health 9:8-16

OTT, M.G., SKORY, L.K., HOLDER, B.B, BRONSON, J.M., and WILLIAMS, P.R. 1983c. Health evaluation of employees occupationally exposed to methylene chloride: Clinical laboratory evaluation. Scand. J. Work Environ. Health 9:17-25

OTT, M.G., SKORY, L.K., HOLDER, B.B, BRONSON, J.M., and WILLIAMS, P.R. 1983d. Health evaluation of employees occupationally exposed to methylene chloride: Twenty-four hour electrocardiographic monitoring. Scand. J. Work Environ. Health 9:26-30

OTT, M.G., SKORY, L.K., HOLDER, B.B., BRONSON, J.M., and WILLIAMS, P.R. 1983e. Health evaluation of employees occupationally exposed to Methylene chloride: Metabolism data and oxygen half-satuaration pressures. Scand. J. Work Environ. Health 9:31-38

PANKOW, D., PONSOLD, W., and MORITZ R.-P. 1985. Zur sauerstofftransportkapazitat des blutes under einflutz von ethanol und dichlormethan. Z. Gesamte Hyg. 31:460-463

PEROCCO, P. and PRODI, G. 1981. DNA damage by haloalkanes in human lymphocytes cultured in vitro. Cancer Lett. 13:213-218

PETERSON, J. 1978. Modeling the uptake metabolism and excretion of dichloromethane by man. Am. Ind. Hyg. Assoc. 39:41-47

PETO, R. 1978. Carcinogenic effects of chronic exposure to very low levels of toxic substances. Environ. Health Perspec. 22:155-159

PRICE, P.J., HASSETT, C.M., and MASFIELD, J.I. 1978. Transforming activities of trichloroethylene and proposed industrial alternatives. In Vitro 14:290-293

PRITCHARD, A. and ANGELO, M.J. 1982. Internal NCA Study. Comparative pharmacokinetic studies of methylene chloride in oil versus drinking water. Report commissioned by the National Coffee Association of USA Inc. August 1982

PRITCHARD, A., DOONAN, B., and ROURKE, R. 1982. Internal NCA Study. Methylene chloride: Effects of microsomal enzymes in mice. Study commissioned by the National Coffee Association of USA Inc.

PUTZ, V.R., JOHNSON B.L., and SETZER, J.V. 1976. A comparative study of the effect of carbon monoxide and methylene chloride on human performance. J. Environ. Pathol. Toxicol. 2:97-112

RANNUG, V., SUUDVALL A., and RAMEL, C. 1978. The mutagenic effect of 1,2-dichloroethane on Salmonella typhimurium. I. Activation through glutathione in vitro. Chem. Biol. Interact. 20:1-16

REDDY, J.K., AZARNOFF, D.L., and HIGNITE, C.E. 1980. Hypolipidaemic hepatic peroxisomal proliferators form a novel class of chemical carcinogens. Nature 283:397

REITZ, R.H., MENDRALA, A.L., PARK C.N., ANDERSEN, M.E. and GUENGERICH, F.P. 1988. Incorporation of in vitro enzyme data into the PB-PK model for methylene chloride (CH_2CL_2): Implications for risk assessment. Toxicol. Lett. 43:97-116

REITZ, R.H., SMITH, F.A., and ANDERSEN, M.E. 1986. (Abstract). In vivo metabolism of 14C-methylene chloride (MEC). Toxicologist 6:260

REITZ, R.H., MENDRALA. A.L. and SCHUMANN, A.M. 1987 (Unpublished). Methylene chloride-glutathione-S-transferase studies: in vitro experiments in humans, mice, rats, and hamsters. For submission to the EPA Science Advisory Board, Dow Chemical Company, Midland, MI.

RIANO, M. and REST. V.M. 1986. Methylene chloride (dichloromethane): Summary of toxicologic data and cancer risk assessment. New York State Department of Environmental Conservation, Albany, NY

RILEY, E.C., FASSETT, D.W., and SUTTON, W.L. 1966. Methylene chloride vapor in expired air of human subjects. Am. Ind. Hyg. Assoc. J. 27:341-348

RODKEY, F.L. and COLLISON, H.R. 1977a. Biological oxidation of 14C-methylene chloride to carbon monoxide and carbon dioxide by the rat. Toxicol. Appl. Pharmacol. 40:33-38

RODKEY, F.L. and COLLISON, H.R. 1977b. Effect of dihalogenated methanes on the in vivo production of carbon monoxide and methane by rats. Toxicol. Appl. Pharmacol. 40:39-47

ROGHANI, M., DASILVA, C., and CASTOGNA, M. 1987. Tumor promotor chloroform is a potent protein kinase C activator. Biochem. Biophys. Res. Commun. 142:738-744

ROSENGREN, L.E., KJELLSTRAND, P., AURELL, A., and HAGLID, K.G. 1986. Irreversible effects of dichloromethane on the brain after long term exposure: A quantitiative study of DNA and the glial cell marker proteins S-100 and GFA. Br. J. Ind. Med. 43:291-299

ROTH, R.P., DREW, R.T., LO, R.J., and FOUTS, J.R. 1975. Dichloromethane inhalation, carboxyhemoglobin concentration, and drug metabolizing enzymes in rabbits. Toxicol. Appl. Pharmacol. 33:427-437

SAMOILOFF, M.R., SCHULZ, S., JORDAN, Y., DENICH, K., and ARNOTT, E. 1980. A rapid simple long-term toxicity assay for aquatic contaminants using the nematode *Panagrellus* *redivivus*. Can. J. Fish Aquat. Sci. 37:1167-1174

SAVOLAINEN, H., PFAFFLI, P., TENGEN, M., and VAINIO, H. 1977. Biochemical behavioral effects of inhalation exposure to tetrachloroethylene and dichloromethane. J. Neuropath. Exp. Neurol. 36:941-949

SCHUMANN, A.M., NITSCHKE, K.D., FOX, T.R., and WATANABE, P.G. 1983. (14C) Methylene Chloride: Pharmacokinetics and Interaction with Hamster and Rat Tissue Macromolecules. Report of the Toxicology Research Laboratory. Dow Chemical Company, Midland, Michigan

SCHWETZ, B.A., LEONG, B.K.J., and GEHRING, P.J. 1975. The effect of maternally inhaled trichloroethylene, perchloroethylene, methylchloroform, and methylene chloride on embryonal and fetal development in mice and rats. Toxicol. Appl. Pharmacol. 32:84-96

SEIDERGARD, J. and PERO, R.W. 1985. The hereditary transmission of high glutathione transferase activity towards transstilbene oxide in human mononuclear leukocytes. Hum. Genet. 69:66-68

SEIDERGARD, J., PERO, R.W., MILLER, D.G., and BEATTIE, E.J. 1985. A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer. Carcinogenesis 7:751-753.

SEROTA, D.G., THAKAR, A.K., ULLAND, B.M., KIRSCHMAN, J.C., BROWN, N.M., COOTS, R.H. and MORGANEIDGE, K. 1986a. A two year drinking water study of dichloromethane on rodents: I. Rats Food Chem. Toxicol. 24:951-958

SEROTA, D.G., THAKAR, A.K., ULLAND, B.M., KIRSCHMAN, J.C., BROWN, N.W.M, COOTS, R.H. and MORGAREIGE, K. 1986b. A two year drinking water study of dichloromethane on rodents: II Mice. Food Chm. Toxicol. 24:959-963

SETTLE, W. 1975 (Abstract). Role of changes in carbon monoxide-hemoglobin finding in methylene chloride toxicity. Fed. Proc. 34:229

SHELDON, T., RICHARDSON, C.R., and ELLIOTT, B.M. 1987. Inactivity of methylene chloride in the mouse bone marrow micronucleus assay. Mutagen. 2(1):57-59

SHUKLA, R.R. and ALBRO, P.W. 1987. In vitro modulation of protein kinase C activity by environmental chemical pollutants. Biochem. Biophys. Res. Commun. 142:567-572

SIMMON, V.F., KAUHANEN, V., and TARDIFF, R.G. 1977. Mutagenic activity of chemicals identified in drinking water. Dev. Toxicol. Environ. Sci. 2:249-258

SIMMON, V.F. and KAUHANEN, V. 1978. In vitro microbological mutagenicity assays of 2-chloroethyl chloroformate. Final report, Contract No. 68-03-11-74. Prepared for U.S. Environmental Protection Agency, National Environmental Research Center, Water Supply Laboratory, Cincinnati, Ohio

SINA, J.F., BEAN, C.L., DYSART, G.R., TAYLOR, V.I., and BRADLEY, M.O. 1983. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat. Res. 113:357-391

SIPES I.G. 1987. Personal communication. University of Arizona

SIVAK, A. 1978. BALBc/-3T3 Neoplastic Transformation Assay with Methylene Chloride (Food Grade Test Specification). Report to the National Coffee Association, Inc. Arthur D. Little, Inc., Cambridge, Massachusetts

SNOW, L., McNAIR, P., and CASTRO, B.C. 1979. Mutagenesis testing of methylene chloride and 1,1,1-trichloroethane in Salmonella strains TA-100 and TA-98. Personal Communication from Northrop Services, Inc., Research Triangle Park, North Carolina. September 19

STEVENS, J.L. and ANDERS, M.W. 1978. Studies on the mechanisms of metabolism of haloforms to carbon monoxide. Toxicol. Appl. Pharmacol. 45:297-298

STEVENS, J.L. and ANDERS, M.W. 1979. Metabolism of haloforms to carbon monoxide. III. Studies on the mechanism of the reaction. Biochem. Pharmacol. 28:3189-3194

STEVENS, J.L., RATNAYAKE, J.H., and ANDERS, M.W. 1980. Metabolism of dihalomethanes to carbon monoxide. IV. Studies in isolated rat hepatocytes. Appl. Pharmacol. 55:484-498

STEWART, R.D. and DODD, H.C. 1964. Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride, and 1,1,1-trichloroethane through the human skin. Am. Ind. Hyg. Assoc. J. 25:439-446

STEWART, R.D. and HAKE, C.L. 1976. Paint-remover hazard. JAMA J. Am. Med. Assoc. 235:398-401

STEWART, F.D., FISHER, T.N., HOSKO, M.J., PETERSON, J.E., BARETTA, E.D., and DODD, H.C. 1972a. Carboxyhemoglobin elevation after exposure to dichloromethane. Science 176:295-296

STEWART, R.D., FISHER, T.N., HOSKO, M.J., PETERSON, J.E., BARETTA, E.D., and DODD, H.C. 1972b. Experimental human exposure to methylene chloride. Arch. Environ. Health 25:342-348

SVIRBELY, J.L., HIGHMAN, B., ALFORD, W.C, and VON OETTINGEN, W.F. 1974. The toxicity and narcotic action of mono-chloro-mono-bromo-methane with special reference to inorganic and volatile bromide in blood, urine and brain. J. Ind. Hyg. Toxicol. 29:383-389

SZABO, S., HORBATH, E., KOVACS, K. and LARSEN, P.R. 1978. Pyrazole-induced thyroid narcosis; a distinct organ lesion. Science 199:1209-10

TAKANO, T. and MIYAZAKI, Y. 1988. Metabolism of dichloromethane and the subsequent binding of its product, carbon monoxide, to cytochrome P-450 in perfused rat liver. Toxicol. Let. 40:93-96

TARONE, R.E., CHU, K.C., and WARD, J.M. 1981. Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6NXC3H/HEN)Fl (B6C3Fl) mice JNCI 66:1175-1181

TASKINEN, H., LINDBOHM, M.-L. and HEMMINKI, K. 1987. Spontaneous abortions among women working in the pharmaceutical industry. Brit. J. Ind. Med. 43:199-205

TAYLOR, G.J., DREW, R.T., LORES, E.M, and CLEMMER, T.A. 1976. Cardiac depression by haloalkane propellants, solvents, and inhalation anesthetics in rabbits. Toxicol. Appl. Pharmacol. 38:379-387

THILAGAR, A.K. and KUMAROO, P.V. 1983. Induction of chromosome damage by methylene chloride in CHO cells. Mut. Res. 116:361-367

THILAGAR, A.K., BACK, A.M., KIRBY, P.E., KUMAROO, P.V., PANT, K.J., and CLARKE, J.J. 1984a. Evaluation of dichloromethane in short-term in vitro genetic toxicity assays. Env. Mutat. 6:418-419

THILAGAR, A.K., KUMAROO, P.V., CLARKE, J.J., KOTT, S., BACK, A.M., and KIRBY, P.E. 1984b. Induction of chromosome damage by dichloromethane in cultured human peripheral lymphocytes, CHO cells, and mouse lymphoma L5178Y cells. Env. Mutat. 6:422

THILAGAR, A.K., BACK, A.M., KIRBY, P.E., KUMAROO, P.V., PANT, K.J., CLARKE, J.J., KNIGHT, R., and HAWORTH, S.R. 1984c (Abstract) Evaluation of dichloromethane in short term in vitro genetic toxicity assays. Journal of the Envir. Mutagen Soc. 6:418-419

THOMAS, A.A., PINKERTON, M.K., and WARDEN, J.A. 1971. Effects of methylene chloride exposure on the spontaneous activity of mice. In Proceedings of the 2nd Annual conference on Environmental Toxicology, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. AMRL-TR-H-120. Paper No. 13. pp. 185-189

THOMAS, A.A., PINKERTON, M.K., and WARDEN, J.A. 1972. Effects of low level dichloromethane exposure on the spontaneous activity of mice. In Proceedings of the 3rd Annual Conference on Environmental Toxicology, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. AMRL-TR72-130 Paper No. 14. pp. 223-227

THORSLUND, T.W., BROWN, C.C., and CHARNLEY, G. 1987. Biologically motivated cancer risk models. Risk Anal. 7:109-119

TOLLEFSON L., LORENTZEN R.J., SPRINGER J., BROWN R.N. 1988. Human and animal risk comparisons. J. Occup. Med. 30(1):18-19

TRUEMAN, R.W., and ASHBY, J. 1987. Lack of UDS activity in the livers of mice and rats exposed to dichloromethane. Environ. Molec. Mutagen. 10:189-195

VAN BLADEREN, P.J., BREIMER, D.D., ROTTEVEEL-SMIJS, G.M.T., and MOHN, G.R. 1980. Mutagenic activation of dibromomethane and diiodomethane by mammalian microsomes and glutathione S-transferases. Mutat. Res. 74:341-346

WEINSTEIN, R.S., and DIAMOND, S.S. 1972. Hepatotoxicity of dichloromethane (methylene chloride) with continuous inhalation exposure at a low-dose level. In Proceedings of the 3rd Annual Conference on Environmental Toxicology, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio. AMRL-72-130. Paper No. 13. pp. 209-222.

WEINSTEIN, R.S., BOYD, D.D., and BACK, K.C. 1972. Effects of continuous inhalation of dechloromethane in the mouse. Morphologic and functional observations. Toxicol. Appl. Pharmacol. 23:660-679.

WEISS, G. 1967. Toxic encephalosis as an occupational hazard with methylene chloride. Zentrabl. Arbeitsmed. 17:282-285

WHITTEMORE, A. 1978. Quantitative theories of carcinogenesis. Adv. Cancer Res. 27:55-58.

WHITTEMORE, A. and KELLER, J.B. 1978. Quantitative theories of carcinogenesis. SIAM Rev. 20:1-30

WINNEKE, G. 1974. Behavioral effects of methylene chloride and carbon monoxide as assessed by sensory and psychomotor performance. In: National Institute for Occupational Safety and Health. Xintaras, C., Johnson, B.L., and DeGroot, eds., Publication DHEW (NIOSH) Publication No. 74-126. pp. 130-144

WINNEKE, G. 1981. The neurotoxicity of dichloromethane. Neurobehav. Toxicol. Teratol. 3:391-395

WINNEKE, G. and FODOR, G.G. 1976. Dichloromethane produces narcotic effects. Int. J. Occup. Health Safety 45:34-37

YESAIR, D.W., JAQUES, P., SHEPIS, P., and LISS, R.H. 1977 (Abstract). Dose-related pharmacokinetics of 14C-methylene chloride in mice. Fed. Proc. Am. Soc. Exp. Biol. 36:998

ZAKHARI, S. 1977. Methylene chloride. In: Non-Fluorinated Propellants and Solvents for Aerosols. Golberg, L., ed. CRC Press, Cleveland

RESULTS OF GENETIC TOXICITY TESTS OF METHYLENE CHLORIDE

The following table lists individual studies of different aspects, of the genetic toxicity of CH_2Cl_2. These investigations include mutation in both prokaryotic and eukaryotic systems, mammalian cell transformation, and indications of DNA damage such as sister chromatid exchange and unscheduled DNA synthesis. Results of each study are summarized in the table, and discussed in the text in Section 6.0. The table is an updated and corrected version of that found in EPA (1985a).

TABLE A-1 Results of Genetic Toxicity Tests of Methylene Chloride
(Adapted from EPA 1985a)

Reference	Test System	Strain	Activation System	Concentration/Results		Comments
Simmon et al., 1977	Salmonella/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	None	(Extrapolated from Figure 17) 0, 50, 100, 200, 400, and 800 µl/9 liter desiccator	(Extrapolated from Figure 17) TA100 Dose (µl) — Revertants/plate 0 — 170 50 — 210 100 — 300 200 — 400 400 — 650 800 — 1350 TA100 revertants	1. Toxicity not reported. 2. Number of revertants observed for TA100 not specified numerically 3. Data not presented for strains other than TA100 4. Purity and source of compound not provided 5. Positive response
Simmon and Kauhanen, 1978	Salmonella/S9 vapor exposure	TA100	Aroclor 1254 induced rat liver microsome S9 mix	0 and 1 ml/9 liter desiccator for 6.5 and 8 hours	Met Act 8h · — Treated 6h·: Control 133 6.5 + 1343 — 130 8 · — 810 — 174 + 912 — 158	1. Toxicity not reported 2. Purity and source of compound not provided 3. Used as a positive control in the testing of 2-chloroethyl-chloroformate. 4. Positive response

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Reference	Test System	Strain	Activation System	Concentration	Results	Comments
Kanada and Uyeta, 1978	Salmonella/S9 and B. subtilis rec assay testing	TA98 TA100	PCB induced rat liver microsome S9 mix	Not reported	CH_2Cl_2 reported negative for both strains in B. subtilis and positive for both in S. typhimurium	1. Results summarized in abstract form. 2. Positive results of "Ames" testing supports reports by other authors using same system.

Jongen et al., 1978 — Salmonella/S9 vapor exposure — TA98 TA100 — Phenobarbital induced rat liver microsome S9 mix

(ppm x 10^3)	TA100[a] +S-9	TA100[a] -S9	TA98[a] +S9	TA98[a] -S9
0	152±19	129±12	21±4	19±5
5.7	379±37	240±32	54±5	44±8
11.4	515±76	40/±47	74±4	56±10
14.1	757±82	582±56	93±9	66±12
22.0	865±82	653±89	123±10	96±11
57.0	1201±191	740±94	149±42	110±42

Comments: 1. Testing conducted in gas tight perspex boxes. 2. Only highest dose exhibited less than 83% survival. 3. Purity of CH_2Cl_2 not reported. 4. Positive response.

[a]Results from three experiments, five plates/dose.

Reference	Test System	PPM Vapor	µmoles/plate[a]	TA1535 -S9	TA1535 +S9	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	Comments
E.D. Barber et al. 1980	Salmonella/S9 vapor exposure	0	0	23	28	23	39	254	264	1. Tested redistilled sample of CH_2Cl_2 >99.9% pure.
		3,600	38	40	36	259	288	752	1152	2. Revertants/nmole at highest dose for TA1535, TA98, and TA100 were
		7,200	76	59	51	441	297	1440	960	0.0006, 0.006, and 0.03, respectively.
		9,100	96	78	70	459	322	2640	1096	3. Data shown for testing in gas tight chamber.
		10,900	115	64	50	741	479	3060	3240	4. Negative response in standard test; positive response in gas light chamber.

[a]CH_2Cl_2 concentration measured by GC/MS in aqueous phase of petri dish

Reference	Test System	Strain	Activation System	Concentration/Results				Comments
				Atmospheric Concentration%		Plate Concentration		
				Theoretical	Actual	µg	Revertants	
McGregor, 1979	Salmonella/S9 vapor exposure	TA1535	None	0	nd	nd	15	1. Purity of CH₂Cl₂ not reported.
				0.5	0.14	245	20	2. Positive response
				1.0	0.33	600, 595, 530	25	
				2.0	0.67	1400	50	
				4.0	1.60	2475	75	
				10	nd	nd	80	
Nestmann et al., 1980	Salmonella/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	Aroclor-induced rat liver S9					1. Data not presented. 2. Negative response in standard test. 3. Positive response in gas tight chamber. Doubling in revertant counts for TA1535; 6-fold increase for TA100.

Reference	Test System	Strain	Activation System	Dose	Results				Comments
					TA100**		TA98		
				(µl/Chamber)	+S9	-S9	+S9	-S9	
Snow et al., 1979	Salmonella/S9 vapor exposure	TA98 TA100	Methylene chloride induced Syrian Golden Hamster liver S9 microsome mix	0	66	63	38	19	1. Purity of CH₂Cl₂ not reported. 2. No information about variability of results. 3. Positive response.
				100	177	142	47	31	
				300	463	274	69	46	
				500	642	468	92	61	
				1000	972	632	39	72	

**Mean calculated from three plates/dose.

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TABLE A-1 (Continued)

Reference	Test System	Strain	Activation System	Dose	Results		Comments
Green, T. 1983	Salmonella/S9 vapor exposure	IA1535 IA100	Rat liver fractions	Dose (% in air) 0 1.4 2.8 5.5 8.3	TA 100 +S9 69±5 283±10 506±27 825±34 1050±88	-S9 - 267±20 462±28 872±27 997±88	1. Preliminary results presented in abstract form. 2. Metabolic studies conducted in rat tissue and TA100. Similar metabolism in both systems. Radiolabel reported to bind to bacterial DNA but not to rat liver DNA. 3. Purity of DCM not reported. 4. Positive response. Author thinks this is due to close proximity of cytoplasmic enzymes and intermediates to DNA in bacteria and that negative responses would be obtained in higher organisms. Positive responses in other tests argue against this. See discussion in text.

Reference	Test System	Strain	Activation System	Concentration/Results	Comments
Hestmann et al., 1991	Salmonella/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	Aroclor 1254-induced rat liver S9		1. Levels of CH_2Cl_2 in exposure chamber related directly to the mutational dose-effect curves of 3 paint removers. 2. Data shown for one paint remover only. Other 2 gave similar response. 3. Purity of CH_2Cl_2 not reported. 4. Positive response for paint removers likely due to CH_2Cl_2

Type	Material Weight (mg)		his+ Revertants/Plate[a]			CH_2Cl_2			Exposure Level (mg/l)	
	Added	Vaporized	TA1535	TA100	TA98	Time Averaged[b]	Max 6h Calculated[c]	Measured	Methanol	Ethanol[d]
Paint remover	0	--	16	144	25	---	---	---	---	---
	203	144	22	310	31	12.7	15.5	13.0	<0.5	---
	370	241	14	433	42	21.9	25.5	23.0	<0.5	---
	790	469	23	563	76	40.1	49.9	45.0	0.7	---
	1435	903	31	785	60	80.2	95.4	86.0	1.9	---
		(ml)								
Mix [90:5:5 v/v/v CH_2Cl_2 methanol ethanol]	0	---	13	154	32	---	---	---	---	---
	0.1	0.1	15	268	43	12.2	12.8	11.5	<0.5	<0.9
	0.2	0.2	24	401	73	26.9	25.3	27.5	<0.5	0.7
	0.4	0.4	25	789	138	50.6	50.8	50.0	0.9	1.1
	0.8	0.8	34	1084	164	94.1	101.0	94.5	2.6	2.7
4NQO (Average values from triplicate plates in 4 experiments)	0.001	---	28	878	162	---	---	---	---	---

(a) Average values from triplicate plates. (b). Determined from an area under curve for concentration against time. (c). Calculated from amount vaporized assuming only CH_2Cl_2 vaporized, in 9l chamber. (d). Maximum measured.

TABLE A-1 (Continued)

Reference	Test System	Strain	Activation System	Dose	Results			Comments
Green, 1983	Salmonella/S9 vapor exposure	TA100	Aroclor-1254 induced rat liver S9, microsomes, and cytosol	% Vapor 0 2.8 5.0 8.4	Revertants +S9 100 458 700 950	-S9 100 386 720 900	+ Cytosol — 490 — —	1. Bacterial and mammalian metabolism similar. 2. Cytosol and glutathione catalyze CH_2Cl_2 to formaldehyde and CO_2. S-chloromethylglutathione is a putative intermediate. 3. CH_2Cl_2 converted to carbon monoxide in the presence of microsomes. Formyl chloride is a putative intermediate. 4. Purity of CH_2Cl_2 not given. 5. Positive response. See entry for Green (1980).
Gocke et al., 1981	Salmonella/S9 vapor exposure	TA1535 TA1537 TA1538 TA98 TA100	Aroclor-1254 rat liver S9	µl/desiccator 0 125 250 500 750	Revertants +S9 30±0 54±7 68±32 105±7 203±32	-S9 40±0 85±7 110±14 195±21 295±7		1. Spontaneous revertants for TA100 too low. 2. No information presented for toxicity. 3. Purity of CH_2Cl_2 not given. 4. Equivocal positive response.
Jongen et al., 1982	Salmonella/S9 vapor exposure	TA100	Aroclor-1254 rat liver S9, microsomes, and cytosol					1. Purity of CH_2Cl_2 not given. 2. Positive response.

	% CH_2Cl_2			
Activation	0	0.35	0.7	1.4
---	150	210	350	550
S9	150	240	410	730
Cytosol	150	220	420	810
Microsomes	150	215	380	610

TABLE A-1 (Continued)

Reference	Test System	Strain	Dose (μM)	% survival	Response/10⁶ Survivors Trp-5 Conversion	Recombination	Total Genetic Alterations	ilv-1 Revertants	Comments
Callen et al., 1980	Saccharomyces cerevisiae	D7	0 104 157 209	100 77 42 --	18 28 107 --	310 190 4490 --	3300 3900 14000 --	2.7 4.4 5.8 --	1. Positive response 2. Active metabolites produced by this system are made intracellularly rather than by an exogenously employed activation system.
Simmon et al., 1977	Saccharomyces cerevisiae suspension test	D3							1. Data not provided but reported negative for mitotic recombination. 2. Strain differences and differences in treatment conditions (i.e., time and temperature) may be the cause of differences between this study and that of Callen et al. (1980) 3. Cytochrome P450 concentration not known. Callen et al (1980) report different yeast strains have different levels.

TABLE A-1 (Continued)

Reference	Test System	Strain	Chemical	Route	# Chromosomes Tested	# Lethals	Corrected Lethals (%)	Comments
Abrahamson and Valencia, 1980	Drosophila sex-linked recessive lethal test	FM6 females, Canton S males	EMS Iris Neg. Controls CH$_2$Cl$_2$	Fed Fed Fed or Inj. Fed Inj	773 2442 94491 14682 8262	44 35 230 34 18	5.69 1.43 0.233 0.204 0.157	1. No precaution taken to design exposure chambers to prevent evaporation of the compound for feeding experiment. 2. No concurrent negative controls reported for the injection experiment. 3. Negative response at dose tested (224 mM).

Reference	Test System	Strain	Chemical		Lethals/Brood (Percent)			Comments
					1	2	3	
Gocke et al., 1981	Drosophila sex-linked recessive lethal test	Basc females, Berlin K males	CH$_2$Cl$_2$ (mM)	0	19/7130 (0.27)	8/5525 (0.14)	13/3416 (0.38)	1. Positive response for Brood 1 indicating CH$_2$Cl$_2$ is mutagenic to sperm in Drosophila. 2. Higher dose used than in test by Abrahamson and Valencia (1980).
				125	16/3632 (0.44)	2/2519 (0.00)	6/1310 (0.46)	
				620	8/1213 (0.66)	3/735 (0.41)	5/1005 (0.50)	
				Total treated	24/4845 (0.50)	5/3114 (0.16)	11/2315 (0.47)	
					P < 0.05			

TABLE A-1 (Continued)

Reference	Test System	Concentration (mol/L)	Mutation Frequency (Lethal mutations/10⁵ loci)	Toxicity (Survival rel. to controls)				Comments
				L2 Juveniles	L2-L3 Molt	L3-L4 Molt	L4 Adult Molt	
Samoiloff et al., 1980	Panagrelus redivivus sex-linked recessive lethal test	CH₂Cl₂ 10^{-8} 10^{-6} 10^{-4}	6.0 10.0 9.8	1.02 1.02 1.00	0.99 1.00 1.00	0.97 0.86 0.88	0.16 0.15 0.17	1. Equivocal positive response. 2. Not dose-related. 3. Some positive controls gave negative (e.g. EMS) or only marginally positive response (e.g. 3-methylcholanthrene). 4. Test system not validated.
		Proflavine 10^{-8} 10^{-6} 10^{-4}	12.5 10.0 28.6					

TABLE A-1 (Continued)

Reference	Test System	Concentration (%)	V79		CHO		Comments
			Mutants/10^5 Survivors	Survival (%)	Mutants/10^5 Survivors	Survival (%)	
Jongen et al., 1981	6-Thioguanine resistance in V79 and CHO cells	DCM 0	2	100	1.9	100	1. Equivocal negative response.
		1	1.8	98	1.8	90	2. Highest dose only resulted in 20% decrease
		2	2	95	1.2	05	in survival. Higher doses up to about 80%
		3	1.7	85	0.9	80	toxicity should be tested.
		4	1.6	80	2.1	73	
		5			2.5	76	
		EMS 0	.2				
		2	13				
		4	33				
Thilagar et al. 1984a	Mutation at thymidine kinase locus in mouse lymphoma C5178Y cells and neo-plastic trans-formation in C3H10T1/2 cells						1. Reported negative in meeting abstract.
							2. Experimental details not given.

		Concentration (%)	Foci/Plate	Survival (%)	Comments
Sivak 1978	Neoplastic transformation in BALB/C-3T3 cells	CH_2Cl_2 0	0.11 ± 0.07	100	1. Food grade purity CH_2Cl_2
		1×10^{-5}	0.28 ± 0.14	60	
		1×10^{-4}	0.47 ± 0.15	58	
		1×10^{-3}	0.11 ± 0.08	71	
		1×10^{-2}	0.40 ± 0.13	66	
		3-MC 0	0.11 ± 0.07	100	
		2×10^{-4}	3.69 ± 0.52	29	
Price et al. 1978	Neoplastic transformation in F1076 cells	0	0		1. Reagent grade purity CH_2Cl_2 was used.
		.16	7		
		1.6	9		

TABLE A-1 (Continued)

Reference	Strain/Tissue	Route of Exposure	Dose	Breaks Chromatid	Breaks Chromosome	Dicentrics	Rings	Exchanges	Comments
Burek et al. 1984	Male and female Sprague-Dawley rat/bone marrow	Inhalation	0	0.9 ± 0.99	0.2 ± 0.42	0	0	0	1. 5 animals/sex/dose.
			500	0.5 ± 0.71	0.2 ± 0.42	0	0	0	2. 200 cells/animals.
			1500	0.5 ± 0.97	0.1 ± 0.32	0	0	0.1 ± 0.32	3. Dose to bone marrow cells may have been low.
			3500	0.7 ± 0.48	0.2 ± 0.42	0	0.2 ± 0.42	0	4. Negative response.

Reference	Test System	Dose	RCG[a]	Breaks Chromatid	Isochromatid	Exchange	Number of Aberrations/Cell	Cells w/ Aberration (%)	Comments
Thilagar and Kumaroo, 1983	Cultured CHO cells	CH_2Cl_2 (µl/ml)							1. Positive response.
		0	100	2	0	0	0.02	2	2. Four experiments yielded similar response.
		2	98.4	4	0	2	0.06	6	
		5	75.3	8	14	8	0.34	26	
		10	66.7	12	34	10	0.56	38	
		TEM							
		1 µg	N.D.	22	30	42	0.96	66	

[a] RCG = Relative cell growth.

Reference	Strain/Tissue	Route of Exposure	Dose ppm	Breaks Chromatid	Chromosome	Dicentrics	Rings	Exchanges	Comments
Gocke et al., 1981	Male and female NMRI mice/bone marrow	i.p. Injection	No. injection x mg/kg	Micronucleated Polychromatic Erythrocytes (%)					1. Probable positive response; authors indicate negative response.
			0	0.19					2. Dose to bone marrow cells may have been low.
			2 x 425	0.19					
			2 x 850	0.35					
			2 x 1700	0.28					

TABLE A-1 (Continued)

Reference	Test System	Dose	Results		Comments
			SCE/Cell Experiment #		
			1	7	
Jonyen et al., 1981	SCE/V79 cells in culture	CH_2Cl_2 (%)			1. Exposure time 1 hour.
		0	0.26 ± 0.02	0.30 ± 0.03	2. Positive response. Significant increases in SCEs (P <0.001).
		0.5	0.40 ± 0.02	---	3. Same type of dose-response observed in experiments 2-6 (data not shown).
		1.0	0.46 ± 0.02	0.47 ± 0.02	4. DMSO did not increase incidence of SCE.
		2.0	0.45 ± 0.03	0.51 ± 0.03	
		3.0	---	0.58 ± 0.03	
		4.0	0.51 ± 0.03	0.61 ± 0.03	

Reference	Test System	Dose	SCE/Cell (X ± SD)	Range of SCE's	M_1	$M_1 + M_2$[a]		Comments
Thilagar and Kumaroo, 1983	Cultured CHO cells	CH_2Cl_2 (µl/ml)						1. Marginal, but not significant increase in SCEs.
		0	10.28 ± 3.17	5-17	0	3	97	2. Three other experiments yielded similar responses.
		2	11.36 ± 3.09	3-19	0	16	04	3. Results not inconsistent with test by Jonyen et al. (1981) where highest dose
		5	12.56 ± 2.95	7-18	6	54	40	was three times greater (i.e., 471 mM vs 156 mM).
		10	12.36 ± 3.35	7-21	4	56	40	
		Triethylene melamine 0.025 µg/ml	47.74 ± 4.76	39-61	4	49	47	

McCarroll et al. 1983 Cultured CHO cells

1. A dose-related increase in SCE's was observed over the dose range 1.8, 3.6, 5.4, and 7.0% CH_2Cl_2 following a 24-hour exposure in the atmosphere.

2. Reported as a meeting abstract; actual results not given.

[a]M_1 - Percentage of cells at first mitosis.
M_2 - Percentage of cells at second mitosis.
M_3 - Percent of cells between first and second mitosis.

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TABLE A-1 (Continued)

Reference	Test System	Dose [*]	Unscheduled DNA Synthesis	Comments
Jongen et al. 1981	V79 cells	0 (%) 0.5 2.0 3.0 4.0		1. Negative results reported. 2. Positive results obtained for 4NQO as a positive control.
Thilager et al. 1984c	Primary rat hepatocytes			1. Reported negative in meeting abstract. 2. Experimental details not given.

Reference	Test System	Dose	S9 Mix	^{3}H-TdR Uptake	Comments
Perocco and Prodi 1981	Primary human lymphocytes	0 2.5 5 10	- - - -	715±24 (dpm) 593±24 491±27 532±31	1. CH_2Cl_2 was negative as an inducer of unscheduled DNA synthesis.
		0 2.5 5 10	+ + + +	612±26 573±35 510±40 537±39	

[*] - % CH_2Cl_2

TABLE A-1 (Continued)

Reference	Test System	Dose	Results	Comments
Osterman-Golkar et al. 1983	E. Coli K39 (λ), prophage induction	10 μl	positive	1. Qualitative Test Procedure 2. Obtained strong positive response (+++ on a scale of + to ++++)

APPENDIX B

DETAILS OF CARCINOGENICITY BIOASSAYS

AND DESCRIPTION OF TUMOR TYPES

The following section describes in detail the results of the chronic bioassays performed using CH_2Cl_2.

B.1 <u>Dow 1980 Inhalation Study in Sprague-Dawley Rats (EPA 1985a and Burek et al. 1984)</u>

Rats were housed no more than three per cage in 8x8x8-foot rooms. During nonexposure periods, animals remained in the chamber rooms with filtered air. Controls were continuously exposed to filtered air.

During the first 2 months of the study, there was an outbreak of sialodacryoadenitis virus infection involving control and exposed rats. This virus has been associated with acute inflammation and diffuse coagulative necrosis of the parotid and submandibular salivary glands and Harderian gland. Inflammatory edema and inflammatory cells are prominent in salivary and Harderian glandular interstitial and periglandular connective tissue. Squamous metaplasia of glandular epithelium occurs in the reparative phase of the disease. High morbidity may occur during outbreaks of disease, but mortality is negligible (Jacoby et al. 1979). Burek et al. (1984) suggested that the combination of viral infection and high exposures to CH_2Cl_2 may have been associated with the salivary gland tumors.

Toward the end of the study, there was a statistically significant increase in mortality in males exposed to 1,500 and 3,500 ppm at 21-22 months and increased mortality in females exposed to 3,500 ppm between 18-24 months. Mortality was high; however, in control groups of both sexes, with 85% mortality in males and 78% in females at 24 months (EPA 1985a). No data were published on the reasons for the poor survival of controls.

Carboxyhemoglobin blood levels were increased in exposed animals, but this elevation was not dose-related.

Neoplasms. As noted in Table B-1, the carcinogenic end points in this study were sarcomas arising in the ventral cervical/salivary gland area in males and benign mammary tumors.

TABLE B-1

SARCOMAS OF THE CERVICAL/SALIVARY GLAND REGION
IN SD MALE RATS
(Burek et al. 1984)

CH_2Cl_2 (ppm)	Incidence
0	1/93 (1%)
500	0/94
1,500	5/91 (5.5%)
3,500	11/88 (12.5%)*
Historical laboratory control	0-2%

* p < 0.05, Fisher's Exact Probability Test

The sarcomas were composed of round to spindle cells with high mitotic activity; local invasion of tissue by tumor cells and necrosis within the tumor were frequent. Areas of different histologic types (fibrosarcoma, neurofibrosarcoma) often occurred within a single tumor; other sarcomas were anaplastic (undifferentiated). All sarcomas involved the salivary glands but did not appear to arise from glandular components. Ultrastructural examination revealed no evidence of epithelial origin (absence of desmosomes or secretory products).

Incidences of benign mammary tumors in both sexes of rats were as follows:

TABLE B-2

BENIGN MAMMARY TUMORS IN SD RATS
(Burek et al. 1984)

| CH_2Cl_2 (ppm) | Males | | Females | | |
	Rats with Tumors	No. Tumors	Rats with Tumors	No. Tumors	Avg. No. Tumor/Rat
0	7/95 (7%)	8/95	79/96 (82%)	165/96	1.7
500	3/95 (3%)	6/95	81/95 (85%)	218/95	2.3
1,500	7/95 (7%)	11/95	80/96 (83%)	245/96	2.6
3,500	14/95 (15%)	17/95	83/97 (86%)	287/97	3.0
Historical laboratory control	10%		80%		

High-dose males had a slight increase in the number of benign mammary tumors compared to concurrent controls and a slight increase in the number of tumors per tumor-bearing rat. Incidences of benign mammary tumors in females were similar in control and exposed rats and comparable to historical laboratory controls. However, the total number of mammary tumors increased in exposed females in a dose-related manner. There was no exposure-related increase in the number of animals with malignant mammary tumors in either sex.

As was the case for all rat and mouse studies listed in Table 8-1, exposure-related nonneoplastic lesions were present in the liver. The primary exposure- and dose-related lesion was hepatocellular vacuolization consistent with fatty change (Table B-3).

TABLE B-3

HEPATOCELLULAR VACUOLIZATION IN SD RATS
(Burek et al. 1984)

CH_2Cl_2 (ppm)	Male	Female
0	17%	34%
500	38%*	52%*
1,500	45%*	59%*
3,500	54%*	65%*

p < 0.05 using Fisher's Probability Test

B.2 <u>Dow 1980 Inhalation Study in Syrian Hamsters (EPA 1985a, Burek et al. 1984)</u>

Animal husbandry and conditions of experimental exposure were similar to those of the Dow 1980 inhalation study in rats. Hamsters were housed three or four to a cage during the first year, then individually for the remainder of the study. An infestation of mange mite (Demodex) occurred during the study in control and treated animals but did not result in increased mortality.

There were no exposure-related increases in mortality in males, although mortality was high at 24 months (82% in control males, 85% in high-dose males). In females, controls had 100% mortality at 24 months, a value higher than any of the exposed groups (95.7%--500 ppm, 89.5%--1,500 ppm, 90.3%--3,500 ppm).

Blood carboxyhemoglobin levels were elevated in all exposed groups at 22 months with a slight dose-related trend in females.

<u>Neoplasms</u>. No exposure-related neoplasms occurred.

<u>Nonneoplastic Lesions</u>. No exposure-related nonneoplastic lesions occurred. Systemic amyloidosis was common in control animals and occurred at a decreased incidence (the decrease was dose-related) in exposed animals.

B.3 <u>Dow 1982 Inhalation Study in Sprague-Dawley Rats (EPA 1985a)</u>.

The toxicology report was not available for review; therefore, this discussion is excerpted from secondary sources (EPA 1985a). This study has recently been published in the literature by Nitschke et al. (1988a).

As the study was conducted at Dow, the majority of animal husbandry and experimental exposure conditions presumably were similar to the previous rat study (Section B.2). However, the study pathologist noted that rats were kept in chambers during the exposure period, then housed in conventional animal rooms overnight and during the weekends. He noted that female rats housed under these conditions often had lower incidences of mammary tumors than rats kept in chambers 100% of the time (Food Solvents Workshop 1984).

A total of 360 male and 492 female Sprague-Dawley rats (Spartan substrain, 6 to 8 weeks old) were used in this study. Groups of 90 rats/sex were exposed by inhalation to 0 (control), 50, 200, and 500 ppm (0, 173, 692, and 1,735 mg/m^3) CH_2Cl_2 (technical grade, lot #TA 05038, with purity of at least 99.5 percent) 6 hr/day, 5 days/week, for 20 (males) or 24 months (females). In addition, 30 extra female rats, identified as 500/0, were exposed to 500 ppm CH_2Cl_2 for the first 12 months of the study and were housed as control rats for the duration of the study (last 12 months). Another 30 female rats, identified as 0/500, were housed in the same manner as control rats for the first 12 months of the study and were exposed to 500 ppm CH_2Cl_2 for the remaining 12 months of the study. To determine the rate of DNA synthesis in the liver, 18 female rats were included in each group. After 6, 12, 15, and 18 months of exposure, five rats of each sex at each exposure level were

sacrificed. In addition, five female rats from each of the 500/0 and 0/500 groups were sacrificed at the 18-month interim necropsy.

Exposure-related increases in mortality did not occur during the study. Mortality in males at 20 months was: control--70%, 50 ppm--70%, 200 ppm--79%, and 500 ppm--66%. Because of the high mortality rate, males were terminated at 21 months of the study. Mortality in females at 24 months was: control--64%, 50 ppm--76%, 200 ppm--67%, 500 ppm--61%, 500/0--72%, and 0/500--52%.

Neoplasms. In females, there was a nonsignificant increase in the number of benign mammary tumors per tumor-bearing rat (Table B-4).

Rats exposed to 500 ppm during the first 12 months of the study ("500/0") had a higher average number of tumors per animal than did the rats exposed to 500 ppm only after 12 months ("0/500").

Nonneoplastic Lesions. An outbreak of sialodacryoadenitis infection (based on clinical signs) occurred after 2 months in exposed and control groups, and lasted approximately 3 weeks. Blood carboxyhemoglobin levels were elevated in all exposed groups. High-dose females had hepatocellular vacuolization (control--4/45 (9%), 500 ppm--11/42 (26%)); high-dose and 500/0 females had an elevated incidence of multinucleated hepatocytes (control--8/70 (11%), 500 ppm--27/70 (39%; $p = 0.0002$ by Fischer Exact Test), 500/0--9/25 (36%; $p = 0.009$ by Fischer Exact Test))

TABLE B-4

BENIGN MAMMARY TUMORS IN SD FEMALE RATS
Dow 1982 Inhalation Study

CH_2Cl_2 (ppm)[2]	No. of rats with tumors/ Total No. of Rats	Total No. of Tumors/ Total No. of Rats	Average No. Tumors per Tumor-Bearing Rat
0	52/70	105/70	2.0
50	58/70	133/70	2.3
200	61/70	135/70	2.2
500	55/70	147/70	2.7
500/0	23/25	60/25	2.6
0/500	23/25	50/25	2.2

B.4 National Coffee Association 1982 Drinking Water Study in Fischer 344 Rats
(EPA 1985a, Serota et al. 1986a)

Rats were caged individually in stainless steel hanging wire mesh cages in a Bioclean laminar flow facility and given the compound in deionized drinking water in water bottles at doses of 5, 50, 125 and 250 mg CH_2Cl_2/kg/day for 104 weeks. An additional group, the recovery group, received 250 mg CH_2Cl_2/kg/day for 78 weeks followed by a 26-week recovery period. Control animals and the recovery group received only dionized water. Complete histopathology was performed on the control groups, high-dose group, and recovery group; liver (target organ) and tumors were examined in the low-dose groups.

Administration of CH_2Cl_2 did not appear to effect mortality; survival was more than 70% in control animals of both sexes at the end of 2 years.

<u>Neoplasms</u>. Exposure-related neoplasms did not occur in male rats. In females, there was an increase in liver tumors (combined neoplastic nodules and hepatocellular carcinomas) when compared to concurrent controls. However, concurrent controls had a lower tumor incidence (zero) than historical laboratory controls (Table B-5).

TABLE B-5

LIVER TUMORS IN FEMALE F344 RATS
Drinking Water Study (EPA 1985a)

CH_2Cl_2 (mg/kg/day)	Neoplastic Nodule	Hepatocellular Carcinoma	Combined
0	0/85	0/85	0/85
0	0/49	0/49	0/49
5	1/85 (1.2%)	0/85	1/85 (1.2%)
50	2/83 (2.4%)	2/83 (2.4%)	4/83 (4.8%)a
125	1/85 (1.2%)	0/85	1/85 (1.2%)
250	4/85 (4.7%)	2/85 (2.4)	6/85 (7.1%)b
250/recovery[c]	2/25 (8%)	0/25	2/25 (8%)
Historical control	19/419 (4.5%)	5/419 (1.2%)	24/419 (5.7%)

[a] $p < 0.05$ Fischer's Exact Test.
[b] $p < 0.01$ Fischer's Exact Test.
[c] Exposed to methylene chloride for 78 weeks and allowed to recover.

Based on concurrent controls, EPA's Carcinogen Assessment Group (CAG) considered the liver tumor data to represent a borderline neoplastic response (EPA 1985a). However, Serota et al. (1986a) considered the findings not significant based on historical control liver tumor incidence, which was higher than concurrent controls (Serota et al. 1986a).

<u>Nonneoplastic Lesions</u>. There were statistically significant (p < 0.05 or p < 0.01) increased incidences of hepatocellular fatty change (125 and 250 mg/kg) and foci of hepatocellular alteration (50, 125, and 250 mg/kg and 250 mg/kg recovery group) in both sexes. These incidiences were analyzed using Fischer's Exact Test.

B.5 <u>National Coffee Association 1982 Drinking Water Study in B6C3F1 Mice (EPA 1985a, Serota et al. 1986b)</u>

Animal husbandry and conditions of experimental exposure were the same as for rats (see Section B.4).

There was no effect of CH_2Cl_2 exposure on mortality, and survival was good in all dosed groups of mice. During the study, control and treated mice developed clinical signs of convulsions, particularly when the mice were weighed. An etiologic agent was not identified.

At necropsy, there was a dose-related increase in gross lung masses, but these were not identified histologically. This finding could indicate that lung masses were not trimmed in from wet tissue; however, the study received an intensive external audit, which did not note the presence of "missed" lung masses (Food Solvents Workshop 1984).

<u>Neoplasms</u>. Exposure-related neoplasms did not occur in female mice. In exposed male mice, there was an increased incidence of liver tumors (Table B-6).

These results were considered borderline evidence of chemically induced neoplasia by the EPA Carcinogen Assessment Group (EPA 1985a). The results were deemed not significant by the National Coffee Association because incidences in exposed groups were within the National Toxicology Program's historical control range as of 1981 (mean of 32%, Tarone et al. 1981). Incidences in historical NTP male controls as of 1986 also were similar: 540/1,784 (30% ± 8%) (NTP 1986).

Nonneoplastic Lesions. Fatty change in liver cells occurred in males and females receiving 250 mg/kg/day (high dose).

TABLE B-6

LIVER TUMORS IN MALE B6C3F1 MICE

CH_2Cl_2 (mg/kg-day)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Combined[d]
0	6/60 (10%)	5/60 (8%)	11/60 (18%)
0	4/65 (6%)	9/65 (14%)	13/65 (20%)
60	20/200 (10%)	33/200 (17%)	51/200 (26%)
125	14/100 (14%)	18/100 (18%)	30/100 (30%)[a]
185	14/99 (14%)	17/99 (17%)	31/99 (31%)[a]
250	15/125 (12%)	23/125 (18%)[c]	35/125 (28%)[b]

[a]EPA (1985a) -- Statistically significant using combined control incidence of 24/125 (p < .05).
[b]Borderline (EPA 1985a).
[c]Fischer's Exact Test, p = 0.0114 compared to the first control group (Serota et al. 1986b)
[d]By trend test, p = 0.03.

B.6 <u>National Toxicology Program 1986 Inhalation Study in Fischer 344 Rats</u>

   <u>(NTP 1986, Mennear et al. 1988)</u>

Rats were housed individually in stainless-steel mesh cages in exposure chambers and remained in the chambers during nonexposure periods.

Sialodacryoadenitis virus/rat corona virus infection occurred during the study as indicated by positive serologic titer results at 19 and 24 months on study. Rats also had titers to a number of other infectious agents: Sendai virus, pneumonia virus of mice (PVM), Kilham rat virus (KRV), and <u>Mycoplasma pulmonis</u>. Of the latter agents, all but KRV are primarily respiratory system pathogens.

Mortality was high in all dose groups of males (66%-82%), with the majority of deaths occurring during the final 16 weeks of the study. There was an unusually high incidence of advanced mononuclear cell leukemia in all groups of male rats. Mortality was significantly increased in high-dose females after 100 weeks on study. Otherwise, there was no correlation between exposure and mortality.

Because of the aberrant elevated incidence of leukemia in control males (Table B-7), NTP could not make an unequivocal conclusion about elevated leukemia incidence in 2,000- and 4,000-ppm females (23/50, 46%, for both exposed groups.)

TABLE B-7

MONONUCLEAR CELL LEUKEMIA IN CONTROL F344/N RATS
NTP (1986)

	Male	Female
Concurrent control	34/50 (68%)	17/50 (34%)
Laboratory histor-ical control	36/100 (36%)	27/99 (27%)
NTP historical control	458/1,727 (27% ± 9%)	307/1,772 (17% ± 6%)

Neoplasms. Male and female rats exposed to CH_2Cl_2 had dose-related increases in incidence of benign mammary tumors (Table B-8), which NTP interpreted as "clear evidence of carcinogenicity" in female rats and "some evidence of carcinogenicity" in male rats.

Dose-related multiplicity of mammary tumors was not discussed in the NTP technical report. Incidence of benign mammary tumors in concurrent control females was lower than in historical laboratory or NTP controls. However, the incidence observed in the high dose female is statistically significant when compared with incidence in either historical laboratory (p - 0.0003) or historical NTP (p - 0.01) controls.

BENIGN MAMMARY TUMORS IN F344/N RATS

NTP (1986)

	CH_2Cl_2 (ppm)			
	0	1,000	2,000	4,000
MALES				
Fibroadenoma, adenoma, or fibroma	1/50 (2%)[d]	1/50 (2%)	4/50 (8%)	9/50 (18%)[a,b,c]
Historical controls:				
Fibroadenoma:				
Laboratory	0/100			
NTP	51/1,727 (3% ± 3%)			
Fibroma:				
Laboratory	6/100 (6%)			
NTP	91/1,727 (5% ± 3%)			
FEMALES				
Fibroadenoma or adenoma	5/50 (10%)	11/50 (22%)	13/50 (26%)	23/50 (46%)
Life Table Test	p<0.001	p - 0.045	p - 0.022	p < 0.001
Incidental Tumor Test	p < 0.001	p - 0.092	p - 0.083	p < 0.001
Cochran- Armitage Trend Test	p < 0.001			
Fisher Exact Test		p - 0.105	p - 0.070	p < 0.001
Historical controls, fibroadenoma:				
Laboratory	16/99 (16%)			
NTP	492/1,772 (28% ± 10%)			

[a] Life Table Test p=0.002
[b] Incidental Tumor Test p=0.008
[c] Fischer Exact Test p=0.008
[d] Cochran-Armitage Trend Test $p \leq 0.001$

<u>Nonneoplastic Lesions</u>. Hepatocytomegaly, hemosiderosis, cytoplasmic vacuolization, and bile duct fibrosis were more frequent in treated than control rats of both sexes, but the incidence did not increase in a dose-related manner.

B.7 <u>National Toxicology Program 1986 Inhalation Study in B6C3F1 Mice (NTP 1986, Mennear et al. 1988)</u>

Animal husbandry procedures and conditions of experimental exposure were the same as for the CH_2Cl_2 rat inhalation study performed at Battelle Pacific Northwest Laboratories (see Section B.6).

Based on serologic titers at the terminal kill, mice had concurrent infections with pneumonia virus of mice (PVM) and mouse hepatitis virus (MHV) during the course of the study.

Mortality was significantly greater than controls in exposed male mice: control--11/50 (22%), 2,000 ppm--26/50 (52%), 4,000 ppm--41/50 (82%). Female high-dose mice had significantly greater mortality (84%) than control (50%) or 2,000 ppm (50%) animals (p < 0.001 for life table trend test for a decreasing survival of male mice with increasing dose; and p = 0.002 for the same test for female mice (NTP 1986)).

<u>Neoplasms</u>. Both sexes of mice had dose-related increases in incidence and multiplicity of alveolar-bronchiolar tumors (adenomas, carcinomas) of the lung (Tables B-9, B-10). Male mice exposed to 4,000 ppm had an elevated incidence

of liver tumors--hepatocellular carcinoma and combined adenoma or carcinoma (Tables B-11, B-12). Female mice had dose-related increases in both hepatocellular adenoma and carcinoma. These findings were interpreted by NTP as "clear evidence of carcinogenicity" in B6C3F1 mice. As noted in the NTP technical report, the incidences of alveolar/bronchiolar neoplasms in the concurrent control groups were lower than the historical laboratory control groups from Battelle and, particularly for the males, lower than historical NTP controls.

Nonneoplastic Lesions. A treatment-related increase in cytologic degeneration occurred in the livers of both sexes of mice. Increased incidences of testicular atrophy in exposed males and ovarian and uterine atrophy in exposed females were considered by NTP to be secondary to the extensive lung and liver neoplasia.

MATHEMATICAL MODELS FOR LOW-DOSE EXTRAPOLATION

Multistage Models

Armitage and Doll (1954) derived a mathematical model for carcinogenesis that assumes a complex, time-dependent process. This model assumes that a cell must undergo a total of k ordered transformations to evolve into a neoplastic cell. If the transformation rates are constant over time and linearly related to exposure, the probability of a tumor response P(d) may be expressed as

$$P(d) = 1 - \exp\left[-\sum_{i=1}^{k} (a_i + b_i d)\right]$$

where k is the number of stages, $a_i > 0$ and is proportional to the background transition rate of the ith stage, $b_i \geq 0$ and is proportional to the transition rate for the ith stage per unit of exposure, and d is a constant exposure rate. The number of stages, k, that have transition rates that are exposure-dependent (i.e. $b_i > 0$) defines the degree of the polynomial relationship. The exponent of the multistage model in this form is a constant times the product of k linear equations. In the resulting polynomial, each of the coefficients associated with terms of order m or less exist (i.e. are greater than zero) and are subject to certain nonlinear constraints. For example, if the coefficient corresponding to the jth power is denoted as C_j and two stages are exposure-dependent, it follows that $C_j > 0$ for $j = 0$, 1, 2 and $C_1 \geq 2(C_0 C_2)^{1/2}$ (Kalbfleish et al. 1983).

<u>Two-Stage Models</u>. Since the original multistage model did not account for the proliferation of preneoplastic cells and only two stages are required to explain the age-dependence of tumor rates, Armitage and Doll (1957) proposed an alternative two-stage model. Their updated model of carcinogenesis is based on the assumption that stem cells become premalignant cells that divide at a constant rate, producing an exponential growth of preneoplastic clones. A preneoplastic cell is then transformed into a cancerous cell, which may ultimately develop into a tumor.

More recently, Moolgavkar and Venzon (1979) generalized the two-stage model by allowing for a variable number of cells at risk over time and a stochastic rather than deterministic growth of preneoplastic cells. The evolving biological basis for this model is discussed in detail in Moolgavkar and Knudson (1981) and Moolgavkar (1986). Thorslund et al. (1987) have extended this form of the two-stage model to incorporate the effects of environmental agents on cell transition rates and first-stage cell proliferation rates. A number of models can be generated from these extensions in which the parameters have a biological basis. Three simple forms of this model that can be used to estimate cancer risk with the limited bioassay data usually available are briefly presented here.

(1) The first form is based upon the assumption that both transition rates are exposure-dependent. This form may be expressed as

$$P(d) = 1 - \exp - \left[(a_1 + b_1 d)(a_2 + b_2 d) \right]$$

C-2

where the terms are defined as for the multistage model. This form may be reparameterized so that the resulting parameters are estimable and have a biological interpretation. The reparameterized model may be expressed as:

$$P(d) = 1-\exp-\left[A[1+(S+I)d](1+Sd)\right],$$

where $1-\exp-A > 0$ and is the background tumor rate, $S > 0$ and is the smallest of the relative exposure-dependent transition rates, and $I \geq 0$ and is the incremental change between the largest and smallest relative transition rates.

(2) The second model is a specific case of the first, which assumes that both transition rates are the same linear function of dose d. As pointed out by Moolgavkar (1986), this situation would occur when two genes on homologous chromosomes that regulate cell growth experience critical point mutations, which would reduce the control on growth. Knudson (1985) lists a number of human cancer types that are consistent with this hypothesis. If these point mutations are linear functions of dose, the dose-response relationship may be expressed as

$$P(d) = \left[1-\exp-A(1+Sd)^2\right].$$

(3) The third simple form of the two-stage model can be used when it is reasonable to hypothesize that preneoplastic cell proliferation occurs. It is based on the assumption that the carcinogenic agent stimulates the growth rate G of transformed first-stage cells proportional to dose. Under

this assumption, the dose-response model has the form

$$P(d) = 1-\exp-\left[2A[\exp(Gd)-1-Gd]/(Gd)^2\right],$$

where $1-\exp-A$ is the background tumor rate and G is the change in the preneoplastic cell growth rate per unit of exposure.

All of the multistage models discussed above have the common property that they are linear in form at low doses, and as a result give conservative estimates of cancer risk.

The multistage model can also be generalized to account for age or duration of exposure. One such formulation for the multistage model may be expressed as

$$P(d,t) = 1-\exp-\sum_{i=1}^{k}\left[(a_i+b_i d)(t-t_o)^k\right],$$

where t is age or duration of exposure and t_o is the latency period. Other formulations are possible, but computer programs to implement their use are not readily available at this time.

Based upon the various multistage models, a number of algorithms or approaches to bound or predict cancer risk can be employed. A brief discussion of some of these approaches, with an indication of the strengths and weaknesses of each, is given in the following sections.

<u>Linearized multistage model (GLOBAL 82)</u>. To avoid computational problems posed by the original multistage model, Guess and Crump (1977) "generalized" the multistage model by giving it a less restrictive polynomial form:

$$P(d) = 1-\exp-\left[\sum_{i=0}^{k} c_i d^i\right],$$

where k is the number of exposure levels used in a bioassay and $c_i \geq 0$ for i = 0, 1, ..., k.

As a first step when using this approach, the maximum likelihood estimates for the C's are obtained. Next, the unique polynomial is derived that gives the maximum risk at a specified level of exposure, subject to a goodness-of-fit constraint. The linear term in this polynomial times an environmental exposure level is, to a very close approximation, the statistical upper bound on cancer risk at low doses.

The advantage of this method is that a unique upper-bound estimate can be routinely obtained. The disadvantages are: (1) the algorithm cannot be used to obtain a stable point estimate of risk for the MLE; (2) only a modified goodness-of-fit test exists for the method; and (3) the mathematical form is based upon an extraneous arbitrary factor, the number of exposure levels used in the bioassay.

<u>Time-to-tumor model (WEIBULL 82)</u>. A time-to-tumor model of the product form:

$$P(d,t) = 1-\exp-\left[\sum_{i=0}^{m} c_i d^i (t-t_o)^k\right]$$

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can be used to estimate cancer risk when the ages at which a tumor is observed are available for individual animals. Such an approach is critical when the death rate due to the agent, independent of the tumor of concern, is exposure level-dependent. For the specific case where $k \geq 1$ and is not restricted to integers and m is equal to the number of exposure levels, the "WEIBULL 82" computer program can be used to obtain cancer risk estimates and their upper bounds. To use this program, one must specify whether a tumor was incidental or the proximal cause of death. The advantages of this approach are:

(1) it supplies a method for adjusting for differential, exposure-induced mortality, and

(2) it gives estimates that are a time-dependent extension of the linearized multistage upper-bound approach.

A number of disadvantages also exist. Among them are:

(1) the model does not have an underlying biological rationale;

(2) it is necessary to classify tumors as incidental or fatal, which in many cases is difficult;

(3) when the presence of a tumor influences the death rate due to a competing cause, a bias of unknown magnitude is created; and

C-6

(4) it must be assumed that the competing risk-adjusted rate at the end of the observation period is a reasonable measure of the lifetime risk.

Direct use of the two-stage model. It is possible to obtain estimates of risk directly using one of the three specific forms of the two-stage model. The advantages of this approach are:

(1) At low doses, the models converge to a linear no-threshold form so that conservative estimates are obtained.

(2) A stable point estimate of risk can be obtained directly.

(3) Several forms of the models have only two parameters that have to be estimated, so that goodness-of-fit tests can be run on the data from the standard bioassay with one control and two exposure groups.

(4) The mathematical form of the model follows directly from one current hypothesis about the mechanisms of cancer induction.

(5) Effects of cell proliferation can be taken into account.

(6) Steep dose-response data can be fitted with the model.

(7) The parameter estimates are biologically meaningful.

It should also be noted that when only two exposure levels exist, the two-stage transition rate model and the linearized multistage model give identical results if the parameter estimates of the latter approach are consistent with the constraints imposed by the multistage theory.

The disadvantages of using the two-stage model are that:

(1) only approximate maximum likelihood estimates can be obtained for the parameters until extensive computer programs that are being developed are completed;

(2) no method exists to adjust for time-to-tumor data; and

(3) a positive estimate of the background tumor rate is required.

APPENDIX D.

HUMAN EPIDEMIOLOGICAL DATA AND RISK ASSESSMENT

As noted in Section 7.2.1, suggestive evidence of an elevated pancreatic cancer mortality rate associated with CH_2Cl_2 exposure was reported in an epidemiological study of Kodak workers (Hearne et al. 1987). These data were not used in a formal risk assessment because (1) the association between CH_2Cl_2 exposure and excess cancer was not statistically significant at a site for which an a priori hypothesis from animal data existed, and (2) a dose-related trend was not observed, (3) confounding factors may have been responsible for the apparent association and (4) the result could be an artifact of multiple statistical testing. Nevertheless, even epidemiological studies which yield negative results in a hypothesis test may be useful in evaluating the plausibility of risk estimates derived from animal studies.

DHS staff undertook two approaches to evaluate the compatibility of the animal and human results. First, a prediction derived by fitting the linearized multistage model to animal data was compared to observed cancer mortality. Second, one of the authors of the epidemiological study supplied additional data that enabled quantitative estimation of risk (Friedlander, 1987). A relative risk model was fitted to these data and the resulting unit risk was compared to the animal-based unit risk.

Comparison of Animal-Based Risk Predictions with Human Epidemiological Data

Hearne et al. (1987) reported that the occupational data in their study were inconsistent with an animal-based risk assessment. Using the EPA's unit risk, they calculated that 14.5 excess lung and liver tumors would be predicted among the exposed workers. However, there is no reason to assume that the most sensitive site in animals will be the most sensitive site in humans.

Therefore, DHS staff compared the predictions based on the lung tumors in mice to the cancer site having the greatest relative risk among workers occupationally exposed: the pancreas. The expected number of pancreatic cancer deaths in this cohort was 3.2 based on rates in the New York State population outside of New York City. Using the upper 95% confidence limit on the unit risk derived (in this document) by fitting the linearized multi-stage model to lung tumor data for female mice in the NTP (1986) study, 9.7 excess cancers were predicted for this cohort (see Table D-1 for calculations). If the 9.7 predicted from exposure are added to the 3.2 expected background pancreatic cancer deaths, a total of 12.9 such deaths would be predicted for this cohort.

Considering that the animal-to-human extrapolation is over two orders of magnitude of exposure, and that the animal and human exposures were temporally dissimilar, this prediction (12.9) is quite close to the observed mortality: eight pancreatic cancer deaths 95% confidence interval - (3.5, 15.8).

In general, DHS staff do not assume that the most sensitive target site is the same organ in humans as it is in the test species used for extrapolation. Nevertheless, staff ace that the conclusion here would remain the same even using the same target site: if the 14.5 excess cancer deaths predicted by Hearne et al. (1987) are added to the 21.8 background expected lung and liver cancer deaths, the resulting prediction is also within a factor of 3 of the observed 14 deaths due to cancers at these sites. Thus, given the confidence limits about the human data, DHS staff believe that the human data could be consistent with animal data, contrary to the assertion of Hearne et al. (1987) that they are inconsistent. Other investigators have reached conclusions similar to those of DHS staff (Mirer et al., 1988, Tollefson et al., 1988).

Calculation of Risk Based on Human Epidemiological Data

The second approach to utilizing the human epidemiological study was to fit a model to the data relating methylene chloride exposure and pancreatic cancer deaths. The data used for this analysis are in Table D-2. A relative risk model was fitted to these data: this model assumes that the excess cancer rate due to the exposure is proportional to the background cancer rate. An absolute risk model was not used because in extrapolating from adult workers to the general population, this model assumes the excess rate to be independent of age; the relative risk model assumes excess risk will follow the same distribution as the background risk, a more plausible assumption.

Details of the model fitting are shown below. The slope was estimated as 0.584 with a variance of 0.1323. The pancreatic cancer death rate was significantly elevated when compared to the control population, however the trend with dose was not, resulting in a large variance for the slope.

Using the background incidence of 0.008 pancreatic cancer deaths based on U.S. 1981 Vital Statistics, the lifetime exposure to 1 ppb CH_2Cl_2 is 9 x 10^{-6}. This value can be compared to unit risks for the same exposure shown in Table 8.7 for the purpose of ascertaining the general consistency between the data sets.

Methods for Fitting Model and Deriving Excess Risk

The relative risk model assumes that the background cancer rate is increased by a proportional amount R per unit of exposure. Under this model

$$E[O_{ij}] = e_{ij} + Rx_i e_{ij} = e_{ij}(1+Rx_i),$$

where $E[\cdot]$ denotes the expectation of a random variable, subscripts i and j denote dose-level and age respectively (i=0 for the control group); and:

x_i = the lifetime continuous equivalent (LCE) in ppm for exposure group i,

e_{ij} = the expected number of cases based upon Kodak controls,

O_{ij} = the observed number of cases, and

R = unknown slope parameter to be estimated from the data.

The observed number of cases, O_{ij}, is assumed to follow a Poisson distribution. The estimate of R and its asymptotic variance were obtained using the maximum likelihood method [see Cramer (1946) for a discussion of the general approach]. The excess risk for an individual with continuous lifetime exposure to x ppm is then:

$$P(x) - P_o Rx$$

where P_o denotes the average lifetime background risk of pancreatic cancer. In the context of the model, P_o can be interpreted as the expected number of cases for the average individual. Values for P_o are obtained from NCI (1981) vital statistics and are based upon the sum of risks up to age 75 for the total U.S. population (Table D-3). A 95% confidence interval is obtained for the risk by substituting $R \pm 1.64[V(R)]^{1/2}$ for R in the risk equations.

Conclusions

In summary, the human epidemiological data are inadequate either to establish or to rule out carcinogenicity of methylene chloride in humans. Nevertheless, several investigators have compared the human epidemiological data with that of the animal risk assessment data to ascertain if they are generally consistent with each other. In this report two procedures were used to compare the human epidemiological data and the animal risk assessment data. Based on these comparisons, DHS staff conclude that the human data could be consistent with animal data.

TABLE D-1

CALCULATION OF PREDICTED EXCESS CANCER DEATHS

AMONG KODAK EMPLOYEES USING LINEARIZED-MULTISTAGE

MODEL FITTED TO LUNG TUMOR DATA IN NTP INHALATION BIOASSAY

		650 ppb	1790 ppb	3810 ppb	
1	Lifetime continuous exposure				
	95% UCL of slope L-M (ppb^{-1})	9×10^{-6}	9×10^{-6}	9×10^{-6}	
2	Individual excess risk	6×10^{-6}	17×10^{-6}	36×10^{-6}	
3A	Number of workers	350	353	310	
3B	Number excess deaths predicted to age 70 if all workers live	2.1	5.9	11.1	
4C	Adjustment for less than lifetime follow-up (from Hearne et al. 1987)	0.21	0.42	0.61	
Total					
5	Number excess deaths	.4	2.5	6.8	9.7

To use any other estimate of slope, take numbers in row 5, divide by L-M slope, multiply by choice of (alternate) slope, e.g., using 1.1×10^{-6}, row 5 has: 0.05, 0.2, 0.7.

The only difference between these numbers and those Hearne et al. (1987) is that the DHS unit risk (slope = 9×10^{-6}) is based on lung tumors only while EPA's (14×10^{-6}) is based on combined liver and lung tumors.

PERSON-YEARS, OBSERVED AND EXPECTED DEATHS FROM PANCREATIC CANCER FOR KODAK EMPLOYEES EXPOSED TO CH_2CL_2
WITH FOLLOW-UP THROUGH 1984

Cohort	Age Group							
	15-24	25-34	35-44	45-54	55-64	65-74	75+	Total
<350 ppm-years $(LCE=x_j=0.65)$:								
Person-years	1,109.9	3,381.7	2,808.3	1,047.4	612.8	306.2	88.2	..
Pancreatic cancer deaths:								
Observed o_{1j}	0	0	0	0	1	0	1	2
Expected e_{1j}	.000	.030	.134	.163	.226	.107	.058	.718
350-749 ppm-years $(LCE=x_j=1.79)$:								
Person-years	0	135	1,737.1	1,986.9	1,334.0	683.8	132.0	..
Pancreatic cancer deaths:								
Observed o_{2j}	0	0	0	1	0	1	0	2
Expected e_{2j}	.000	.001	.083	.310	.493	.239	.087	1.213
750+ ppm-years $(LCE=x_j=3.81)$:								
Person-years	0	0	165.8	1,112.3	1,643.5	1,005.2	174.8	..
Pancreatic cancer deaths:								
Observed o_{3j}	0	0	0	0	1	3	0	4
Expected e_{3j}	.000	.000	.008	.174	.607	.351	.116	1.256

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PERSON-YEARS, OBSERVED PANCREATIC CANCER CASES AND

RATES FOR KODAK EMPLOYEE CONTROL GROUP FOLLOW-UP THROUGH 1984

| | | Pancreatic Cancer | |
| | | Age-Specific | |
Age Group	Person-Years	Deaths	Rate
15-24	56,887	0	0
25-34	113,789	1	0.88×10^{-5}
35-44	83,627	4	4.78×10^{-5}
45-54	76,934	12	15.60×10^{-5}
55-64	67,713	25	36.92×10^{-5}
65-74	42,910	15	34.96×10^{-5}
75+	22,689	15	66.11×10^{-5}

APPENDIX E

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL

The Andersen et al. (1987) physiologically-based pharmacokinetic model (PBPK) consists of a series of mass balance differential equations describing the concentration of CH_2Cl_2 in five compartments, organs or tissue groups, i.e., the lungs, the liver, fat tissue, richly perfused tissues (e.g., kidney, brain and viscera), and slowly perfused tissue (e.g., skin and muscle) and a sixth compartment for gas exchange. The lung compartment is placed between the gas exchange compartment and the systemic arterial blood supply (Figure E-1). This model provides a mathematical description for the absorption, concentration, metabolism and elimination of CH_2Cl_2 to determine the effective dose at the animal target organs.

With this model CH_2Cl_2 may enter the body by inhalation in the gas exchange compartment, or by ingestion with absorption directly into the liver compartment. It is assumed that absorption of ingested CH_2Cl_2 occurs by a zero-order process entering the liver at a constant hourly rate.

This PBPK model requires three types of biochemical and physiological data: (1) partition coefficients for the different types of tissues, (2) physiological constants for blood flow and tissue volumes, and (3) biochemical constants for the critical biotransformation pathways.

The partition coefficients for liver, muscle and fat tissues were experimentally determined _in vitro_ by measuring the distribution of CH_2Cl_2 using a tissue homogenate. The tissues were homogenized as a 1:3 w/v

mixture using 0.9% saline and then partition coefficients determined with a closed vial equilibrium technique for various tissues including blood. The authors took the ratio of the air/blood to air/tissue coefficients to derive the tissue/blood partition coefficients. Blood/air partition coefficients were measured for mouse, rat, and human blood. Tissue/air partition coefficients for mouse and human tissue were not determined and were assumed to be equal to those measured in the rat. Physiological constants for tissue volume and blood flow were obtained from the published literature, and enzyme activities were obtained either from the literature, from _in vitro_ studies, or through computer optimization of results from closed inhalation chambers. For apportioning the the enzymatic activities between liver and lung, the authors selected values from Lorenz (1984) using 7-ethoxycoumarin for the MFO pathway and 2,5-dinitrochlorobenzene for the gluthathione S-transferase (GST) pathway. Andersen et al. (1987) used a computer curve-fitting technique in the optimization procedure to obtain the "whole tissue" kinetic constants for KM, VMAX and KF. However, since there was no human data for KF, the cytosolic GST rate constant, allometric scaling of body weight to the 0.7 power from mouse, hamster and rat data was used to obtain a human value. A cross-species comparison is conducted with this model.

The model is designed to estimate the tissue concentration of CH_2Cl_2 (or metabolites) in lung and liver for a given animal or human exposure. Liver and lung are assumed to be able to metabolize CH_2Cl_2 by both the cytochrome P450 (MFO) and cytosolic GST pathways. The P450 pathway is considered to be saturated at the experimental doses of the NTP (1986) bioassay, while the GST pathway was presumed not saturated, but first order. In this model, the

GST-pathway is assumed to be the only source of a carcinogenic intermediate.

DHS staff are concerned about the method used to derive the tissue partition coefficients since these values may substantially influence the final result. The use of tissue homogenate destroys the integrity of critical cell membranes and may significantly distort the true partitioning of materials. In a review of this model, the EPA concluded that the model overestimates postexposure blood concentration of CH_2Cl_2 by inhalation and suggested that this may be due to the data from the in vitro equilibrium studies (EPA 1985b). Additionally, the EPA noted that the MFO and the GST activities selected by Andersen et al. may not reliably reflect the appropriate rates or specific activity of CH_2Cl_2 in each tissue. For example, the specific activity of GST varies significantly depending on the substrate used for the assay, there is approximately a 15-fold difference between specific activities of 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene.

The DHS staff believe that this model requires experimental validation. The authors could have used noncarcinogenic endpoints, e.g., acute toxicity, to demonstrate the model's ability to predict biological responses. The computer optimization procedures, based on the loss of CH_2Cl_2 from closed chamber inhalation studies, used to derive the whole tissue kinetic constants is inexact. The fit of the model to the experimental data is a circular argument, since the fit was optimized to the experimental data, and it does not accurately reflect the model's ability to predict the experimental data.

DHS staff are aware that _in vitro_ studies of CH_2Cl_2 GST studies in humans, mice, rats and hamsters are in progress at several different laboratories. This may help to clarify the role of the GST pathway in humans as compared to mice. DHS staff believe that the MFO pathway also produces reactive metabolites that may contribute to the observed tumorigenic response, and the focus on the GST pathway may be inappropriate. However, the basic purpose of the PBPK model, to incorporate additional data to improve the exposure assessment, is sound. The model represents a scientifically plausible estimate of human exposure. Consequently, a risk estimate, based on the PBPK dose is presented in Section 8.
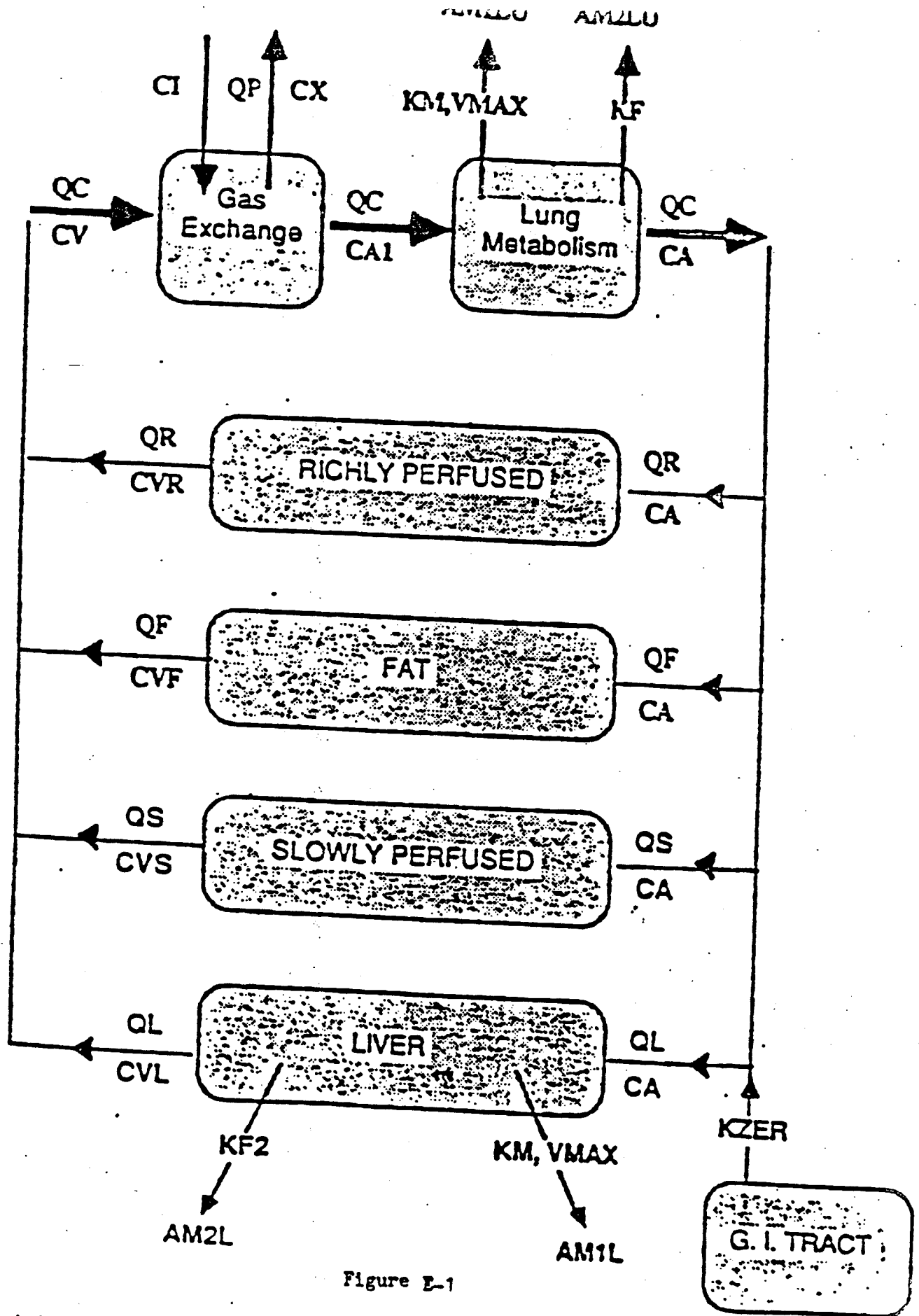
CI QP CX   KM,VMAX   KF

QC
CV → Gas Exchange   QC / CA1 → Lung Metabolism   QC / CA →

QR   RICHLY PERFUSED   QR
CVR   CA

QF   FAT   QF
CVF   CA

QS   SLOWLY PERFUSED   QS
CVS   CA

QL   LIVER   QL
CVL   CA

KF2   KM, VMAX   KZER

AM2L   AM1L   G. I. TRACT

Figure E-1

After: Andersen et al. 1987

LEGEND

AM1L - Amount of CH_2Cl_2 metabolized by MFO in liver.

AM2L - Amount of CH_2Cl_2 metabolized by gluthathione S-transferase (GST) in liver.

AM1LU - Amount of CH_2Cl_2 metabolized by MFO in lung.

AM2LU - Amount of CH_2Cl_2 metabolized by GST in lung.

CA   - Concentration of (mg/l) CH_2Cl_2 (mg/l) in arterial blood.

CA1 - Concentration of (mg/l) CH_2Cl_2 (mg/l) in blood leaving gas exchange compartment.

CI   - Concentration of CH_2Cl_2 (mg/l) in inhaled air.

CV   - Concentration (mg/l) of CH_2Cl_2 in mixed venous blood.

CVL - Concentration (mg/l) of CH_2Cl_2 in venous blood leaving liver.

CVF - Concentration (mg/l) of CH_2Cl_2 in venous blood leaving fat compartment.

CVS - Concentration (mg/l) of CH_2Cl_2 in venous blood leaving slowly perfused tissues.

CVR - Concentration (mg/l) of CH_2Cl_2 in venous blood leaving richly perfused tissues.

CX   - Concentration (mg/l) of CH_2Cl_2 in alveolar air.

KF   - First-order rate constant for lung GST pathway.

KF2 - First-order rate constant for liver GST pathway.

K_m - Michaelis-Menten constant for MFO pathway.

KZER - Zero-order rate of input of CH_2Cl_2 to liver.

QC   - Cardiac output (l/hr).

QF   - Blood flow through fat compartment (l/hr).

QL   - Blood flow through liver compartment (l/hr).

QR   - Blood flow through richly perfused compartment (1/hr).

QS   - Blood flow through slowly perfused compartment (1/hr).

V_{max} - Maximum velocity of metabolism by MFO.

(Andersen et al. 1987)

TABLE E-1

QUALITATIVE EVALUATION OF PARAMETERS AND ASSUMPTIONS

IN THE PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL[a]

Assumptions	Mouse Data[b]	Human Data[c]
Model structure is reasonable	B[d]	C
MFO saturates at high concentrations	A	C
MFO saturation concentration identified —	B	D
Identification of ultimate carcinogen(s)	D	F
Restriction of metabolism to lung and liver	C	F
Restriction of lung and liver as target organs	B	F

Input Parameters	Mouse Data	Human Data
Partition coefficients	D	D
Concentration of CH_2Cl_2 in alveolar air	B	B
First-order rate constant for lung GST pathway (KF)	C	F
First-order rate constant for liver GST pathway	C	F
Michaelis-Menten constant for MFO pathway (KM)	C	D
Maximum velocity of metabolism by MFO	C	D
Zero-order rate of input of CH_2Cl_2 from GI tract to liver	C	D
Cardiac output.	B	B
Ventilation rate	B	B
Tissue weights	B	B
Tissue volumes	B	B
Blood flow through fat compartment	C	C

Calculated Parameters	Mouse Data	Human Data
Blood flow through liver compartment	C	C
Blood flow through richly perfused compartment	C	C
Blood flow through slowly perfused compartment	C	C
Amount of CH_2Cl_2 metabolized by MFO in liver	B	D
Amount of CH_2Cl_2 metabolized by GST in liver	B	D
Amount of CH_2Cl_2 metabolized by MFO in lung	C	F
Amount of CH_2Cl_2 metabolized by GST in lung	C	C
Concentration of CH_2Cl_2 in arterial blood	B	C
Concentration of CH_2Cl_2 in blood leaving gas exchange compartment	C	D
Concentration of CH_2Cl_2 in inhaled air	A	A
Concentration of CH_2Cl_2 in mixed venous blood	B	D
Concentration of CH_2Cl_2 in venous blood leaving liver	C	F
Concentration of CH_2Cl_2 in venous blood leaving fat compartment	D	F
Concentration CH_2Cl_2 of in venous blood leaving slowly perfused tissues	D	F
Concentration of CH_2Cl_2 in venous blood leaving richly perfused tissues	C	D

[a] This table reflects a subjective rating of the confidence DHS staff have in the values, parameters and assumptions used in the PBPK model of Andersen et al. 1987 as applied by EPA (1987a,b).

[b]Mouse data refers to test data or assumptions used by CPSC and EPA in applying the PBPK model. This evaluation refers to the high-to-low dose adjustment used by CPSC (Cohn 1987, EPA 1987a).

[c]Human data refers to test data, extrapolated data or assumption used by EPA (1987a,b) in applying the PBPK model for their estimate of risk from CH_2Cl_2 exposure.

[d]A indicates high confidence in value or assumption, F indicates low confidence in value or assumption.

CALCULATION OF HUMAN EQUIVALENT DOSAGES

To extrapolate the absorption of an inhaled substance across species, there are two alternate baseline assumptions: (1) the carcinogenic agent is a water-soluble gas and is absorbed in proportion to the amount of air inhaled; or (2) the agent is not water-soluble; thus, after equilibrium is reached the rate of absorption is proportional to the metabolic rate (EPA 1985a). Methylene chloride is moderately water-soluble (2 g/100 ml); thus, it is likely to be absorbed by the lungs to a high degree in both humans and rodents. Andersen et al. (1987) directly measured the blood/air partition coefficient in B6C3F1 mice and in humans as 8.29 and 9.7, respectively. Klassen (1986) considers a blood-gas solubility of about 1.2 as the demarcation between the two solubility cases defined by low solubility, thus perfusion limited, and gases with high solubility and hence ventilation limited. Thus, CH_2Cl_2 should be considered as a very soluble gas at low concentrations and the application of an interspecies correction based on surface area appears appropriate. The EPA treated CH_2Cl_2 as a water-soluble agent and estimated the risks to humans at low doses using the interspecies conversion formula based on surface area (EPA 1985b). The draft update (EPA 1987b) also used the surface area correction although it is used primarily to account for interspecies differences in CH_2Cl_2 pharmacodynamics.

F.1 Conversion of Rodent Exposures to Lifetime mg/kg/day

The inhalation rates for the rodents were estimated using the following formulas (EPA 1985b):

For mice: $I = 0.0345 \, [wt(kg)/0.025(kg)]^{2/3} \, m^3/day$

For rats: $I = 0.105 \, [wt(kg)/0.113(kg)]^{2/3} \, m^3/day$

Inhalation rates were calculated using the NTP (1985) average weights for mice and rats (both sexes) at the midpoint (51 weeks) of the bioassay, since the NTP report does not give the average animal weight over the whole study period (Table F-1).

TABLE F-1

ESTIMATED INHALATION RATES FOR NTP (1986)
TEST ANIMALS (EPA, 1985b)

	Weight at Bioassy Midpoint (kg)	Estimated Inhalation Rate (m^3/day)
Male Rat	0.462	0.268
Female Rat	0.278	0.191
Male Mouse	0.037	0.0448
Female Mouse	0.032	0.0407

Dose conversion factors can be calculated between the NTP dosing schedule (6 hr/day, 5 days/week) and a continuous mg/kg/day exposure assuming a lifetime exposure:

Continuous exposure

= (Experimental Dose) x (6 expt hours/day ÷ 24 hours/day) x (5 expt days/week ÷ 7 days/week)

= (Experimental Dose) x (0.17857)

An inhalation exposure in ppm can be converted to an estimated inhaled dose in mg/kg (assuming 1 ppm $=$ 3.47 mg/m^3) as shown in the following example:

Average inhaled dose for mouse breathing 2000 ppm CH_2Cl_2

$=$ (2000 ppm) x (3.47 mg/m^3/ppm) x (0.0407 m^3/day) x (0.17857) $\div$ (0.032 kg)

$=$ 1576 mg/kg-day

F.2 Calculation of Human Equivalent Doses

Equivalent human CH_2Cl_2 doses are calculated using the Carcinogen Assessment Group (CAG) methodology for well-absorbed vapors. The CAG assumed that humans and animals exposed to equal doses of a carcinogen on a $(mg/kg)^{2/3}$ basis over equivalent proportions of a lifetime will encounter the same degree of cancer risk. This implies that a rodent with weight W_R, exposed to a dose of D mg/kg/day and a human with weight W_H, exposed to a dose of $D\left(W_H/W_R\right)^{-1/3}$ mg/kg/day encounter the same lifetime cancer risks (EPA 1985b).

Using this information, the surface area correction, based on an inhaled rodent dose can be calculated by the following formula where C is the exposure concentration and BR refers to the breathing rate of humans (H) or rodents (R).

Estimated Human Dose Equivalent

$= C \times \left(BR_R/W_R\right) \times \left(W_H/W_R\right)^{-1/3} \times \left(W_H/BR_H\right)$

$= C \left(BR_R/BR_H\right) \times \left(W_H\right)^{2/3} / \left(W_R\right)^{2/3}$