NCEA-S-0570

TOXICOLOGICAL REVIEW

OF

CHLOROETHANE

(CAS No. 75-00-3)

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

July 1999

NOTICE

This document is a **preliminary draft.** It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency position on this chemical. It is being circulated for peer review on its technical accuracy and science policy implications.

U.S. Environmental Protection Agency
Washington, DC

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(CAS No. 75-00-3)

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FOREWORD

The purpose of this Toxicological Review (ToxR) is to support the hazard identification and dose-response assessment for cancer and noncancer effects (the oral reference dose [RfD] and the inhalation reference concentration [RfC]) from chronic exposure to chloroethane (CE). Supportive CE subchronic studies also are included. The ToxR is a review and analysis of data supporting the chemical or toxicological nature of CE and supports the Integrated Risk Information System (IRIS) Summary document. The ToxR characterizes each relevant study with regard to overall confidence in the quantitative and qualitative aspects of hazard. This analysis considers knowledge gaps, uncertainties, and quality of data, while highlighting the limitations of the individual studies and providing a guide to the risk assessment process.

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

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
BE	Bromoethane
BM	Bromomethane
BMC	Benchmark Concentration
CE	Chloroethane
CDNB	1-Chloro-2,4-dinitrobenzene
CHO	Chinese hamster ovary
CIIT	Chemical Industry Institute of Toxicology
CM	Chloromethane
EROD	Ethoxyresorufin O-dealkylase
GD	Gestation day
GSH	Glutathione
HDT	Highest dose tested (in a bioassay)
HEC	Human equivalent concentration
HPRT	Hypoxanthine Phosphoribosyltransferase
IM	Iodomethane
MDT	Maximum tested dose (in a bioassay; same as HDT)
MTD	Maximum tolerated dose (ascertained in a subchronic bioassay)
MN	Micronucleus
MNL	Mononuclear cell leukemia
NPSH	Non-protein sulfhydryl
NTP	National Toxicology Program
p-NP	p-Nitrophenyl hydroxylase
PBPK	Physiologically based pharmacokinetic
PROD	Pentoxyresorufin O-dealkylase
RfC	Reference concentration
RfD	Reference dose
ToxR	Toxicological review
SECys	S-Ethyl-L-cysteine
SEG	S-Ethyl glutathione
SENAYCys	S-Ethyl-N-acetyl-cysteine
UDS	Unscheduled DNA synthesis

1. INTRODUCTION

This document (ToxR) presents a complete compilation and analysis of available information on the toxicity of chloroethane (CE) in experimental exposure studies to animals. No human studies are known to exist. CE is a simple halohydrocarbon (Figure 1). In an attempt to establish relative safe environmental exposure levels, the quantitative oral reference dose (RfD) and inhalation reference concentration (RfC) values shall be developed from applicable non-cancer toxicological responses to CE where the data are sufficient.

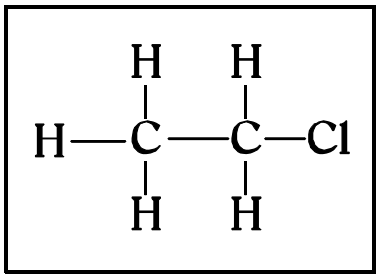

Figure 1. Chloroethane chemical structure.
Chloroethane is a small, gaseous, hydrophobic molecule. C-1 is susceptible to nucleophilic attack due to the polarity of the C–Cl bond because of the electronegativity of the Cl atom relative to the C-1 carbon.

Toxicological analysis of chronic exposure studies leads to the derivation of the RfD and/or RfC that provide information on long-term toxic effects other than carcinogenicity. The RfD assumes that thresholds exist for certain toxic effects such as cellular necrosis, but not for other toxic effects such as some carcinogenic responses. The RfD, expressed in milligrams per kilogram per day (mg/kg/day), is an approximation of the daily exposure to humans that is likely to result in no appreciable risk of deleterious effects over a lifetime of continuous exposure. The inhalation RfC is analogous to the oral RfD and considers toxic effects to the respiratory system (portal-of-entry) and extrarespiratory, or systemic, effects expressed in milligrams per cubic meter (mg/m^3).

The carcinogenicity assessment provides information on aspects of the carcinogenic risk assessment for the agent in question which includes the U.S. EPA classification, and quantitative estimates of risk from oral exposure and from inhalation exposure. The classification reflects a weight-of-evidence judgment of the likelihood that the agent may be a human carcinogen, or not, and the conditions under which any potential carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor*, resulting from the application of a low-dose extrapolation procedure, is presented as the risk per milligrams per kilogram per day $[(mg/kg/day)^{-1}]$. The risk is the quantitative estimate in terms of either risk microgram per liter $[(\mu g/L)^{-1}]$ drinking water or risk per microgram per cubic meter $[(\mu g/m3)^{-1}]$ air breathed. The third form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000.

Hazard identification and dose-response assessment for CE follow the general risk assessment principles for established by the National Research Council (1983). EPA guidelines used in the development of this assessment include the following:

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1. Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1986)
2. *Proposed* Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a)
3. Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991)
4. Guidelines for Reproductive Risk Assessment (U.S. EPA, 1996b)
5. Guidelines for Neurotoxicity Risk Assessment (proposed) (U.S. EPA, 1995b)
6. Methods for Derivation of Inhalation Reference Concentrations and application of Inhalation Dosimetry (U.S. EPA, 1996)
7. Guidelines for Mutagenicity Risk Assessment (U.S. EPA, 1986c)
8. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994)
9. Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988a)
10. Use of the Benchmark Dose Approach in Health Risk Assessment (U.S. EPA, 1995a)
11. Science Policy Council Handbook: Peer review (U.S. EPA, 1998b)

The literature search strategy for CE is based on the CASRN and at least one common name, and includes the following databases: HEAST, RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. The current IRIS file for this chemical (U.S. EPA, 1998a) and the ATSDR toxicological profile (ATSDR, 1997) was also used as a resource.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Common synonyms of chloroethane (CE) include ethyl chloride, monochloroethane, Kelene, moriatic ether, narcotile, hydrochloric ether, Chloryl, Chloryl Anesthetic, Dublofix, and NCI-C06224. Some relevant physical and chemical properties of CE are listed below (U.S. EPA, 1988b):

CASRN: 75-00-3
Empirical formula: C_2H_5Cl
Structural formula: CH_3CH_2Cl
Molecular weight: 64.5
Specific gravity: 0.897 (at 20°C)
Vapor pressure: 1,000 mm Hg (at 20°C)
Vapor density: 2.22 (air = 1.0)
water solubility: 5,710 mg/L
Melting point: –138.7°C
Boiling point: 12.3 °C at 760 mm Hg
Log K_{ow}: 1.43
Chloroethane gas conversion factors:
1 ppm = 2.64 mg/m^3, 1.0 mg/m^3 = 0.38 ppm

CE is a colorless gas at room temperature, with a sweet taste and a pungent ether-like odor. CE is flammable. Even under increased pressure and lowered temperatures it is a volatile and mobile liquid. The explosion limits are 3.8% up to 14.8% by volume in air, which means air concentrations of 38,000 ppm or more can ignite. This indicates the upper limit in testing CE in bioassays. Combusted CE forms phosgene ($COCl_2$), among other products. CE reacts with steam to form corrosive oxidizing materials. Under ambient conditions, CE is an extreme fire and explosion risk at higher concentrations.

CE is used primarily as an intermediate in the production of perfumes, tetraethyl lead (a decreasing use), ethylcellulose, ethylbenzene, alkyl catalysts, and pharmaceuticals. In the past, CE was used as a general anesthetic (loss of sensation and consciousness) or a narcotic (producing stupor) (Lawson, 1965; Cole, 1967). In recent times, however, CE's medical application has become limited to use in topical skin analgesic sprays, for example, for the temporary relief of sports injuries or the discomfort associated with ear piercing. CE has also been used on a limited basis as a solvent (e.g., for elemental phosphorous, fats, waxes, acetylene, and a number of resins) and a refrigerant (ATSDR, 1997).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

3.1.1. Gastrointestinal Absorption

Though no information is available on the intestinal absorption of CE in humans, a report by Dow Chemical Co. (1992) addressed the potential for the compound to induce toxic effects via the oral route in laboratory animals. CE was administered in a single gavage dose of either 37 or 1,750 mg/kg ^{14}C-CE in corn oil. The animals were sacrificed 48 hr after dosing. An alternative regimen involved the administration of seven daily doses of the unlabeled compound at 37 mg/kg, followed on day 8 by 37 mg/kg of ^{14}C-CE, before termination. Overall recovery of radioactivity was good and ranged 87–93% of the administered dose. Most of the counts (77–89%) were in exhaled as $^{14}CO_2$ or as unchanged CE. Recovery in feces was only 1.44%. This supports the conclusion that CE absorption from the gastrointestinal tract is nearly quantitative. This uptake is consistent with the ability of many lipid-soluble xenobiotics, such as CE, to cross the brush border (and other biological membranes) with great facility.

3.1.2. Respiratory Absorption

The same physicochemical characteristics that favor absorption of the compound at the intestinal mucosa might be expected to facilitate absorption at the alveolar membrane. Morgan et al. (1970) investigated respiratory absorption: they studied a human volunteer who took one breath of ^{38}Cl-labeled CE via the mouth, held his breath for 20 seconds, then exhaled. This was repeated. Only 18% of the counts (radioactivity) was exhaled after two exhalations, thus by inference 82% was retained and adsorbed. The constituents of the exhaled breath were not analyzed. It was further noted that an additional 30% of the CE counts were exhaled during the first hour. Only small amounts were excreted in the urine. Although this experiment was not quantitative, it shows that pulmonary CE retention in the first hour is > ½ of the initial inhaled ^{38}Cl-labeled CE counts. This suggests that CE is more likely to be absorbed in the lung than to be retained within the alveolar lumen.

Respiratory absorption has also been studied in laboratory animals. Groups of 10 female B6C3F1 mice and 10 female F344 rats were exposed by inhalation to 150 (a low dose) or 15,000 ppm (a high dose) of ^{14}C-CE (0.14-2.25 µCi/mg CE) for 6 hr (Dow Chemical Co., 1992). No males were studied. Half the animals were sacrificed immediately after dosing, while the other

half were maintained in metabolic cages for 48 hr. While these animals were metabolizing CE, urine, feces, and exhaled gases were collected. Animals were sacrificed after 48 hr and selected tissues were analyzed for radioactivity. The tissue distribution of CE or CE metabolites is presented in Table 1.

At 150 ppm, female mice and rats, respectively, exhaled 42% and 54% of the counts as CO_2, 35% and 32% in the tissues and carcass, 16% and 10% in the urine, 6% and 3% in the feces, and < 2% in the breath as unchanged CE. So, at the low dose, a substantial portion of the inhaled CE input was metabolized by both species, and there were comparable counts in the various compartments in both species. These data support those of Morgan et al. (1970) by indicating that CE can be readily absorbed at the alveolar membrane.

However, with exposure at the highest dose treated (HDT), 15,000 ppm, the relative distributions shifted (Table 1). In both mice and rats, respectively, expired CO_2 decreased from "150 ppm levels" to 32% and 19%, tissues and carcass decreased to 16% and 8%, urine increased to 38% in the female mouse but showed no change at 9% in the female rat, feces remained unchanged at 7% in mice but decreased to 2% in the rat, and expired unchanged CE in the breath

Table 1. Distribution of recovered radiolabeled chloroethane in female rats and mice 48 hr after inhalation exposure[a]

Mode of excretion/ deposition	Relative percentage of radioactivity recovered (%)			
	Female mouse		Female rat	
	150 ppm	15,000 ppm	150 ppm	15,000 ppm
Expired CE	1.72 ± 0.53	6.96 ± 1.75	1.12 ± 0.32	62.81 ± 1.32
Expired CO_2	41.76 ± 11.35	31.60 ± 6.84	53.57 ± 2.34	19.17 ± 0.98
Urine	15.86 ± 4.10	38.37 ± 9.13	9.66 ± 1.09	8.68 ± 1.22
Feces	6.02 ± 1.91	7.05 ± 4.84	3.15 ± 0.16	1.60 ± 0.66
Tissue/carcass	34.65 ± 12.79	16.02 ± 2.04	32.03 ± 2.82	7.64 ± 0.97

[a]Values are the means ± SD for five animals in each exposure group. Males were not tested.
Source: Dow Chemical Co. (1992).

showed relatively large increases in the mouse, to 7% (4-fold), and to 63% in the rat (56-fold). On a per microequivalent basis the authors reported a 49-fold increase in nonmetabolized CE in the breath in the mouse and a 700-fold increase in the rat (Dow Chemical Co., 1992). Thus, the

compartmental recoveries did not increase in proportion to CE exposure. These disproportions, and the high amount of parent CE exhaled at the HDT, suggest that CE metabolic disposition is saturated at 15,000 ppm compared to 150 ppm.

3.2. DISTRIBUTION, METABOLISM, AND EXCRETION

There is no information on the distribution, metabolism, and excretion of CE in humans in the literature. However, toxicological data that shed light on these issues have come from animal studies involving (1) inhalation of radiolabeled CE, and (2) in vivo and in vitro experiments in which the relevance of certain putative catabolic mechanisms has been evaluated following challenge with CE. Taken together, these findings have identified some CE intermediates and excretory products, thereby pointing to the possible mechanism(s) that may be involved in CE's metabolism (Figure 2).

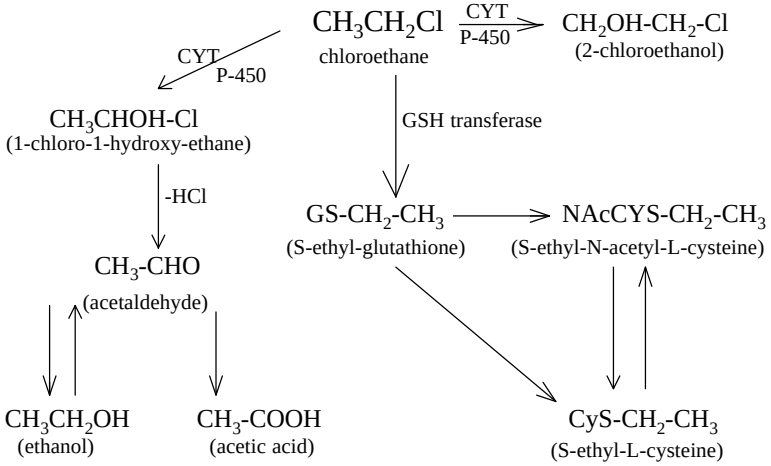

Figure 2. Proposed Metabolic Pathways for Chloroethane. Scheme presented in Fedtke et al., 1994b).

Dow Chemical Co. (1992) drew a contrast between the disposition of products of CE metabolism after inhalation of low (150 ppm) versus high (15,000 ppm) concentrations of the radiolabeled compound by female F344 rats or female B6C3F1 mice (Table 1). The higher concentration of CE appeared to saturate the metabolic processes, resulting in an increase in the proportion of unchanged compound that was exhaled. At the HDT, this CE exhalation was especially marked in rats (62.81% of recovered radioactivity) versus mice (6.96% of recovered radioactivity). These data in Table 1 suggest that female B6C3F1 mice may have a greater capacity to metabolize CE at 15,000 ppm than female F344 rats. Whether as CO_2 or as CE, most of the exhaled counts and those collected in urine were recovered in the first 24 hrs, thereby showing rapid CE metabolism. After 48 hr, the primary target tissues appeared to be ovary, adrenals, and skin (Dow, 1992). By contrast, the Dow research team noted a lack of selective

retention of counts in the uterus, an organ identified as an important site of potential carcinogenic responses to the compound in female B6C3F1 mice (Section 4.2.2.2.3.4, p. 23).

Reduction. Dow Chemical Co. (1992) explored the effect of inhalation CE exposure on tissue glutathione (GSH) content in female F344 rats and female B6C3F1 mice. GSH is a reducing agent often employed in cells to metabolize xenobiotics (Figure 4). GSH content was measured by analyzing the non-protein bound free sulfhydryls (NPSH). Dow researchers exposed female rats and mice to 150, 3,000, 6,000 (mice only), or 15,000 ppm unlabeled CE for 3 or 6 hr. Effects were seen only at 15,000 ppm CE, which suggests a threshold for GSH depletion (Table 2). During this exposure period (at 15,000 ppm CE), the GSH decreased in mice and rats below normal levels. Mice, for example, showed GSH depletions in the following tissues: liver (21%), kidney (56%), lung (32%), and uterus (55%). Mouse brain and adrenals did not show GSH decreases. Blood showed the largest absolute decrease, 870 → 618 nmol GSH/mg blood. Blood can account for significant amounts of [GSH] changes in tissues, or could reflect systemic GSH changes, or both. The above were the only tissues sampled for GSH. Recovery to control levels and overshooting to excessive GSH tissue concentrations occurred 18 hr after CE exposure in mice (Table 2). The rat data pointed to GSH decreases in the liver (65%), ovaries (57%) and adrenals (32%) (Table 3). The authors discussed that a relationship between CE-induced GSH depletion and the induced toxicity is plausible and a suggested pathway is presented in Figure 3 .

Fedtke et al. (1994a,b) sought to explain the biochemical mechanism(s) by which CE is catabolized and the processes by which CE induces metastatic endometrial uterine tumors in B6C3F1 mice but not in F344 rats. Using an analogous protocol to that employed by the NTP (1989a), these workers exposed groups of male and female F344 rats and B6C3F1 mice to 0 or 15,000 ppm CE via inhalation, 6 hr/day for 5 days.

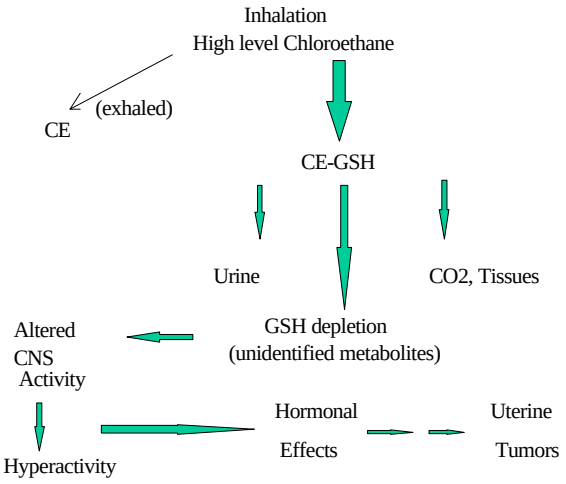

Figure 3. Proposed metabolic scheme for chloroethane disposition and toxicity in mice and rats following a high-level inhalation exposure. Source: This scheme is adapted from a scheme proposed by the Dow Chemical Co., 1992.

Table 2. GSH levels in female B6C3F1 mouse tissues after in vivo inhalation exposure to CE: specific GSH levels at the completion of exposure and after an 18-hr recovery

Exposure for 6 hr at 15,000 ppm

Tissues	Mouse tissue GSH levels (nmoles/mg tissue)[a]					
	Control			Exposed		
	Time after incubation					
	0 hr	18 hr	0:18 ratio	0 hr	18 hr[b]	0:18 ratio
Liver	5.49 ± 0.37	4.73 ± 1.21	1.22	1.18 ± 0.27	5.50 ± 1.29***	0.21
Kidney	2.80 ± 0.31	3.33 ± 0.28*	0.84	1.65 ± 0.04	3.62 ± 0.16***	0.46
Brain	1.62 ± 0.09	1.55 ± 0.11	1.05	1.30 ± 0.09	1.48 ± 0.12	0.88
Lung	1.74 ± 0.23	1.85 ± 0.12	0.94	0.56 ± 0.11	2.33 ± 0.05***	0.24
Ovary	1.68 ± 0.04	1.60 ± 0.03	1.05	0.94 ± 0.10	1.59 ± 0.17*	0.59
Adrenal	2.00 ± 0.47	1.69 ± 0.23	1.18	1.36 ± 0.13	1.87 ± 0.17	0.73
Uterus	1.48 ± 0.42	1.20 ± 0.65	1.23	0.82 ± 0.27	1.67 ± 0.25**	0.49
Blood	870.99 ± 96.23	999.26 ± 132.33	0.87	618.00 ± 68.58	872.50 ± 39.26***	0.71

[a]Exposure to inhaled CE preceded tissue analysis for GSH levels. Recovery tissues were analyzed after exposure and 18 hr of nonexposure. Values are the means of eight liver samples, four kidney samples, two adrenal and ovary samples, and four samples of all other tissues.

[b]Statistical comparisons (0 hr versus 18 hr) were done by Dow using the Student's t-test, * <0.05, ** <0.01, *** <0.001.

Source: Dow Chemical Co. (1992).

Table 3. GSH levels in rat tissues after in vivo inhalation exposure to CE-specific GSH levels at the completion of exposure and after an 18 hr recovery

Exposure for 6 hr at 15,000 ppm

Rat tissue GSH levels (nmol/mg tissue)						
	Control			Exposed		
Time after incubation	**0 hr**	**18 hr**	**0:18 ratio**	**0 hr**	**18 hr[b]**	**0:18 ratio**
Liver	5.53 ± 0.41	5.75 ± 0.37	0.96	3.58 ± 0.38	5.51 ± 0.70***	0.65
Kidney	3.91 ± 0.23	3.72 ± 0.24	1.05	3.04 ± 0.16	3.81 ± 0.19***	0.80
Brain	1.60 ± 0.05	1.56 ± 0.10	1.03	1.45 ± 0.10	1.44 ± 0.11	1.01
Lung	1.68 ± 0.08	1.76 ± 0.06	0.95	1.32 ± 0.07	1.69 ± 0.28*	0.78
Ovary	2.56 ± 0.18	2.89 ± 0.35	0.89	1.45 ± 0.39	2.72 ± 0.51	0.53
Adrenal	2.42 ± 0.02	2.82 ± 0.05*	0.86	0.77 ± 0.11	3.45 ± 0.55*	0.22
Uterus	0.98 ± 0.13	1.27 ± 0.17*	0.77	0.67 ± 0.10	1.01 ± 0.30	0.66
Blood	805.88 ± 43.28	N/D	-	1160.00 ± 36.74	1006.76 ± 84.07*	1.15

[a]Exposure to inhaled CE preceded tissue analysis for GSH levels. Recovery tissues were analyzed after exposure and 18 hr of nonexposure. Values are the means of eight liver samples, eight kidney samples, two adrenal and ovary samples, and four samples of all other tissues.

[b] Statistical comparisons (0 hr versus 18 hr) were done by Dow using the Student's t-test, * <0.05, ** <0.01, *** <0.001.

Source: Dow Chemical Co. (1992).

In one study, Fedtke et al. (1994b) examined the ability of GSH to conjugate CE in a reductive conjugation reaction, an example of which is shown in Figure 4. The enzymatic nature of the CE-GSH reaction was investigated in an in vitro protocol featuring the addition of cytosolic preparations from mice or rats to a mixture of CE and GSH. Cytosolic GSH concentrations (as measured by NPSH) were measured in 105,000 x g supernatant centrifuge preparations from liver, lung, kidney, and uterus in control and CE-exposed rats and mice. Also measured were the most likely CE-GSH reaction product, S-ethyl glutathione (SEG), and an enzyme catalyzing the synthesis of the SEG, GSH-S-transferase. Finally, the appearance of the putative SEG metabolites, S-ethyl-N-acetyl-cysteine (SENACys) and S-ethyl-L-cysteine (SECys), was monitored in the urine of control and CE-exposed rats and mice.

GSH-metabolite results were as follows (Fedtke et al., 1994b): In rats, GSH was

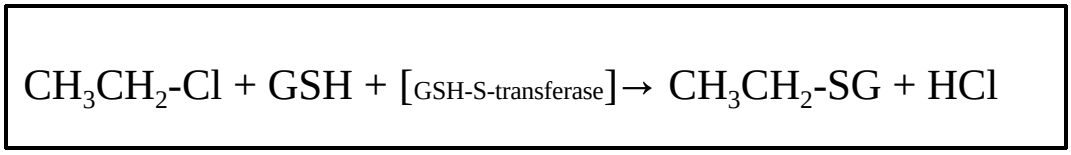

Figure 4. Reductive conjugation of chloroethane with glutathione (GSH). The same reaction is thought to take place with other methyl and ethyl halides.

decreased compared to controls in male liver ($p<0.01$), female uterus and kidney ($p<0.01$), and the lung of both sexes ($p<0.01$). In mice, significant GSH decreases were observed also in the uterus and kidney ($p<0.01$). In rats, similar amounts of SEG were measured in both exposed and control preparations, thus no effect. In mice elevated levels of SEG were measured in liver cytosols ($p<0.01$).

Table 4 summarizes GSH S-transferase enzyme activities in various tissues in mice and rats that were treated with 15,000 ppm CE or were air controls. The GSH S-transferase is measured by use of the nonspecific substrate CDNB, which measures total GSH S-transferase activity in tissue preparations; the use of CDNB may obscure any specific isozymic GSH S-transferase changes. The comparison of S-transferase total activities showed that rats had consistently higher activities than mice (Fedtke et al., 1994b). This does not agree with an earlier Dow study finding that depletions in mice were –80% and in rats were –35% after CE treatment, suggesting higher transferase activity in mice (Pottenger et al., 1992). When the activities were compared within species pre- and posttreatment, the only biologically significant changes were in the livers of female rats and female mice (Fedtke et al., 1994b).

Table 4. The effect of chloroethane exposure on baseline cytosolic GSH-transferase enzyme activity in rats and mice

Species/sex	Tissue	Controls	CE exposure
		(µmol/min-mg)	(µmol/min-mg)
F-344-m	Liver	0.61 ± 0.04	0.61 ± 0.04
	Lung	0.11 ± 0.03	0.10 ± 0.01
	Kidney	0.19 ± 0.02	0.15 ± 0.01 [a]
F-344-f	Liver	0.54 ± 0.05	0.74 ± 0.10 [a]
	Lung	0.17 ± 0.01	0.12 ± 0.02 [b]
	Kidney	0.17 ± 0.01	0.16 ± 0.01
	Uterus	0.26 ± 0.04	0.33 ± 0.16
B6C3F1-m	Liver	3.09 ± 0.12	3.07 ± 0.45
	Lung	0.33 ± 0.05	0.40 ± 0.09
	Kidney	0.55 ± 0.05	0.52 ± 0.04
B6C3F1-f	Liver	1.14 ± 0.23	1.87 ± 0.14 [b]
	Lung	0.48 ± 0.02	0.32 ± 0.06 [a]
	Kidney	0.70 ± 0.02	0.63 ± 0.01 [b]
	Uterus	0.62 ± 0.06	0.72 ± 0.07

[a] $p < 0.05$ versus controls.
[b] $p < 0.01$ versus controls.

Source: Fedtke et al. (1994b). This paper also tabulated the excretory kinetics of the conjugate SEG.

metabolites, SENACys and SECys, and demonstrated elevated specific amounts (in µmol/kg body weight) of SENACys in the urine of mice compared to rats (Table 5). SECys was undetected in the urine of exposed rats, though the compound was present in the urine of both exposed and control mice (Table 6).

Taken together, the data presented by Fedtke et al. (1994b) and Pottenger et al. (1992) make the case that reductive GSH conjugation constitutes at least one important pathway for CE

Table 5. Excretory kinetics of S-ethyl-N-acetyl-cysteine in CE-exposed F344 rats and B6C3F1 mice [a]

Urine collection time interval (hr)	F-344 (m) (µmol/kg bw)	F-344 (f) (µmol/kg bw)	B6C3F1 (m) (µmol/kg bw)	B6C3F1 (f) (µmol/kg bw)
0–7	19.2 ± 7.6	27.0 ± 5.0	93.4 ± 36.7	102.1 ± 14.7
7–24	50.1 ± 8.6	54.2 ± 5.1	44.1 ± 33.0	27.1 ± 8.9
24–31	17.5 ± 12.2	8.1 ± 11.5	155.6 ± 45.7	58.6 ± 5.6
31–48	55.7 ± 16.0	89.7 ± 9.2	31.2 ± 16.5	6.3[b]
48–55	16.4 ± 5.6	24.7 ± 2.4	169.3 ± 30.6	105.5 ± 49.4
55–72	41.9 ± 8.7	59.8 ± 7.7	43.9 ± 28.7	11.3 ± 10.4
72–79	13.1 ± 1.4	20.7 ± 14.5	127.9 ± 29.5	99.7 ± 47.1
79–96	43.8 ± 16.3	55.0 ± 10.0	35.2 ± 12.9	36.1[c]
96–103	6.9 ± 2.2	5.6 ± 6.2	34.8 ± 15.8	75.6 ± 38.6

[a] Data are from Fedtke et al. (1994b) and are the mean ± SD.

[b] Data from one group only.

[c] Data from two groups only.

Table 6. Excretory kinetics of S-ethyl-L-cysteine in CE-exposed B6C3F1 mice [a]

Urine collection time interval (hr)	B6C3F1 (m) (µmol/kg bw)	B6C3F1 (f) (µmol/kg bw)
0–7	46.6 ± 19.4	23.9 ± 3.5
7–24	42.3 ± 33.7	19.5 ± 9.7
24–31	112.8 ± 15.0	28.0 ± 7.1
31–48	31.6 ± 11.1	8.5[b]
48–55	46.8 ± 24.7	33.7 ± 5.6
55–72	28.8 ± 10.3	6.3 ± 3.2
72–79	43.5 ± 18.2	25.3 ± 2.4
79–96	18.7 ± 8.6	8.4 ± 6.9
96–103	9.3 ± 3.1	17.8 ± 8.7

[a] Data are taken from Fedtke et al. (1994b) and are the mean ± SD.

[b] Data from one group only.

metabolism (see Figure 1, p. 1). Fedtke et al. (1994b) further discussed their results in the context of CE's ability to induce uterine tumors in B6C3F1 mice, and speculated that CE-induced tumor formation might be a consequence of alterations in normal cellular GSH pools, GSH conjugation, and GSH-related metabolites.

Oxidation. Fedtke et al. (1994b) also examined the potential for CE to be oxidized via one or more of the cytochrome P-450-dependent metabolic pathways. Liver microsome preparations from CE-exposed or control F344 rats and B6C3F1 mice were measured for their ability to oxidatively metabolize CE to acetaldehyde in vitro. Also measured were the specific activities of p-nitrophenyl hydroxylase (p-NP), a marker enzyme for cytochrome P450IIE1; ethoxyresorufin O-dealkylase (EROD), a marker for cytochrome P450IA; and pentoxyresorufin O-dealkylase (PROD), a marker for cytochrome P450IIB. Samples of blood and urine from exposed and control animals were also analyzed for acetaldehyde.

In the in vivo phase of the study, all animals lost weight after exposure to 15,000 ppm CE 6 hr/day for 5 days, though differences between exposed and control animals were not statistically significant. Absolute and relative weights of the major internal organs were likewise unchanged as a result of exposure to CE, except for the uterine weights of CE-exposed female B6C3F1 mice, which averaged about 65% of controls. The 35% loss in uterine weight is considered toxicologically significant. The appearance of acetaldehyde in the urine of exposed animals reflected species-specific differences. In male mice, the urine acetaldehyde concentration ranged from 15.4 – 70.1 µmol/L urine CE treated versus 7.6–20.3 in air male controls. In female mice, there was less of an effect: 11.6–17.0 µmol/L for CE-treated versus 0–18.1 in air-treated controls. In rats, acetaldehyde concentrations in urine were at or below the limit of detection (2 µmol/L). These results suggested that at 15,000 ppm CE the P450 oxidative is not normally a major CE pathway in the rat but is employed in the mouse responses.

In experiments that explored the capacity of liver microsomes from exposed or control animals to break down CE in vitro with concomitant acetaldehyde formation, the presence of an NADPH-generating system in the incubation mixture was shown to be essential for oxidative activity (Fedtke et al., 1994a). Research showed that there were significant increases in treated versus control rates of NADPH-dependent CE oxidative metabolism in microsomal preparations from female rat liver ($p<0.05$), male mice ($p<0.05$), and female mice ($p<0.01$) compared to their unexposed controls. The oxidative rates of the treated mice were about twice those for the treated rats. These general metabolic responses were complemented by the increased specific rates of P450IIE1 (p-NP activity) in female rats and both sexes of mice ($p<0.01$). This indicates that CE induces its own oxidative metabolism. However, the activities of microsomal P450IA (EROD activity) and P450IIB (PROD activity) either decreased or remained unchanged in response to CE. The role of liver microsomal cytochrome P450IIE1 in the metabolism of CE was

confirmed by the use of the specific P45IIE1 inhibitor, 3-amino-1,2,4-triazole. This inhibitor decreased the in vitro oxidative metabolism of CE by 75% in the rat and 100% in the mouse, and correspondingly decreased the microsomal reaction of p-NP (i.e., P450IIE1 enzyme) by 57% in the rat and 62% in the mouse.

Gargas et al. (1990) has described a physiologically based pharmacokinetic (PBPK) model in male F344 rats for chlorinated methanes and ethanes that included CE. The metabolism of CE is characterized kinetically as proceeding via a combination of a saturable and a first-order process: (1) the first-order component might be due to GSH conjugation (Fedtke et al., 1994b), and (2) the saturable component might be due to the activity of the cytochrome P450IIE1. The saturable component would be expected to convert CE initially to 2-chloroethanol or 1-chloro-1-hydroxy-ethane and then on to 2-chloroaldehyde and acetaldehyde (Figure 1, p. 1). Doubts have been expressed as to whether oxidation is likely to be of specific etiological significance in the onset of CE-induced tumors in the uterus of female B6C3F1 mice (Fedtke et al., 1994b). These authors also speculate that other dehalogenation mechanisms might be involved, on the basis of an earlier report (Van Dyke and Wineman, 1971). The latter observed ^{36}Cl-chloride formation by dechlorination of ^{36}Cl-labeled CE in rat hepatic microsomes in either the presence or absence of NADP, and considered the data to indicate the existence of both enzymatic and nonenzymatic dechlorination mechanisms for CE.

In conclusion, how the CE metabolic pathways are linked to the observed toxicity (fetotoxicity and uterine cancers) is unknown. The suggestion of metabolic saturation and implied nonlinear kinetics suggests further dosimetry work on CE toxicity should be fruitful.

4. HAZARD IDENTIFICATION

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

4.1.1. Oral Exposure

No reports have been identified that describe toxicological effects in humans arising from oral exposure.

4.1.2. Inhalation Exposure

Short-term exposure to CE in human beings has occurred through the compound's use as a general anesthetic. However, in recent times, the widespread use of CE has been superseded by more effective and manageable anesthetics.

A considerable amount of information has accumulated on CE's acute neurological and other pharmacological effects stemming from its former use as a general anesthetic. For example, Lawson (1965) pointed to the compound's ability to induce rapid anesthesia at a vapor concentration of 4% (40,000 ppm). Maintenance of anesthesia with CE alone was considered to be difficult because of the compound's rapid expiration via the lungs (Section 3.1.2, p. 4). Accordingly, the suitability of its sole use seems to be limited to short operations or procedures. Cole (1967) discussed his own extensive use of CE as an anesthetic, in which the CE was used predominantly mixed with nitrous oxide (N_2O) or as an intermediate agent between fast-acting intravenous thiopentone and the slower-acting trichloroethylene. Both authors point to the compound's capacity to induce respiratory stimulation followed by depression, with attendant fluctuations in systolic blood pressure and pulse rate (Lawson, 1965; Cole, 1967).

Dobkin and Byles (1971) drew attention to the capacity of CE to form explosive mixtures in air at concentrations in the effective pharmacological and anesthetic range. Similarly, the blood concentrations achieved during anesthesia appeared to be too close to those associated with respiratory failure (20 to 30 mg % versus 40 mg %). The danger of CE overdose in anesthesia is great. Other potential side effects are the fall in blood pressure, considered to occur through depression of vasomotor centers, and the peripheral vasodilation of blood vessels (Dobkin and Byles, 1971). Subsequent vagal depression causes tachycardia, with bradycardia being a sign of overdose. More moderate effects of CE-induced anesthesia include moderate salivation and, on recovery, nausea and vomiting.

Reports of the toxicological consequences of exposure to subanesthetic concentrations of CE center on case studies of persons deliberately sniffing the compound for hallucinogenic purposes. The amounts of CE involved in such cases are ill-defined. A 28-year-old woman who had sniffed 200 to 300 mL of CE from her coat sleeve for 4 months showed the following neurological symptoms: ataxia, tremors, nystagmus (involuntary movements of the eyeball), scanning dysarthria (speech difficulties), diadochokinesis of the arms (alternate extension and flexion of each of the arms back and forth, or pronation and supination of the arms), sluggish lower limb movements, and hallucinations (Hes et al., 1979). Similar symptoms were described for a 52-year-old man who had a history of abusing solvents, barbiturates, and alcohol over 30 years (Nordin et al., 1988). In the period immediately before hospitalization, he was reported to have inhaled about 100 mL CE on a daily basis over a 4-month period. Despite suffering a dramatic fall in blood pressure and a grand mal seizure 12 hr after admission, the patient was able to recover from all symptoms (short-term memory loss, visual hallucinations, neuropathy of the lower extremities, plus some clinical chemistry fluctuations) during a 6-week period. The authors attributed the neurological symptoms to the abuse of CE and a response to subsequent withdrawal.

4.1.3. Dermal Exposure

CE has been used as a pain-killing spray for such conditions as fibrositis, dysmenorrhoea, causalgia, and renal colic because it can cause a local rapid lowering of temperature, thereby acting as a surface analgesic (Lawson, 1965). CE has been used in sports such as American football to relieve local traumatic pain. In a recent report, Bircher et al. (1994) described a patient with an allergic contact reaction to CE, with sensitization to dichlorodifluoromethane (Freon 12). Immunohistochemical analysis identified responses that were consistent with a T-cell-mediated allergic reaction.

4.2. ACUTE, SUBCHRONIC, AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

A 7- or 14-day oral CE palatability study was conducted in F344 rats; it investigated acute toxicology of aqueous CE (Pottenger et al., 1995). The F344 rats were administered at either 0 or 0.57 g CE/100 g water (570,000 ppm), which is at the practical solubility limit of CE in water at room temperature. Toxicology parameters investigated were body weights, body weight gain,

food and water consumptions, gross pathology, selected organ weights, histopathology, clinical chemistries, and hematology. Rats (5/sex) consuming water at this high dose for 7 days were only modestly affected (within 15% of controls), showing little effect on palatability. At 14 days water consumption (10 rats/sex) was decreased to 81% of controls for males and 76% for females thus showing palatability effects. At 14 days feed consumption and body weight decreases were noted, but were within 10% of control values. All other parameters were normal. Thus, consumption of CE at high water concentrations (0.57g/100 g) for 14 days did not produce significant subchronic toxicological effects. The NOEL for CE dissolved in water may be estimated to be 297 mg/kg bw/day for male rats and 361 mg/kg bw/day for female rats (Pottenger et al., 1995). No oral chronic CE study exists.

4.2.2. Inhalation Exposure

4.2.2.1. *Landry Inhalation Studies*

A report by Landry et al. (1982) described the acute inhalation exposure of six F344 rats/sex/group and two male beagle dogs/group to CE for 6 hr/day, 5 days/week for 2 weeks. Concentrations applied were 0, 1,590, 3,980, or 9,980 ppm and duration-adjusted values are 0, 800, 1,900, and 4,700 mg/m^3. Landry et al. (1982) observed daily clinical signs and measured serial body weights before, during, and after the 2-week exposure period. Initial and terminal blood and serum samples were measured for routine hematological and clinical chemistry parameters in rats. Dogs were measured before and after CE exposure for hematology. All animals received a complete gross pathological examination at necropsy, with a full range of tissues and organs processed for histopathological evaluation.

Other than transient behavioral excitement, there were few compound-related effects in the dogs due to CE at these exposures. Similarly, except for a slight lethargy in the high-dose rats, there were no clinical signs, body weight changes, gross necropsy, or histopathological effects due to treatment. Hematology, urinalysis, and clinical chemistry fluctuations were unremarkable in male rats. There was, however, a statistically significant decrease in BUN in female rats at the two highest exposures, but it is not interpretable to any toxic effect because of the lack of any associated histopathological changes in the kidneys. There were increases in the relative liver weights of male rats at the two highest concentrations. The authors considered the observed changes to be minor, and to probably represent adaptive rather than toxicological changes. The subchronic study identified the highest level of exposure (9,890 ppm) as a free-standing NOAEL, equivalent to a NOAEL(ADJ) for extrarespiratory effects of 4,700 mg/m^3.

In a separate section of the study (Landry et al., 1982), six male F344 rats/group were subjected to a single 6-hr exposure at nominal CE concentrations of 0, 1,600, 4,000, or 10,000 ppm to analyze the effects of CE on liver NPSH concentration. Five B6C3F1 mice/group were exposed to 0 and 4,000 ppm CE only. For mice and rats decreased cellular NPSH (GSH) was observed. Levels of 88% and 89% of control at 4,000 and 10,000 ppm CE were observed for rats, and 64% of controls were observed at 4,000 ppm CE for mice. Statistical significance was observed at 4,000 and 10,000 ppm.

In an unusual protocol, Landry et al. (1989) exposed seven B6C3F1 mice/sex/group to actual concentrations of 0, 250, 1,247, or 4,843 ppm CE for 23 hr/day for 11 days (duration-adjusted exposure values are 0, 630, 3,200, and 12,200 mg/m^3). Animals were observed daily for clinical signs; on day 12 a blinded neurobehavioral observation battery was conducted. Terminal body weights were measured, then blood samples were collected to measure hematological and clinical chemistry parameters. At sacrifice, animals were subjected to a gross pathological examination. Slides of sections of brain, heart, liver, kidney, thymus, and testes from the control and high-dose groups were examined for histopathological lesions.

In general, for doses 250–4,843 ppm there were no clinical signs of exposure, neurobehavioral manifestations, body weight changes, clinical chemistry, or hematological responses at any of the CE concentrations tested. Apparent compound-related effects were limited to increases in the relative liver weights in both sexes exposed at the highest CE concentration (4,843 ppm). This change has been associated with an increase in the size of the liver noted in some animals in this group, and with an increase in the incidence of hepatocellular vacuolization evident in 4/7 mice/sex exposed to this concentration. The authors did not consider any of the observed histopathological or relative weight changes in the liver to be correlated to CE. Accordingly, the Landry study defined a free-standing NOAEL of 4,843 ppm. It is perhaps notable that exposures of 250 ppm and 1,247 ppm do not show *any* effects, neurological or clinical.

4.2.2.2. *Principal Study Performed by the U.S. National Toxicology Program*

The most comprehensive study on the inhalation toxicology of CE in mice and rats is that sponsored by the U.S. National Toxicology Program (NTP, 1989a). Groups of F344 rats and B6C3F1 mice of both sexes were exposed to CE vapor (whole body) for periods of 2 weeks (acute), 13 weeks (subchronic), or 2 years (chronic). Other acute studies are described that usually explored the anesthetic properties of CE.

4.2.2.2.1. ***NTP acute study.*** A single exposure experiment (19,000 ppm for 4 hr) was part of the range-finding exercise that resulted in concentrations of 0 and 15,000 ppm being chosen for the chronic portion of the study. In this acute study, all rats and mice (5/sex/group) survived the single exposure for 4 hr at 19,000 ppm CE with no concurrent or subsequent clinical signs. Similarly, those animals (5/sex/group) exposed for 10 days at 19,000 ppm CE and held 2 weeks survived for the duration of the study. Among the rats, there were no compound-related effects of weight gain, whereas for the mice, body weights of exposed animals were greater than those of controls. Overall, no mice or rats in this portion of the study displayed clinical signs, and gross necropsy and histopathological findings indicated an absence of CE-related effects. Hence, in the NTP study, CE produced no apparent acute effects at a high dose of 19,000 ppm.

4.2.2.2.2. ***Subchronic study.*** The subchronic portion of the NTP study featured the administration of 0, 2,500, 5,000, 10,000, or 19,000 ppm CE to 10 F344 rats and B6C3F1 mice/sex/group, 6 hr/day, 5 days/week for 13 weeks (NTP, 1989a). Duration-adjusted exposures in units of mg/m^3 were 0, 1,180, 2,360, 4,710, and 8,950 mg/m^3, respectively. No exposure-related clinical signs or gross or histopathological lesions were evident in either rats or mice in this study.

Possible compound-related consequences of exposure were limited to comparatively minor fluctuations in body and liver weights. Thus, for both males and females in 13 weeks, slight decreases in body weights were noted at HDT, i.e., 19,000 ppm CE. Statistically significant increases in relative liver organ weights were observed in male rats (+14%) and female mice (+18%) exposed at the HDT (8,950 mg/m^3), however, male mice exposed to 4,710 mg/m^3 CE displayed a significant decrease in liver weight. Based on the increases in relative liver weight in male rats and female mice, this study identified a NOAEL(HEC) of 4,710 mg/m^3 and a LOAEL(HEC) of 8,950 mg/m^3 (HDT). It is notable that 2,500 ppm produces no significant effect. Benchmark concentration modeling was not conducted on liver weight because it was not excessive and monotonic increases with concentration were not observed.

4.2.2.2.3. ***Chronic study.*** Male and female F344 rats and B6C3F1 mice (50/sex/group) were exposed to 0 or 15,000 ppm CE (39,570 mg/m^3) for 6 hr/day, 5 days/week for 103 weeks (rats) or 100 weeks (mice). The time-adjusted dosage is 39,570 $mg/m^3 \times 6/24 \times 5/7 = 7{,}070\ mg/m^3$. The particular concentration of 15,000 ppm was chosen and was based on an apparent lack of toxicity in the subchronic portion of the study, and on concerns for potential flammability and explosion at higher concentrations. Clinical signs were observed daily, while body weights were recorded weekly for the first 12 weeks, then monthly. A complete histological examination was carried out on all animals dying prematurely and on those animals surviving to term.

4.2.2.2.3.1. *F344 Rat Toxicological Results in the NTP Study*

Male F344 rat survivals in control (16/50) and CE-exposed (8/50) groups were low after 103 weeks, with no statistically significant difference between control and treated group. The NTP authors suggested that an unusually high incidence of mononuclear cell leukemia in both groups likely contributed to poor male rat survivals (NTP, 1989a). In contrast, female rats showed good survival in control (31/50) and CE-treated groups (22/50) at study termination; and there was no statistical difference between the groups. A slight decrease in mean body weight gain (4–8%) in the male rats compared to controls was observed after wk. 33 of chronic exposure, and the mean body weights of female rats were 5–13% lower than controls from wk. 11 to the end of the study (NTP, 1989a). At termination, the mean body weight of exposed female rats was reduced by10% compared with concurrent controls. No remarkable clinical signs were observed in the exposed animals, and no CE-induced nonneoplastic lesions were observed even at this high dose. This level of weight loss is not considered to to be a critical toxicological effect.

A number of uncommon skin tumor types were observed in exposed male F344 rats (Table 7). The total tumor response in male F344 rat skin seems to show that skin and certain skin appendages are displaying a cancer response. Because skin under the fur is exposed to CE in the inhalation chamber during the 102 weeks, there is some dermal exposure.

When compared with the concurrent control incidence, that is, 5/49 (10%) versus 8/46 (17%), the male rat malignant whole skin response is not statistically increased (p=0.23). The first skin tumor, a subcutaneous fibroma, occurred at 79 weeks in the treated group. Moreover, the rates are not significantly increased at 15,000 ppm CE when adjusted for animals dying before the first skin tumor. The comparison, in this case, is 5/42 (12%) versus 8/42 (19%), p=0.27. When the male rat skin tumors of the treated group are compared with those of the historical inhalation controls from the same testing laboratory, there is a statistically significant increase in epithelial cancers: 2/300 (0.7%) versus 8/46 (17.4%), $p=2 \times 10^{-6}$. Similarly, when NTP controls from noninhalation historical experiments are compared with the treated group (28/1,936 [1.4%] versus 8/46 [17.4%], $p=8 \times 10^{-5}$), there is also a statistically significant increase in epithelial skin tumors.

Historical incidence rates can be characterized. For example, tumor incidences may be subjectively ranked: (1) incidence rates <0.5% are rare, (2) incidences occurring >0.5% but <2% may be considered uncommon, and (3) incidences >2% are generally common to aging test rodents. These definitions are operational, not absolute, and they represent expert judgment. In this bioassay, the historical malignant skin tumor incidence is 0.7%, and NTP incidence is 1.4% where both are designated as uncommon tumor incidences. On the other hand, the observed control skin incidence is 10% (5/49) (Table 7). Comparing either the observed or historical control incidences to the treated group incidences leads to different conclusions: there is a

statistically significant increase when historical skin controls are considered, but not when the study concurrent control is considered as the reference control.

In the female rats, brain astrocytomas occurred at a low incidence of 3/50 (6%) (Table 7). In analyzing the significance of this low-incidence brain tumor, it is known that astrocytomas are not common in most strains of rat or in humans. So, even low incidences could be a sign of carcinogenicity. There is extra concern when astrocytomas do occur because such a tumor type in the brain has fatal implications in rodents and humans. When compared statistically with the concurrent control (0/50 [0%] versus 3/50 [6%]), the response yields statistical insignificance (p=0.12), which suggests that there may be no effect. The same may be stated when the adjusted rates are examined by subtracting the number of animals dying before the first astrocytoma appears (52 weeks): 0/46 versus 3/49, p = 0.12.

When rare tumors occur, the tumor rates require special consideration. Uncommon or rare tumor incidences may not indicate a statistical increase when compared with their respective concurrent control incidences. This is because the number of trials (i.e., the number at risk in the control and treated groups) is small, ≈50/sex/group, and a larger number of animals (in this case, at the 95% level of confidence, ≈150/sex/group) is needed to statistically score a rare tumorigenic event. Accordingly, when the observed incidence (3/50) is compared with historical pooled control incidence (1/297) from the same testing laboratory (Battelle Pacific Northwest Laboratories), the statistically significant increase in astrocytomas is p=0.01 (Table 7). Note that the larger denominator affects the statistical inference in the case of uncommon or rare tumors. Similarly, when the observed 3/50 astrocytomas in female F344/N rats are compared with the incidence of all experimentally discovered astrocytomas in NTP studies (23/1,969), the statistical significance is p=0.02 (Table 7).

The 3/50 (6%) astrocytoma response in female F344/N rats is statistically significant when compared with historical controls, but not when compared with the concurrent controls. The observed and historical control incidences present different conclusions; that is, a statistically significant increase in astrocytomas is seen when historical controls are considered, but not when the study concurrent control is considered.

Further analysis shows, however, that Battelle Pacific Northwest Laboratories had a singular prior incidence of 3/50 (6%) astrocytomas in a female concurrent control group of F344/N rats. This singular control brain tumor incidence happens to be commensurate with the brain response in the 15,000 ppm CE group (Table 7). Thus, if a past concurrent control incidence can reach as high as 3/50 (6%), the apparent statistical significance of the dosed group

Table 7. Tumors of F344/N rats at 2 years

Sex	Controls	15,000 ppm chloroethane	Estimate of *p* value[a]
Males	Keratoacanthoma = 4/49 (8%) Fibroma = 1/49 (2%) *Total = 5/49 (10%)*	Basal cell carcinomas = 3/46 (7%) Keratoacanthoma = 2/46 (4%) Squamous cell carcinoma = 1/46 (2%) Trichoepithelioma = 1/46 (2%) Lip, squamous cell carcinoma = 1/46 (2%) *Total = 8/46 (17%)*	0.23
	Adjusted to first appearance of tumor (79 weeks) (42 males) Tumor incidence = 5/42 (12%)	Adjusted to first appearance of tumor (79 weeks in treated group) (42 males) Tumor incidence = 8/42 (19%)	0.27
	Skin Historical controls = 2/300 (inhalation) (0.7%)	See above, 8/46	2.0×10^{-6} [b]
	Skin Historical controls = 30/1,936 (noninhalation) (2%)	See above, 8/46	1.3×10^{-6} [b]
Females	Astrocytomas = none in controls	astrocytomas = 3/50 (6%)	0.12
	Adjusted to animals on test at 0 weeks (46 females) Tumor incidence = 0/50 (0%)	Adjusted to first appearance of tumor at 52 weeks (49 females) tumor incidence = 3/49 (6.1%)	0.12
	Historical astrocytoma controls = 1/297 (inhalation studies) (0.3%)	See above, 3/50	0.01 [b]
	Historical astrocytoma controls = 23/1,969 (all studies) (1.1%)	See above, 3/50	0.02 [b]

[a] The *p* value is the likelihood (probability) that the assumption of a positive cancer effect is in error. Usually $p \leq 0.05$ is taken as a reasonably significant level of certainty to continue to assume there is a positive cancer effect.

[b] Designates statistical significance in a Fischer's exact test comparison. Data taken from NTP report no. 346 (NTP, 1989a).

response—also an incidence of 3/50 (6%)—becomes less important. Moreover, in past NTP studies, the average astrocytoma incidence is 0.9% (18/1,969) and the range is 0% to 6% in female F344/N rats. Here, too, it is observed that an incidence level of astrocytoma cancers as high as 6% may be observed in concurrent controls.

It is determined, then, that this female rat astrocytoma effect may be real, but if so is marginal. Sensitivity analysis indicates that only one more rat with an astrocytoma would have shifted the concern to a significant response. Therefore, the female rat brain response is designated as equivocal evidence for CE carcinogenicity.

4.2.2.2.3.2. ***B6C3F1 Mouse Toxicological Results in the NTP Study.***

In male B6C3F1 mice, survivals were significantly reduced compared to controls after wk.42. The same was true for female mice after week 81. Thus, because of the low survival rates the NTP mouse study was terminated earlier than protocol called for. The mice were terminated at the 100th week, at which time survivals were 28/50 control versus 11/50 CE in exposed males and 32/50 control versus 2/50 CE in exposed females. The high mortality in the male mice was attributed to a greater than normal incidence of nonneoplastic urogenital lesions observed in both the control and exposed males, although the exposed mice were more severely affected. In female mice, the majority died as a result of CE-induced carcinomas of the uterus, endometrium, myometrium, and complications of metastasis, as further discussed in Section 4.2.2.2.3.4, p. 23.

Female mice exhibited a characteristic hyperactivity during the daily periods of exposure, a transient response to treatment because activity returned to normal at the end of each exposure period (NTP, 1989a). There was no effect on body weight in either sex, and no other exposure-related clinical signs or nonneoplastic lesions were observed. Based on this absence of noncarcinogenic toxic effects, the single concentration tested (7,070 mg/m^3) was a NOAEL for female mice. Benchmark concentration modeling could not be conducted because only one exposure level was tested.

Because of poor survivals in mice, the murine portion of the study was terminated at week 100. Many of the male mice died prematurely from urogenital infections, thereby reducing the power of the male group results to detect late-developing neoplasms (NTP, 1989a). Survival until termination was 28/50 in male controls and 11/50 in 15,000 ppm males. Notwithstanding male mouse results, there were no significant cancer increases—except possibly an increase in lung adenomas and/or carcinomas. Lung cancer incidence was 5/50 in controls versus 10/48 at 15,000 ppm (p = 0.11). The poor survivals in the male B6C3F1 mice force the conclusion that the male mouse is inadequate to determine carcinogenicity.

Survivals in female mice were 32/50 in controls and 2/50 in 15,000 ppm. The study diagnosis was that female mice died early because of aggressive carcinogenicity (NTP, 1989a). Life-shortening is a primary element in assessing carcinogenicity. Treated female mice had a high incidence of primary tumors in the uterine endometrium (Table 8). High incidence is another primary element in assessing carcinogenicity. These lesions occurred in almost all females tested: 43/50 of CE-exposed female B6C3F1 mice

Table 8. Incidence of tumors in female B6C3F1 mice after exposure to CE for 2 years

Effect	Incidence of hyperplasia/tumors		*p* [a]
	Controls	CE-exposed	
Uterine hyperplasia and cytstic hyperplasia	41/49	6/50	3×10^{-5} [b]
Uterine carcinoma	1/49	43/50	$<10^{-8}$
Uterine carcinoma[c]	1/46	43/48	$<10^{-8}$
Uterine lymphomas	1/49	7/50	0.03
Systemic lymphomas	5/49	10/50	0.14
Hepatic combined adenomas and carcinomas	3/49	8/48	0.025

[a]As determined using Fischer's exact test.
[b]Negative correlation biologically. Uterine lining in aging females normally show hyperplasia, but CE exposure demonstrates an obliteration of the normal hyperplasia due to the dispersed metastatic carcinomas.
[c]Corrected for time to first tumor at 67 weeks which as a uterine tumor.

Source: NTP (1989a).

versus 0/49 in controls. These endometrial tumors showed a remarkable capacity for metastasizing. Aggressive metastasis is another primary element of carcinogenesis evolving into the malignant state of cancer. Secondary cancer sites (16 total) included, out of 50 starting female mice, lung (23), ovary (22), lymph nodes (18), kidney (8), adrenal gland (8), pancreas (7), urinary bladder (7), mesentery (7), spleen (5), heart (4), colon (2), and stomach, gall bladder, small intestine, ureter, and liver (1 each).

Other carcinogenic effects of treatment included increased incidences of combined adenomas and carcinomas in the livers of female mice (8/48 versus 3/49 in controls; p=0.025). There were also increases in hematopoietic cancer involvement with CE treatment, including

increases in a number of white cell types in bone marrow, lymph nodes, spleen, and thymus. Though these effects were difficult to differentiate from the metastatic impacts of the primary carcinogenic effect, they lend support to the powerful carcinogenic effects of CE in female B6C3F1 mice. It has been concluded that there is clear evidence of the carcinogenicity of CE in female B6C3F1 mice (NTP, 1989a; Holder, 1998).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Exposure

No reports of studies were identified that addressed the reproductive/developmental toxicity of CE when administered via the oral route.

4.3.2. Inhalation Exposure

4.3.2.1. *Principal Study*

Scortichini et al. (1986) reported the findings of an inhalation reproductive toxicity/teratological study on 120 pregnant female CF-1 mice exposed to nominal exposure concentrations of 0, 500, 1,500, or 5,000 ppm CE for 6 hr/day on gestation days (GD) 6–15. This is 10 days of CE exposure for the dams. All animals were sacrificed on GD 18. Mean concentrations were found to be 0, 491 $\pm$ 37 ppm, 1,504 $\pm$ 84 ppm, or 4,946 $\pm$ 159 ppm which convert to 0, 1,300, 4,000, and 13,000 mg/m^3, respectively. These values were not duration adjusted, in accordance with current EPA practice. As indicators of the compound's potential maternal toxicity, the dams were observed for clinical signs: body weights and food and water consumption were measured every 3 days, and at necropsy, dam liver weights and gravid uterine weights were recorded. As indicators of possible developmental toxicities and teratological effects of CE, fetal observations included the number and position of fetuses in utero, the number of live and dead fetuses and the number of resorption sites, the weight and sex of each fetus, and the incidence of any gross external alterations or cardiac abnormalities. Half of each fetal litter were necropsied to look for visceral abnormalities and skeletal alterations. Serial sections of the head were made in a subset of fetuses.

Observations showed no maternal toxicity from CE inhalation exposure as measured by clinical signs, food and water consumption, body weight, and liver weight. Nor were there any CE-related changes in reproductive performance: pregnancy rate, resorption rate, litter size, fetal

sex ratio, or fetal body weights. By contrast, in examining the possible teratological effects of CE, a number of effects appeared sporadically. Cervical ribs appeared in the exposed fetuses 1/257, 1/299, 6/311, and 4/242 (p trend = 0.13). On a per litter basis (2/22, 1/25, 5/26, 4/22), the response of cervical ribs was not statistically significant (p = 0.31). Exposures to higher levels of CE might have produced significant rib malformation. There was, however, an increase in the incidence of delayed fetal foramina closure (DFFC) of the CF-1 mouse skull bones (Scortichini et al., 1986). This developmental delay, viewed at at GD 18, is a retardation of a small frontal area of ossification of the skull. This is not to imply that the foramina will not ultimately close in exposed CF-1 mice. Thus, this is a fetotoxic effect—not a teras—and possibly represents a CE-induced skeletal variation. In Table 9, the data show that at 4,946 ppm that 5 fetuses (4%) were affected from a total of 5 litters (23%), compared with 1 fetus in 1 litter for each of the lower exposure groups, including the control group. The average historical control incidence of this DFFC variance is 0.2% with a range of 0–1.2% in CF-1 mice (Figure 5). At the HDT, the incidence of fetotoxicity of 5/116 fetuses (4.39%) falls outside this range of historical controls. Comparison of the HDT incidence to the upper historical control incidence (3/245) yields p = 0.074; the marginality of this HDT effect is also indicated by the pairwise comparison to control incidence (1/126) by the Fischer's exact test, p = 0.077 (Figure 5). However, supporting the concept of DFFC as a CE-related effect was the statistically significant trend in skull foramina (p<0.05), using various nonparametric trend tests. The apparent effect is weak in intensity. This study should hve been followed by a similar study in the 2,000–12,000 ppm CE range to see if there was a DFFC dose response.

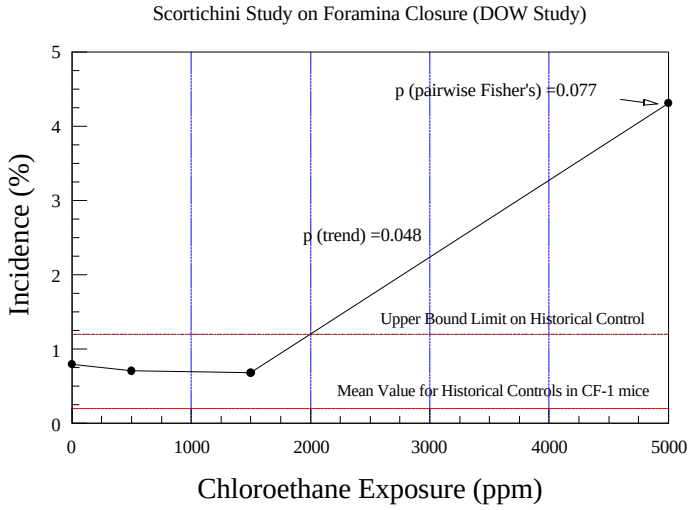

Figure 5. Occurrence of delayed foramina closure in skulls of CF-1 mice. Dams were treated with 0, 491, 1,504, or 4,946 ppm CE by inhalation for 6 hrs/day on days 6–15 of gestation. Similar statisitcal results were obtained when incidence on a litter basis was considered rather than on an individual basis as shown in this figure.

Although there is insufficient evidence to unequivocally resolve the dose-response issue, this is the lowest-dose critical effect suggested by CE exposure of all noncancer studies reviewed in this document. Therefore, the middle dose or subthreshold exposure concentration of 3,970

Table 9. Chloroethane inhalation teratology in CF-1 mice: incidence of fetal alterations among litters of mice

Chloroethane concentration (ppm)					
		0	**500**	**1,500**	**5,000**
Number of fetuses (number of litters) examined					
External examination		258 (22)	299 (25)	311 (26)	242 (22)
Soft-tissue examination		132 (22)	156 (25)	164 (26)	126 (22)
Skeletal examination		257 (22)	299 (25)	311 (26)	242 (22)
Bones of the skull		126 (22)	142 (24)	147 (25)	116 (22)
Percent affected (numbers affected)					
External observations					
Cleft palate	*Fetuses*	0.4 (1)	1 (3)	0.3 (1)	0.4 (1)
Litters		5 (1)	12 (3)	4 (1)	5 (1)
Exencephaly	*F*	0	0	1 (2)	0
	L	0	0	8 (2)	0
Micrognathia	*F*	0	0	0	0.4 (1)
	L	0	0	0	5 (1)
Chloroethane concentration (ppm)					
		0	**500**	**1,500**	**5,000**
Microphthalmia	*F*	0.4 (1)	0	0	0
	L	5 (1)	0	0	0
Soft tissue observations					
Dilated renal pelvis and ureter	*F*	0	1 (1)	0	0
	L	0	4 (1)	0	0
Pale spleen	*F*	0	0	1 (1)	0
	L	0	0	4 (1)	0
Pale foci on liver	*F*	1 (1)	0	0	0
	L	5 (1)	0	0	0

Table 9. Chloroethane inhalation teratology in CF-1 mice: incidence of fetal alterations among litters of mice (continued)

Chloroethane concentration (ppm)					
		0	**500**	**1,500**	**5,000**
Dilated ventricles of brain	*F*	0	1 (1)	0	0
	L	0	5 (1)	0	0
Intraventricular hemorrhage	*F*	1 (1)	0	0	0
	L	5 (1)	0	0	0
Skeletal observations					
Skull -delayed ossification	*F*	2 (3)	6 (9)	4 (6)	2 (2)
	L	9 (2)	29 (7)	20 (5)	9 (2)
Foramina	*F*	1 (1)	1 (1)	1 (1)	4 (5)[a]
	L	5 (1)	4 (1)	4 (1)	23 (5)
Irregular pattern of ossification	*F*	0	0	0	1 (1)
	L	0	0	0	5 (1)
Vertebrae					
Delayed ossification	*F*	1 (3)	0.3 (1)	1 (2)	0
	L	14 (3)	4 (1)	8 (2)	0
Centra delayed ossification	*F*	0	0.3 (1)	0.3 (1)	0
	L	0	4 (1)	4 (1)	0
Atlas forked	*F*	1 (2)	1 (2)	1 (2)	1 (2)
	L	9 (2)	8 (2)	8 (2)	5 (1)
Fused	*F*	0	0	0.3 (1)	0

Table 9. Chloroethane inhalation teratology in CF-1 mice: incidence of fetal alterations among litters of mice (continued)

Chloroethane concentration (ppm)					
		0	**500**	**1,500**	**5,000**
	L	0	0	4 (1)	0
Ribs					
Delayed ossification	*F*	0	0.3 (1)	0	0
	L	0	4 (1)	0	0
Forked	*F*	0	0.3 (1)	0	0
	L	0	4 (1)	0	0
Fused	*F*	0	0.3 (1)	0	0
	L	0	4 (1)	0	0
Cervical	*F*	1 (2)	0.3 (1)	2 (6)	2 (4)
	L	9 (2)	4 (1)	19 (5)	18 (4)
Sternebrae					
Delayed ossification	*F*	4 (11)	6 (18)	4 (12)	2 (5)
	L	27 (6)	48 (12)	23 (6)	14 (3)
Fused	*F*	5 (12)	6 (18)	7 (23)	5 (13)
	L	46 (10)	28 (7)	54 (14)	36 (8)
Staggered	*F*	0	0.3 (1)	0.3 (1)	0.4 (1)
	L	0	4 (1)	4 (1)	5 (1)
Irregular pattern of ossification	*F*	1 (2)	0	0	0
	L	9 (2)	0	0	0
Misshapen	*F*	0.4 (1)	0	0	0
	L	5 (1)	0	0	0

[a]$p<0.05$ using a censored Wilcoxon test.

Source: Scortichini et al. (1986).

mg/m^3 (1,504 ppm) is characterized as a NOAEL for the fetotoxic foramina effect, with the HDT = 13,000 mg/m^3 (4,946 ppm), which is the LOAEL.

4.3.2.2. *Supporting Reproductive or Teratological Studies*

In an earlier reproductive/teratological study 8–10 pregnant female CF-1 mice were exposed to 0, 5,000, 10,000, or 15,000 ppm CE for 6 hr/day on GDs 6–15 (Dow Chemical Co., 1985). Among the responses investigated were the number of litters, number of implantation sites/dam, number of live fetuses/litter, resorptions/litter, percentage implantations resorbed, and the ratio of resorptions to litters with resorptions. Most exposed mice displayed stereotypical behavior characterized by repetitive running, and significant decreases in body weight on GD 16 and decreased body weight gains on GDs 10–16 were observed at all CE doses. However, there were no compound-related reproductive, developmental, or teratological effects in any treatment group.

Breslin et al. (1988) reported an estrous cycling study in B6C3F1 mice. A vaginal lavage technique measured estrous cyclicity, following CE exposure of two groups of 10 female mice to 0 or 15,000 ppm for a minimum of 14 consecutive days (three estrous cycles). Before exposure, the groups had been acclimated to the inhalation chambers until their estrous cycles stabilized to a regular estrus periodicity. All animals were also monitored for clinical signs, body weight changes, and reproductive pathology and histopathology at termination. No effects on behavior, gross pathology, or histopathology were observed in the 15,000 ppm group, but mean body weight gain was significantly increased ($p<0.05$). The mean length of the estrous cycle in exposed mice was 5.6 days, significantly longer than the pre-exposure duration for the same group (5.0 days) and for the corresponding control group (4.5 days). The authors noted that, in some animals, the estrous phase was lengthened, while in others it was the diestrous phase that was affected. Consequently, they attributed the observed effects to a generalized stress reaction rather than to any specific reproductive CE effect, but a direct exposure-related effect of CE on neuroendocrine function cannot be ruled out. Thus, assuming that CE does have the ability to disrupt the estrous cycle of mice, these data would point to a duration-adjusted free-standing LOAEL of $\approx$7,071 mg/m^3 = LOAEL(HEC).

Bucher et al. (1995) sought to explain why CE induces a lengthened estrous cycle in B6C3F1 mice. Because CE (Section 4.2.2.1.3.4) and bromoethane (BE) (Section 4.7.1) both cause murine uterine tumors, an uncommon B6C3F1 tumor, it was decided to look for a hormonal basis for the chemical carcinogenesis. Serum levels of estradiol and progesterone were measured in haloethane-exposed and control female mice. Female mice (30/group) were exposed to 15,000 ppm CE, 400 ppm BE, or filtered air as controls for 6 hr/day over a duration of 21 days

to monitor hormone levels. Vaginal smears were determined and daily cell cytology was done. Body weights of animals in the study were recorded once a week, and, at termination, blood samples were obtained via cardiac puncture for hormone analysis. At necropsy, the liver, uterus, pituitary, adrenal glands, and ovaries were removed, the liver and uterus were weighed, and the organs were fixed for histopathological examination.

In line with the data reported by Breslin et al. (1988), Bucher et al. (1995) observed a slight but statistically significant increase (+ 0.4 days) in the mean duration of the estrous cycle in mice exposed to CE. However, there were no consistent concomitant hormone treatment-related changes in serum estradiol or progesterone. Likewise, there were no CE-induced clinical signs, body weight gains, or changes in uterine weight. The latter observation is in contrast to that of Fedtke et al. (1994a), who reported an overall 35% reduction in uterine weight as a result of a similar level of CE exposure in this animal model. Taken with the absence of any consistent compound-related effects on the duration of individual estrous stages, the lack of any changes in the serum concentrations of estradiol or progesterone due to CE or BE exposure suggests that the minimal alteration of the estrous cycle described by Breslin et al. (1988) and Bucher et al. (1995) is unlikely to represent a major mechanism by which the haloethanes perturb uterine metabolism to cause cancer.

4.4. OTHER TOXICITY STUDIES

4.4.1. Acute Toxicity Studies

4.4.1.1. *Neurotoxicity*

In many older acute or subchronic inhalation experiments, narcotic or anesthetic doses of CE gas were administered and the doses were often uncertain. In such experiments, use was often made of a saturated substrate (e.g., cotton) that generates a high, but unknown, flow to the nose. In one such CE inhalation study, the rat cerebral cortex demonstrated decreased respiration, but the thalamus and white matter did not appear affected upon gross examination (Seller, 1938). Rats, mice, and rabbits were each anesthetized with CE; acetylcholine was then extracted from the respective frozen brain tissues. Each showed increased acetylcholine levels as a result of CE anesthesia (Sayers et al., 1929). Mice were administered 30,000 or 60,000 ppm CE for up to 1 hr via inhalation (Neal et al., 1964). After 25 minutes, 17% of the mice in the 60,000 ppm group had become anesthetized, but no anesthesia occurred in the 30,000 ppm group.

It was generally found that > 35,000 ppm CE causes primary CNS and circulatory effects (Lazarew, 1929; Henderson, 1930).

CE anesthesia for 60 min caused a decreased sedimentation rate of RBCs from rabbits, followed by a period of accelerated sedimentation rate, reaching its maximum 3 hr after anesthesia was initiated and then normalizing in 12–14 hr (Hinko, 1934). Rats were anesthetized with 54,000 ppm CE for 5 min, and subsequently O_2 consumption and CO_2 excretion were decreased significantly and the body temperature fell by 2.5 °C (Hattori, 1957). Rats were anesthetized for 2 hr with CE, after which occurred a disappearance of liver glycogen, a decrease in acid phosphatase levels, and increases in alkaline phosphatase and succinic dehydrogenase levels (Heller et al., 1966). A two-hr inhalation LC_{50} value of 60,632 ppm for rats and mice has been reported (Troshina, 1966).

Guinea pigs were exposed to CE via inhalation at concentrations ranging from 10,000 to 240,000 ppm for times ranging from 5 to 810 min (Sayers et al., 1929). An unsteady gait appeared after 25 min at 20,000 ppm. Some deaths occurred at exposures of 15,300 ppm and higher. At exposures ≥20,000 ppm, pulmonary congestion, hemorrhage, and edema were observed in gross pathology. At 87,000 ppm for 130 minutes, violent shaking occurred in one pig, and after 270 minutes, rales were heard in several guinea pigs. At 127,000, 142,000, and 153,000 ppm for 1 minute, there was complete loss of equilibrium, a running movement, and scratching. Abdominal walls seemed distended and convulsion of the intestines was observed. After 15 to 20 min, struggling became less violent; respiration became shallow, rapid, and convulsive; and death occurred in 30 to 40 min. At 232,000 and 240,000 ppm, there was loss of equilibrium in 30–60 seconds, and in 5 min. animals lost consciousness.

The effects of CE on feline brain blood flow were studied in the cortex and the medulla oblongata (Tokita, 1953). CE gas increased feline brain blood flow. The avoidance flexion reflex was tested, during administration of CE, on a super-maximal single electrical stimulation to the hind limb of intact rats (Hiraiwa, 1952). Changes in the flexion reflex curves were observed.

Dogs and rats were examined for neurological behavior at 0, 1,600, 4,000, and 10,000 ppm CE inhaled 6 hr/day, 4 days/wk, for 2 wk (Landry et al., 1982). Dog examinations were performed 2 days prior to exposure and at exposure end. Dogs were examined for gait, posture, mental status, cranial nerve reflexes, postural reactions, spinal cord reflexes, muscle tone, and pain perception. An ophthalmoscopic exam was also performed. No reactions were seen in dogs except for some hyperactivity. Only hyperactivity in exposed rats was observed by Landry et al. (1982). Although these were acute observations, longer exposure to CE than 2 weeks may have produced different results.

The well-described capacity of CE to induce anesthesia in human beings (Lawson, 1965; Cole, 1967) and case reports of the abuse of the compound for hallucinogenic purposes at

subanesthetic concentrations (Hes et al., 1979; Nordin et al., 1988) may be consistent with evidence of the neurotoxicity of CE that has accumulated from animal studies. For example, when sublethal CE concentrations (0, 5,000, 10,000, or 15,000 ppm CE for 6 hr/day on GDs 6-15) were explored in neurotoxicity experiments, increased physical activity in female mice was observed at all doses (Dow Chemical Co., 1985). Most exposed mice displayed stereotypical behavior characterized by repetitive running. Similarly, hyperactivity was reported in female B6C3F1 mice exposed to the high dose of 15,000 ppm CE for 6 hr/day, 5 days/week for 2 years (NTP, 1989a). Hyperactivity was also observed by Pottenger et al. (1992); the observed depletion of GSH pools (using buthionine sulfoximine) blocked hyperactivity, thus showing it was GSH mediated (Pottenger et al., 1992). The hyperactivity was *not* apparent in mice exposed to the similar concentrations in the subchronic portion (13 weeks) of the NTP study, raising doubt as to whether the response was compound-related or a more generalized or uncontrolled response to stress.

4.4.1.2. *Immunotoxicity*

There have been a number of studies in animals that do not produce immunotoxicological results. For example, 438 ppm for 10 days of inhalation was negative (Schmidt et al., 1972). Likewise, 10,000 ppm for 2 weeks' murine and canine exposures was immunotoxicologically negative (Landry et al., 1982). Moreover, the 1988 NTP studies of 19,000 ppm for 2 weeks or 13 weeks, and 15,000 ppm for 104 weeks were all negative immunotoxicologically. There is one study where 5,305 ppm CE for 8.5 weeks, as well as a lower exposure of 216 ppm for 24 weeks, caused reduced leukocytic phagocytes in rats (Troshina, 1966). This study did not report sufficient experimental details and has not been validated.

4.4.1.3. *Cardiac Sensitization*

CE-anesthetized dogs showed increased cardiac sensitivity to epinephrine, as demonstrated by ventricular tachycardia (Morris et al., 1953). In CE-anesthetized dogs, cardiac irregularities observed are asystole, ventricular standstill, and ventricular tachycardia (Haid et al., 1954). Dogs were either anesthetized with CE only or in combination with atropine, an anticholinergic drug (Bush et al., 1952). They observed electrocardiographic changes that suggested two mechanistic CE effects on the heart: (1) a direct depression of cardiac tissues and (2) a cardiac inhibition resulting from vagus nerve stimulation. Beagle dogs were exposed while conscious to high concentrations of CE 5 minutes after an intravenous injection of 0.008 mg/kg epinephrine. The treatment resulted in an exacerbated incidence of epinephrine-induced

arrhythmias, marked by ventricular fibrillation and tachycardia. Exposures to 40,000–50,000 ppm CE were not well tolerated, as the dogs entered the excitatory stages of anesthesia. These investigators concluded that the dogs were susceptible to cardiac sensitization that was induced by CE. CE-induced cardiac sensitivity has not been thoroughly tested in the dog with a chemically-related series of chlorinated solvents. CE has been generally classified as a weak cardiac sensitizer in the dog, at least at high doses (Reinhardt et al., 1971).

4.4.1.4. *Dermal Effects*

Eye irritation has been reported by exposing human volunteers to short exposures of 40,000 ppm CE but not to 20,000 ppm (Sayers et al., 1929). The eye may be the only surface tissue that responds adversely to CE. Histopathological effects of the dermis (canine and murine, 10,000 ppm or 19,000 ppm for 2 weeks) were negative (Landry et al., 1982; NTP, 1989a). It has been noted: 1) after 48 hr inhalation exposure to 15,000 ppm CE (saturating metabolic conditions), the B6C3F1 mice show more metabolism than F344 rats, and 2) the primary target rat tissues appeared to be ovary, adrenals, and skin (Dow, 1992). If there are acute dermal toxicological effects in the rat, these may build up during chronic exposure. NTP F344 rats treated with CE by inhalation for 2 years did have more skin tumors: basal cell carcinomas = 3/46 (7%), keratoacanthoma = 2/46 (4%), squamous cell carcinoma = 1/46 (2%), trichoepithelioma = 1/46 (2%), lip and squamous cell carcinoma = 1/46 (2%) (NTP, 1989a). This total is 8 skin cancers in 46 rats (17%) in the CE inhalation group versus 5 in 42 (12%) in concurrent control versus 2/300 in historical controls.

4.4.1.5. *Kidney Effects*

Kidney responses in the rat show no effects at low doses (438 ppm for 10 days) (Gohlke and Schmidt, 1972). Other than a decreased BUN there were no renal effects at 4,000 ppm and 10,000 ppm by inhalation for 11 days (Landry et al., 1982). NTP showed no adverse kidney effects at 19,000 ppm for 13 weeks (1989a). Moreover, exposure at 9,625 ppm for 6½ months showed no kidney histological effects (unrefereed study by Adams et al., 1939).

In guinea pigs at high levels (40,000 ppm for 9 hr), CE shows kidney congestion and degeneration (Sayers et al., 1929). Exposures at 15,000 ppm for 2 years seem to promote mouse tubular regeneration and glomerulosclerosis, albeit mild, while rats were without renal effects (NTP, 1989a).

4.4.2. Genotoxicity

As described in NTP (1989a), a methodological variation is necessary to quantitatively examine the effects of volatile chemicals such as CE in the Ames test. NTP solved this problem by introducing CE into sealed desiccators through the vacuum valves, thereby gassing the plates of *S. typhimurium* bacteria tester strains. Another innovation involved the use of a gas sampling bag as an exposure vessel (Araki et al., 1994). Using these techniques NTP reported CE-induced gene reversion in the *S. typhimurium* base substitution strain TA1535, with or without S-9 metabolic activation. Negative results were obtained in strains TA100 or TA98 (NTP, 1989a). The positive result was confirmed for TA1535 as well as a related strain, TA1537 (Araki et al., 1994). These authors also observed CE-induced gene reversion by CE in *E. coli* WP2 uvrA.

A difference between the genotoxicity of CE in vitro versus in vivo test systems was demonstrated by Ebert et al. (1994), who compared CE's effects in a hypoxanthine phosphoribosyltransferase (HPRT) test using Chinese hamster ovary (CHO) cells, an in vivo/in vitro unscheduled DNA synthesis (UDS) assay in female B6C3F1 mice, and an in vivo micronucleus (MN) test in male and female B6C3F1 mice. Positive evidence of CE's genotoxicity was obtained in the in vitro test system, but the compound appeared to have no ability to induce UDS or MN in vivo. This result caused the authors to question whether CE possesses clastogenic potential and to speculate on what other combination of mechanisms (other than genotoxicity) might be involved in the compound-induced carcinogenicity of the uterus.

Therefore, based on the totality of the genotoxicity/mutagenicity evidence, CE may be considered to be a positive mutagen based on its strong gene reversion effects in certain strains of *S. typhimurium* and *E. coli*. However, the absence of positive genotoxic effects of CE in vivo leaves open the question of the compound's carcinogenic mechanism in animal studies.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NON-CANCER EFFECTS AND MODE OF ACTION—ORAL AND INHALATION

Data gaps limit the toxicological (and carcinogenic) evaluations of CE. For example, first, there is no published information on the toxicity of CE when chronically administered via the oral route. Second, there is no two-generation CE exposure reproduction study. Thirdly, most of the well-documented toxicological effects of CE, that have been described, have resulted from frequent exposures to comparatively high concentrations, i.e., 15,000 ppm CE, but no inhalation studies identified effects at concentrations lower than 250 ppm (660 mg/m^3) (Landry et al., 1989). It is plausible to infer that CE is not very toxic at low exposure levels because the noncancerous

CE effects observed at high doses appear to be limited to marginal changes in fetotoxicity, body weight loss, mild nephrosis, and changes in uterine and liver weights. It is reasonable to hypothesize that any toxicological effects of the compound at the intermediate to lower CE levels (and so far untested) would be even more mild, and perhaps cease. This hypothesis cannot be validated however, with the present data. The interpretation of noncancer effects did not follow the Agency's risk assessment guidelines for developmental toxicity.

4.5.1. Primary Effect

4.5.1.1. *Reproductive and Developmental Toxicity*

Reproductive organ and fetal developmental CE effects have been shown in various experiments. For example, at higher CE concentrations in the dog, the uterus has been noted to respond with decreased muscle tone and lessened contraction force (Van Liere et al., 1966). Moreover, in an acute study Fedtke et al. (1994a) reported a 35% decrease in relative uterine weight in B6C3F1 mice, but not in F344 rats, exposed to 15,000 ppm (39,570 mg/m^3) CE 6 hr/day for 5 days. These workers also found decreased GSH levels in the uterus of CE-exposed, mice and rats. An NTP chronic cancer bioassay has demonstrated quite clearly in mice that the uterus is the primary organ site for carcinogenesis at 15,000 ppm CE; male rats had skin tumors and female rats had brain tumors, both marginal (NTP, 1989a). The above findings are consistent with the hypothesis that the uterus is a primary CE target tissue in rats and mice.

Breslin et al. (1988) observed a statistically significant lengthening of the estrous cycle (+0.6 days) in B6C3F1 mice exposed to 15,000 ppm (39,600 mg/m^3) for 6 hr/day for a minimum of 14 consecutive days (3 estrous cycles), although no single phase of the cycle appeared to be uniquely affected. Bucher et al. (1995) also found a statistically significant increase (+0.4 days) in the mean duration of the estrous cycle in B6C3F1 mice exposed to 15,000 ppm (39,600 mg/m^3) 6 hr/day for 3 weeks.

Evidence points to a perturbation of fetal skeletal development in pregnant CF-1 mice (Table 9, p. 26; Scortichini et al., 1986). These authors reported an apparent statistically significant (by trend only, p =0.048) increased incidence of delayed foramina ossification closure in the skulls of fetal CF-1 mice, but only at the highest exposure of 4,946 ppm CE (13,000 mg/m^3) and not at lower doses of 0, 491, or 1,504 ppm CE (Figure 5, p. 25). This HDT effect likely represents a weak but true fetotoxic response to CE exposure because it was manifested in the absence of maternal toxicity by all measures. It is the lowest dose (4,946 ppm) and shortest time representing a critical toxicological effect for CE. Therefore, the fetuses of CF-1 dams exposed by inhalation during organogenesis to 4,946 ppm CE (13,200 mg/m^3) represent a

sufficiently significant response in delayed fetal foramina closure (DFFC) and are employed herein as the basis for deriving the noncancer RfC.

4.5.2. Secondary Effects

4.5.2.1. *Weight Loss*

As noted in Section 4.2.2.1.3.1, a slight (4–8%) decrease in mean body weight gain in male rats treated at 15,000 ppm CE compared with controls was observed after week 33 of chronic exposure, with the mean body weights of female rats 5–13% lower than controls from week 11 to the end of the study (NTP, 1989a). At bioassay termination, the mean body weight of exposed female rats was reduced by 10% compared with controls. No food consumption data were described in the study report. Neither clinical signs nor other CE-induced nonneoplastic lesions were observed. This suggests that the observed weight loss may have been compound-related and not simply a consequence of food aversion. Because the extent of the weight loss (10%) is at the threshold that EPA considers to be toxicologically significant, the response is thus considered a secondary noncancer effect. On the basis of decreased body weight in female rats at the single exposure level tested, this study identified a LOAEL(ADJ) for CE of 7,070 mg/m^3, a concentration that would represent a NOAEL in males.

4.5.2.2. *Hepatotoxicity*

Increased relative liver weight in response to CE exposure at 19,000 ppm for 13 weeks was observed in both sexes of B6C3F1 mice (NTP, 1989a). In addition, slight increases in mean relative liver weights with a possibly related increase in the degree of hepatocellular vacuolization were reported by Landry et al. (1989) in mice exposed to 5,000 ppm for 23 hr/day for 11 consecutive days. Similarly, statistically significant increases in relative liver weights were also observed in male rats exposed to 4,000 or 10,000 ppm for 6 hr/day, 5 days/week for 2 weeks (Landry et al., 1982). However, these liver changes appeared to be unaccompanied by any evidence of compound-related histopathology.

Combining these inferential findings of CE's hepatotoxicity with the observation of a moderate elevation of the activity of alanine aminotransferase (ALT) in the serum of the 52-year-old man who had a history of CE sniffing along with other substance abuse activities (Nordin et al., 1988) suggests that the liver may be a CE target organ at high exposures, although, in general, few instances of CE-related histopathology of the liver or changes in clinical chemistry components have been identified.

CE effects were alluded to in a Russian report in which rats were exposed daily (4 hr/day) to 570 mg/m^3 (220 ppm) for 6 months (Troshina, 1966). This exposure was reported to result in perturbed hepatic function and lipid degenerative changes, along with decreased arterial blood pressure and some dystrophic changes to the lungs. However, these findings remain uncorroborated by other workers and have generally been discounted because of inadequate reporting (ATSDR, 1997; U.S. EPA, 1998c).

4.5.2.3. *Neurotoxicity*

A range of neurotoxicological responses to CE have been reported in human beings and in laboratory animals, with a wide range of doses. Thus, CE can induce anesthesia in humans at higher doses (Cole, 1967; Dobkin and Byles, 1971; Lawson, 1965), and hyperactivity in mice (NTP, 1989a), even as low as 5,000 ppm (Dow Chemical Co., 1985). However, these transient effects may not be appropriate as the basis for developing an RfC because of the acute reversible nature of the responses. More sensitive measures of central nervous system effects have not been observed, although Landry et al. (1989) conducted a neurobehavioral observation battery for mice exposed to concentrations of CE up to 5,000 ppm for 23 hr/day for 11 consecutive days. In general, mechanisms of anesthesia are not well understood, though it is likely that the observed effects are due to the direct action of high concentrations of the parent compound on nervous tissue.

4.5.3. Mode of Action of Toxic Effects

It is difficult to determine the exact nature of CE toxicity in each responding tissue. It also is difficult to know if there is a interconnecting mode of action among tissues. Section 3.2 discusses what is known on CE metabolic issues, that may underlie the mode of toxic action.

Many haloethanes and halomethanes are conjugatively reduced by GSH. Specifically, it is known that CE binds GSH to form SEG because the conjugate (SEG) has been directly measured, as have GS-synthetase enzyme activities of the GS-ethyl formation reaction (Fedtke et al., 1994b). Other SEG-induced metabolites SENACys and SECys have been demonstrated in elevated amounts following CE exposure that is a further indication of the reductive conjugative pathway. At high doses, such as 15,000 ppm CE, the metabolism by GSH conjugation (Figure 4) can become saturating. When this happens, oxidation of CE to acetaldehyde (and other oxidation products) occurs by the P450 metabolic route (Ivanetich and Van Der Honert, 1981). This oxidation occurs more in the mouse than the rat (cf. p. 13). The uterus is a target organ of CE in the mouse, among others, and may respond by lowering GSH to below normal levels, thereby

depleting GSH pools, which in turn leads to oxidation with acetaldehyde. The oxidative products are known to react with cellular macromolecules (Morris, 1997; Behrens et al., 1988). This in turn can lead to toxicity.

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

Although no data exist that document a tumorigenic effect of CE in human beings, there are consistent lines of evidence indicating that CE is a carcinogen in animal test systems. These include:

(1) **Chemical carcinogenesis**. The clear-cut demonstration of CE's causality in uterine carcinogenicity in female B6C3F1 mice (uterine incidence: 43/50 = 86%), which is relevant because uterine cancer is rare to uncommon in B6C3F1 mice occurring at an uncommon rate of 4/1,371 (0.29%) in a limited population (NTP, 1989a; IARC, 1992).

(2) **Mutagenesis.** CE is mutagenic in it's capacity to induce gene reversion in certain strains of *S. typhimurium*.

(3) **Structural Activity Relationship.** BE, a structural CE analogue, induces similar tumorigenic effects in the uterus of B6C3F1 mice as does dichloroethane.

(4) **Metabolism.** CE's ability to lower GSH pools, similar to methyl halohydrocarbons CH_3Cl and CH_3CH_2Br, and oxidative metabolism proceeding via acetaldehyde. CM may be a carcinogen too forming the renal cortex and papillary cystadenocarcinomas in male mice exposed. BE causes uterine tumors and marginally other tumors: lung, pheochromocytomas of the adrenals, and brain.

Section 4.2.2.1.3 gives a detailed summary of the principal carcinogenesis study (NTP, 1989a). As noted, the primary effect was the high incidence of uterine carcinomas in female B6C3F1 mice (43/50 at 15,000 ppm vs. 0/49 in controls). The tumors were of endometrial origin and showed a profound capacity for metastasizing. First the cancers moved to the neighboring myometrial tissue and from there disseminated to such secondary tissue sites as lung, ovary, lymph nodes, kidney, adrenal gland, pancreas, mesentery, urinary bladder, spleen, heart and, to a lesser extent, colon, stomach, gall bladder, liver, small intestine, and ureter. The complications arising from these CE-induced tumors are considered to be the cause of the poor survival in these female B6C3F1 mice (NTP, 1989a). Thus, in addition to metastasis, life-shortening tumor effects

were observed in female mice, providing further emphasis on the severity of CE chemical carcinogenesis.

Survival was poor in the male B6C3F1 mice and tumorigenic responses could not be inferred because of low statistical power. There was a borderline tumorigenic response in the lung, but the B6C3F1 tumor incidence data in male mice are considered inadequate to determine potential carcinogenicity in humans.

As noted in Section 4.2.2.1.3.3, marginal increases in some uncommon skin tumors in male F344 rats were not persuasive enough to unequivocally designate the compound as a carcinogen in the rat animal model, but the rat skin response is suggestive of tumorigenesis. The female F344 rat brain astrocytoma response, an uncommonly occurring response, was also equivocal because of its low incidence (3/49) versus controls (0/46), but the rat brain response is suggestive. Table 11 summarizes all the relevant tumor-forming effects of CE from the NTP (1989a) study in F344 rats and B6C3F1 mice. It is concluded: CE is clearly carcinogenic in female B6C3F1 mice, but the evidence for CE carcinogenicity in male and female F344 rats is equivocal.

Taken as a whole, the mutagenicity and metabolic data that have been amassed for CE are consistent with the findings of carcinogenicity but some information is lacking (Figure 2). For example, well-documented positive point mutations have been obtained in Ames tests (NTP, 1989a; Zeiger et al., 1992; Araki et al., 1994), but CE does not induce in vivo clastogenic responses in the same strain of mice (B6C3F1) in which the uterine carcinomas were described (Ebert et al., 1994). GSH links with the ethyl group of CE in an elimination reductive conjugation pathway. If CE is dosed high enough, GSH pool levels likely become limiting for further ethylation of CE. GSH pools likely become rate-limiting for other detoxification reactions too (Figure 2, p. 6). In this way the excess CE then would be forced to flow through oxidative metabolism (oxidation in mice is twice that of rats) with acetaldehyde being an intermediate (Figure 1, p. 1). This can cause cancer at high internal intermediate mutagenic doses; whether excessive systemic SEG or acetylaldehyde are involved in the mode of carcinogenic action, or both, is not known. CE induces its own metabolism, likely p-450 enzyme P450IIE1, which happens primarily in the liver.

Table 10. Toxicity/carcinogenicity of chloroethane in experimental studies						
Species/strain	**Sex/number**	**Route of exposure**	**Dosing regimen**	**Principal effects**	**NOAEL/ LOAEL**	**Reference**
Carcinogenicity						
Rats/F344	M and F 50/group	Inhalation	0 or 15,000 ppm, 6 hr/day for 2 years.	Uncommon skin tumors in males at incidence 8/46; malignant astrocytomas in females (3/50).	N/A	NTP (1989a)
Mice/B6C3F1	M and F 50/group	Inhalation	0 or 15,000 ppm, 6 hr/day for 2 years.	Uterine tumors in females (43/50).	N/A	
Noncancer toxicity						
B6C3F1 Mice	F 10/group 3 groups	Inhalation	0 or 15,000 ppm CE for 6 hr/day for 5 days.	Reduction in relative and absolute (–35%) uterine weights in females.	39,570 mg/m^3	Fedtke et al. (1994a)
Rats/F344	M and F 50/group	Inhalation	0 or 15,000 ppm, 6 hr/day, 5 days/week for 2 years.	Reduction in body weight gain in females.	39,570 mg/m^3 (L)	NTP (1989a)
Mice/F344	M and F 50/group	Inhalation	0 or 15,000 ppm, 6 hr/day, 5 days a weeks for 2 years.	No significant toxic effects other than transient hyperactivity during dosing.	39,570 mg/m^3 (N)	
Mice/CF-1	F, 30/group	Inhalation	0, 491, 1,504, or 4,946 ppm, 6 hr/day on GDs 6-15.	Marginal increase in delay of foramina closure in the fetal skull.	4,000 mg/m^3 (N)	Scortichini et al. (1986)

Table 10 is continued on the following page

Table 10. Toxicity/carcinogenicity of chloroethane in experimental studies						
Species/strain	**Sex/number**	**Route of exposure**	**Dosing regimen**	**Principal effects**	**NOAEL/ LOAEL**	**Reference**
Rats/F344	Males only, not females, 10/group	Inhalation	0, 2,500, 5,000, 10,000, or 19,000 ppm, 6 hr/day, 5 days/week, for 13 weeks.	Increases in relative liver weight in males at the highest dose.	50,122 mg/m^3 (N)	NTP (1989a)
Mice/B6C3F1	Females only, not males, 10/group	Inhalation	0, 2,500, 5,000, 10,000, or 19,000 ppm, 6 hr/day, 5 days/week, for 13 weeks.	Increases in relative liver weight in females at the highest dose.	50,122 mg/m^3 (N)	NTP (1989a)
Rats/F344	M and F 6/group.	Inhalation	0, 1,590, 3,980, or 9,980 ppm, 6 hr/day, 5 days/week, for 2 weeks.	No biologically significant effects at any dose level.	26,300 mg/m^3 (N)	Landry et al. (1982)
Mice/B6C3F1	M and F 7/group	Inhalation	0, 250, 1,247, or 4,843 ppm, 23 hr/day for 11 days.	No biologically significant effects.	12,200 mg/m^3 (N)	Landry et al. (1989)
Mice/B6C3F1	F 10/group	Inhalation	0 or 15,000 ppm, 6 hr/day for 14 days.	Elongation of the estrous cycle.	39,570 mg/m^3 (L)	Breslin et al. (1988)
Mice/B6C3F1	F 30/group	Inhalation	0 or 15,000 ppm, 6 hr/day for 21 days.	Elongation of the estrous cycle.	39,570 mg/m^3 (L)	Bucher et al. (1995)

Table 11. Summary and Conclusions of Tumorigenesis in Rats and Mice

Sex	F344 rats	B6C3F1 mice
Males	*Marginal evidence* ! Skin tumors	*Inadequate evidence*
Females	*Equivocal evidence* ! Brain tumors	*Clear evidence* ! Uterine tumors that metastasized to 16 secondary organ sites. ! Liver response (weak). ! Hematopoietic response in a number of tissues and lymph nodes. (Profound life shortening associated with the primary effect)

Source: NTP (1989a). Two year bioassay for chemical carcinogenesis in B6C3F1 mice and F344 rats.

Evidence in support of the carcinogenicity of CE is also provided by similar long-term experimental studies that were carried out on its structural analogue, BE (NTP, 1989b). When challenged with concentrations of BE at 100, 200, and 400 ppm, female B6C3F1 mice responded with the formation of uterine squamous cell carcinomas, adenomas, and carcinomas, in direct analogy to CE. The uterine responses were 4/50, 5/47, and 27/48 for 100, 200, and 400 ppm BE exposure groups, respectively, versus an incidence in controls of 0/50. Although not statistically significant, 1,2-dichloroethane administered by gavage produced adenocarcinomas of the uterus in 3/49 mice at 148 mg/kg and 4/47 mice at 229 mg/kg in 78 weeks (NTP, 1978). Related chlorohydrocarbons that do not cause increased uterine tumors are 1,1-dichloroethane, 1,1,1-trichloromethane, 1,1,2-trichloroethane, 1,1,1,2-tetrachloroethane, 1,1,2,2-tetrachloroethane, pentachloroethane, and hexachloroethane (all referenced in NTP, 1989a). Other analogues such as methylchloride cause only cystadenomas and adenomas of male mice. Methylbromide seems not to be carcinogenic; dibromoethane does produce uterine cancers and also produces alveolar/bronchiolar carcinomas in male and female mice, as well as hemangiosarcomas, fibrosarcomas in the subcutaneous tissue, nasal carcinomas, and mammary adenocarcinomas in female mice.

The similar carcinogenic responses in female B6C3F1 mice to some structural analogues of CE, i.e., BE, and 1,2-dichloroethane in separate assays, provide support for two concepts: (1) uterine effects associated with these compounds are unlikely to have come about by chance alone, and (2) these effects may be brought about by metabolically related mechanisms. Although the present database for CE carcinogenicity is limited in animals, evidence supporting carcinogenicity classification are adequate to classify CE.

Based on the criteria set forth in the current *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 1986), a weight-of-evidence classification of B2 is indicated. That is, CE is *a probable human carcinogen* based on no evidence in human beings and adequate evidence in animals with medium confidence. Categorizing CE according to the weight of evidence approach proposed by the *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 1996) would derive a *likely human carcinogen by the inhalation route* classification for CE.

4.7. OTHER HAZARD IDENTIFICATION ISSUES

4.7.1. Possible Structural-Activity Relationships

As discussed in Section 4.6, BE and chloromethane (CM) are structural analogues of CE. Bromoethane can deplete GSH, much like CE and CM, and therefore is normally detoxified primarily by reductive conjugation to GSH (Khan and O'Brien, 1991). Excessive amounts of deplete GSH and become oxidized to unresolved acetaldehyde (from BE and CE) and formaldehyde (from BM and CM). "Unresolved" refers to the lack of metabolic steady state and a buildup of the toxicant intermediate. The toxicity of these analogues may be used to explore and illuminate the toxicity and mechanism of action of CE.

For BE, the toxic process has been thoroughly documented in an NTP study of the combined acute, subchronic, and chronic toxicology and carcinogenicity (NTP, 1989b). Consistent with the lethality data at high doses (5,000-10,000 ppm) of BE, subchronic exposure to lower concentrations of BE induced profound indications of noncarcinogenic toxicity, including clinical signs such as tremors, atrophy in some major organs and tissues (e.g., the thigh, skeletal muscle, and lung), and degeneration of the sex organs (uterus in rats; ovary in mice) (NTP, 1989b). Interestingly, the histopathologic findings of the 14-week subchronic studies at single exposures of 1,600 ppm were not observed at lower doses and frequent exposure at 400 ppm in the 2 year study, which suggests a threshold for these high-dose (1,600 ppm) events. Inhalation concentrations of 0, 100, 200, and 400 ppm were chosen for the 2-year BE study in contrast to 15,000 ppm for CE.

Though BE displays greater toxicity than CE, especially in the nasal passages and lungs, a comparison of the carcinogenic endpoints of the two halogenated hydrocarbons implies a domain of commonality in toxicity mechanisms. That is, the most sensitive carcinogenic response to BE was the incidence of uterine cancers of female B6C3F1 mice: 0/50 at 0 ppm, 4/50 at 100 ppm ($p = 0.06$), 5/50 at 200 ppm ($p = 0.03$), and 28/48 ppm ($p < 10^{-8}$) at 400 ppm BE. This same uterine carcinogenic response was also reported for mice exposed to CE: 0/50 at 0 ppm versus 43/50 (p

$< 10^{-8}$) at 15,000 ppm (NTP, 1989a). Other notable cancer sites for BE were lung, pheochromocytomas, bronchiolar, nasal, and low-level granular-type tumors and gliomas in the brain; other cancer sites for CE included the liver. The tumor spectra share the uterus, but the other carcinogenic sites are different. The conclusion of carcinogenicity drawn for BE supports the chemical carcinogenesis hypothesis for CE.

A recent review by Bolt and Gansewendt (1993) has collated much of the available information on the toxicity, carcinogenicity, and underlying metabolism of potential CE structural analogues, e.g., CM, BM, and iodomethane (IM). Examination of available data for CM may be expected to shed light on the toxic potential of CE if a sufficiently similar pattern of toxic responses and possibly related metabolic processes are revealed. In this context, CM appears to behave in a similar manner to CE in the Ames test, inducing positive responses +/- S9 in the *S. typhimurium* strain TA 1535 (and TA 100). Though CM failed to induce genotoxic responses in a number of in vivo tests, a positive dominant lethal response was observed in male F344 rats.

The key information linking the carcinogenic potential of CE and CM comes from an unpublished study carried out at the Battelle Memorial Institute on behalf of the Chemical Industries Institute of Toxicology (CIIT), which was cited by Bolt and Gansewendt (1993). As described in the review, Battelle exposed 30 F344 rats and B6C3F1 mice/sex/group to 0, 50, 225, or 1,000 ppm CM via inhalation, 6 hr/day, 5 days/week for 2 years. Though characterized by poor survival among the groups, the study revealed an increase in tumors consisting of cystadenomas and adenomas of the renal cortex and papillary cystadenocarcinomas in male mice exposed to the highest CM concentration.

There appear to be striking analogies between the metabolic fates of CM and CE. For example, following rapid uptake via the lungs, important metabolic products of CM were identified as formaldehyde, formic acid, and carbon dioxide, with part of the material being incorporated into the C_1-pool (tetrahydrofolic acid) of intermediate metabolism (Kornbrust and Bus, 1982; Landry et al., 1983). An important outcome of these experiments was the demonstration that the incorporation of ^{14}C from CM into major structural macromolecules such as DNA occurred as a consequence of normal protein synthesis rather than as a result of methylation (Kornbrust et al., 1982).

Bolt and Gansewendt (1993) combined four lines of evidence into an argument that may explain the carcinogenic consequences of CM in male B6C3F1 mice in biochemical terms:

(1) demonstration of GSH-linked sulfhydryl derivatives in the urine of CM-exposed rats (Landry et al., 1983)

(2) the depletion of NPSH content in the liver, lung, and kidneys of exposed F344 rats (Dodd et al., 1982)

(3) the demonstration of both P450-mediated oxidative and GSH-mediated reductive pathways for the metabolism of CM (Kornbrust and Bus, 1982)

(4) the inhibition of the acute toxicity of CM in male B6C3F1 mice by GSH depletion (Chellman et al., 1986).

Bolt and Gansewendt (1993) used the latter inhibition data to develop a cancer mechanistic hypothesis for CM. They assumed a relationship existed between GSH depletion and cancer because the exposure CM levels for the onset of kidney tumors in male B6C3F1 mice and the depletion of kidney GSH levels are comparable. Decrementing GSH can switch CM from the reductive pathway, which is used principally for CM metabolism, to also include oxidative pathway, i.e., catabolism by P450 of CM to formaldehyde, formate, and CO_2. Excessive formaldehyde can cause cancer (Morgan ,1997; Morris, 1997; Monticello and Morgan, 1997). GSH depletion can likewise cause a paucity of the cofactor GSH for formate dehydrogenase, the enzyme inactivating formaldehyde. This effect can promote the formation of DNA-protein crosslinks in susceptible target organs.

As shown in Table 12, lines of evidence that draw parallels between the metabolism of CE and CM include (1) the duality of CE metabolism, in which reductive metabolism through binding to GSH and oxidative metabolism mediated by cytochrome P450IIE1 are featured,

Table 12. Common metabolic features of chloroethane and chloromethane: potential relevance to tumor formation in experimental studies

Mechanisms	Chloroethane	Chloromethane
GSH conjugates	S-ethyl-N-acetylcysteine (SENACys) S-ethyl-cysteine (SECys)	S-methyl-cysteine (SMCys) S-methylglutathione
Oxidative metabolism	Cyt P450IIE1	CytP450IIE1
Oxidative products	Acetaldehyde	Formaldehyde
Tumor sites	Uterus in B6C3F1 ♀ mice Liver in B6C3F1 ♀ mice	Kidney in B6C3F1 ♂ mice
route of exposure	Inhalation	Inhalation
LOAEL	15,000 ppm	1,000 ppm
Tumor ineffective doses	Not tested at lower doses	0, 50, 225 ppm

(2) the depletion of GSH that occurs in target tissues in response to CE exposure, and (3) the formation of the formaldehyde homologue acetaldehyde during the oxidative metabolism of CE.

If CE and CM share similar mechanisms for tumorigenesis, the occupational exposure information that has accumulated for CM in humans may also be of relevance to the potential carcinogenicity of CE. In general, CM data show a marked diversity in the ability of persons to

metabolize CM, and in the extent of the toxic response elicited by the compound. Bolt and Gansewendt (1993) discuss a number of findings that point to the existence of different population subgroups defined by their ability to metabolize CM. Persons with lower rates of GSH conjugation might be expected to be at greater risk of tumor formation arising from either CM or CE exposure.

4.7.2. Possible Gender Differences

A report by Griesemer and Eustis (1994) summarized the findings of NTP with regard to the sex- and tissue-specific onset of carcinogenicity observed throughout their series of 2-year toxicity/carcinogenicity studies. A total of 1,760 untreated control groups from 440 studies using F344 rats and B6C3F1 mice (approximately 88,000 animals, from which 3.5 million tissues were examined microscopically) have contributed data on the gender-specific background rates of tumor formation. These data apply both to major organs such as lung, liver, and kidney, and to gender-specific organs, such as uterus, ovary, and testis. Of particular interest to the potential carcinogenicity of CE (and BE) is the markedly low background frequency of uterine tumor formation in B6C3F1 mice (0.3%). Based on the high incidence of uterine tumors at the single CE concentration tested (15,000 ppm), and on the dose-dependent increase in response to challenge with BE, the conclusion may be reasonably drawn that the occurrence of these uterine tumors has direct etiological association with the target compounds. Because CE and BE cause female uterine cancers to the greatest extent of their carcinogenicity, the mode of action of CE and BE may be highly gender specific.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

A chronic RfD can not be determined for CE in water because no chronic oral CE studies exist

At water-saturating concentrations (0.57 g/100 g) CE oral intake *ad libitum* for 14 days did not demonstrate significant toxicological effects (Pottenger et al., 1995). The *acute* NOEL for water is 297 mg/kg bw/day for F-344 male rats and 361 mg/kg bw/day for female rats.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect - With Rationale and Justification

As discussed in Section 4.5, the noncancer effects of CE exposure in experimental studies were fetotoxicity, weight loss, neurotoxicity, hepatotoxicity, and immunotoxicity. Of these effects, the fetotoxicity effect (Scortichini et al., 1986) occurs at the lowest level of CE exposure in the current animal database and is the "critical" toxic effect, the designation of critical effect being a judgment step in EPA's RfC risk assessment methodology.

5.2.2. Methods of Analysis

5.2.2.1. *Principal Study*

Scortichini et al. (1986) reported a statistically significant increase in the delay of frontal foramina closure (DFFC) of the progeny of CF-1 mice exposed to a mean concentration of 4,946 ppm CE. This fetotoxicity is covered in more detail in Section 4.3.2.1, p. 24. At 4,946 ppm CE there were 5 affected skulls/116 skulls examined (incidence = 4.3%), representing 5 litters/22 litters examined (incidence = 22.7%). It is notable that the effect was scattered in five different litters. The lower CE exposures produce responses at 1,500 ppm (1/147) and 491 ppm (1/142) that were the same as the control (1/116 = 0.9%). The Scortichini study fetotoxicity at 4,946 ppm CE (13,057 mg/m^3) is the noncancer critical effect LOAEL for CE, and 1,500 ppm CE (3,970 mg/m^3) is the NOAEL.

5.2.2.2. *Primary Supporting Study*

F-344 rats and B6C3F1 mice were both exposed for 5 days to 15,000 ppm or air controls (Fedtke, 1994a). There was a loss in body weight in both species, 3.7% in rats and 16.4% in mice. Because the body weight differences between air and CE exposure were not significant, the weight losses were considered stress related. At autopsy at study end the liver, lung, and kidney were normal in weight and appearance. Remarkably, the CE-treated mice, but not the rats, had decreased uterus weights (mean absolute and mean relative). The decrease was about 35%. Moreover, the uterus is the site for carcinogenesis in mice, but not in rats. GSH pool reduction in mice, but not rats, can be the basis of the mode of action causing significant acetaldehyde oxidant intermediate to cause toxicity. Therefore, the 35% uterine weight decrease at 15,000 ppm for 5 days is the primary supporting noncancer effect of CE.

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

In developing RfCs from observed NOAELs in experimental studies, human equivalent concentrations (HEC) for extrarespiratory effects are derived by factoring the time-adjusted NOAELs with the ratio of the animal/human blood gas partition coefficients (λ_A / λ_H). For human exposure, it is assumed that in time equilibrium is attained for blood/air (b/a) concentrations. When blood gas partition coefficients are unavailable for an experimental animal, or when $\lambda_A > \lambda_H$, then a default ratio of 1 is used (U.S. EPA, 1994). Human blood:air partition coefficients of 1.9 (Morgan et al., 1970), and 2.69 (Gargas et al., 1989) have been reported. A rat blood:air partition coefficient of 4.08 has been reported (Gargas et al., 1988; 1989). Because both reported values for humans are lower than the rat partition coefficient, a default ratio of 1 is used to calculate the HEC. Thus, LOAEL(HEC) = LOAEL(ADJ) × (λ_A/λ_H) = 13,057 mg/m^3 × (1) = 13,057 mg/m^3. Thus, for the fetotoxic effect, the LOAEL(HEC) = 13,057 mg/m^3, and NOAEL(HEC) is 3,970 × 1 = 3,970 mg/m^3.

The above data set, 1/126, 1/142, 1/147, and 5/116 (on a skull-examined basis) is not corrected for continuous exposure. A reasonably good fit (p=0.87) at doses 0, 500, 1,500, and 5,000 ppm was obtained using software designed to estimate the benchmark dose employing the Weibull model (U.S. EPA, 1998d). The Weibull model, $p(d) = 1 - \exp\{-\alpha - \beta*(d)^{\gamma}$, was used. This dichotomous model predicted that, on the probability of a fetus being affected and for a benchmark response (BMR) of 10% incidence, the BMC is 17,832 mg/m^3 (6,754 ppm CE). This BMC for 10% is just above a LOAEL and provides a dependable reference concentration (Allen et al., 1994a). The BMDL (benchmark dose lower limit) at 10% is 13,421 mg/m^3 (5,084 ppm CE).

The litter quantal model reduces all fetal incidence data to a question of whether any fetuses in a litter are affected, while the above fetal model allows the use of fetal data grouped by dose. Because there were no interlitter biases reported and the dams are the units that are treated, the litters are considered the representative biological units of CE-induced fetotoxicity. The litter data 1/22, 1/24, 1/25, and 5/22 (Table 9) were also modeled using the probability of a litter being affected. Good fits were obtained at 0, 491, 1,504, and 4,946 ppm CE (*p*-values 0.88). The BMC for 10% extra risk was chosen for litters and is based on the results for generic quantal models in Allen et al. (1994b). The BMC for litters is estimated to be 10,634 mg/m^3 (4,028 ppm) using the Weibull model; the lower 95% confidence limit (BMLD) is 4,240 mg/m^3 (1,606 ppm CE). The polynomial model [$P(d) = 1 - \exp\{-q_0 - q_1*(d) - q_2^*(d)^2 ... -q_k*(d)^k\}$] was little different in results. These litter results are more conservative than the above per-skull basis results. The BMLD for litters (4,261 mg/m^3) may be used as a substitute for a NOAEL when a NOAEL cannot be estimated. There is a NOAEL of 3,970 mg/m^3 but the BMLD will be used to estimate the RfC, as will the NOAEL. Both results will be compared below.

To establish an RfC, a number of uncertainty factors must be accounted for. The NOAEL or the BMLD is divided by a complex factor and therefore the acceptable inhalation level is lowered to accommodate the areas of uncertainty. The factor includes the major areas of uncertainty that necessitate accommodation. The net result is the establishment of an acceptable inhalation exposure level that is the highest concentration that takes the combination of these factors conservatively into account.

An uncertainty factor of 10 is considered for variations in sensitive subpopulations within populations, and a further factor of 10 is used for interspecies extrapolation. An uncertainty factor of 3 is used for extrapolating from a LOAEL to a NOAEL. A full factor of 10 is used for database deficiencies to account for the lack of a multigeneration reproductive study, and because no evaluation of reproductive function following long-term exposure is available. These uncertainty components combine (10 × 10 × 3 × 10) to an overall uncertainty factor (UF) of 3,000.

No modifying factor for this noncancer fetotoxic effect is proposed, therefore, the RfC is obtained directly as follows:

- Method I RfC = BMLD ÷ 3,000
  RfC = 4,261 mg/m^3 ÷ 3,000 = 1.4 mg/m^3 (0.54 ppm CE)
  RfC = 1.4E0 mg/m^3
- Method II RfC = NOAEL/3,000
  RfC = 3,970/3,000 = 1.32 mg/m^3 (0.50 ppm CE)
  RfC = 1E0 mg/m^3

The traditional method of estimating a dose to which one may reference as relatively safe (RfC) is to factor down the noncancer NOAEL by the UF. With the fetotoxic effect, the RfC = 1E0 mg/m^3 (rounded off) and will be the recommended value. The RfC in Method I employing a fit to the data, establishment of the 95% LCL on dose, and factoring down by the UF yields essentially the same answer (which is a good check).

5.3. CANCER ASSESSMENT

5.3.1. Qualitative Cancer Assessment in Animals

No human cancer data exists on CE. Indirect evidence for the carcinogenicity of CE was observed in laboratory animal studies in a single NTP study (NTP, 1989a). In the bioassay 50 B6C3F1 mice and 50 F344 rats/sex/group were exposed via inhalation to only one dose: 15,000 ppm CE, 6 hr/day, 5 days/week for 2 years. This is a nonstandard protocol because normally there are 2 or 3 doses. Tumorigenic responses in male B6C3F1 mice were compromised by poor survival and the onset of urogenital infections. The B6C3F1 female mice responses were quite remarkable because of the strong carcinogenic response: uterine carcinogenicity in 43 female mice of 50 put on test (86%). This incidence is relevant because uterine cancer is an uncommon cancer site in B6C3F1 mice. The human historical incidence for uterine cancer is approximately 0.006 %, making it the seventh most common female human cancer (Parkin et al., 1999). The historical rate for B6C3F1 mice is 0.29%, an uncommon but not rare cancer in mice bioassayed so far (NTP, 1989a; IARC, 1992). The uterine incidence ratio of the CE-treated B6C3F1 group to the historical group is 86%/0.29% = 297-fold response.

The primary endometrial tumors metastasized to 16 secondary organs or tissue sites. The females died early compared to concurrent controls due to tumors indicating aggressive carcinogenic progression. These considerations represent clear evidence of CE's carcinogenicity in female B6C3F1 mice (NTP, 1989a). In addition, the F344 rat incidence of marginally tumorigenic responses in males (various skin tumors) and females (astrocytoma brain tumors) was suggestive of CE's broader spectrum of possible animal carcinogenic responses.

Supporting CE's chemical carcinogenesis is the structural analogue evidence of carcinogenicity comparing CE to BE, particularly because of the same organ site specificity of primary uterine tumors for the haloethanes. This structure-activity relationship between the haloethanes lends credence to the weight-of-evidence classification of CE's carcinogenicity. Also, the comparison of CE to CM suggests similar mode of action likely leading to carcinogenicity.

Thus, even though the cancer in female B6C3F1 mouse is in only 1 sex of 1 species, and not in the male mouse or in either sex of rat, the response is nonetheless very high in incidence,

malignancy, and life-shortening effects. This constitutes compelling carcinogenicity evidence in B6C3F1 mice. Accordingly, by combining all of the evidence for CE's mutagenicity, animal carcinogenicity, and similar animal carcinogenic responses of its structural analogues, a weight-of-evidence classification for human hazard potential can be inferred. CE is *a probable human carcinogen* (Category B2) based on no evidence in humans, and a sufficient evidence in animals (U.S. EPA, 1986). Categorizing CE according to the newer *Proposed Guidelines for Carcinogenic Risk Assessment* would designate CE as *a likely human carcinogen by inhalation* (U.S. EPA, 1996).

5.3.2. Quantitative Cancer Assessment in Animals

5.3.2.1. *Considerations in Quantitative Cancer Assessment*

The absorption and distribution of metabolized CE seems to be nonlinear at high doses of CE (cf. p. 6). The metabolism of the halohydrocarons CE, BE, dichloroethane, and EDB likely proceeds under normal circumstances by a reductive conjugation pathway mediated by GSH and various specific glutathionetransferases (Fedtke, 1994a; Commandeur et al., 1995). Methyl chloride and methyl bromide are metabolized similarly by GSH (Bolt and Gansewendt, 1993). When exposures of these halohydrocarbons exceed the capacity of the reduction pathway enzymes, a lesser used pathway, oxidation, becomes more predominant. Specifically, CE is oxidized via a P450 pathway through acetaldehyde (a toxic compound itself when in excess), acetic acid, and finally to CO_2 and H_2O as terminal oxidation products (Fedtke, 1994b). It is difficult to hypothesize that this change in metabolism, in any combination, occurs by a linear dose-response process, yet the present database is not informative enough to discern nonlinerarity. The shape of the total metabolic curve, and perhaps the coupled carcinogenicity, is unknown. Experiments studying CE cellular binding sites, GSH depletion kinetics, and acetaldehyde kinetics could be useful.

Based on positive animal studies, the derivation of a *potential* human cancer risk is based on two aspects of extrapolation: 1) the extrapolation from high animal doses in the observable range to low animal doses, and 2) the inference that humans will react metabolically similar to the chemical as the test animals. In the first extrapolation, curve fitting models are used that are appropriate to the kind of data in the bioassay (U.S. EPA, 1996a, p. 17,992). Extrapolation to low environmental ranges, commensurate with human exposure, is done on the fitted curve of the test animal dose-response. The second extrapolation assumes the route of exposure, comparative metabolism, and target organ mode of action are similar for test animals and humans. Ideally, the selection of an extrapolation dose-response model is guided by the mode of metabolic action. For CE the absorption and distribution of metabolized CE seem to be nonlinear at high CE (cf. p. 6).

More kinetic data points are needed to establish this issue. Further, not enough is known about the relationship of CE metabolism, whatever it's true kinetic response with dose, and the apparent CE cancer outcome in the uterus. Unless it is known that current metabolic evidence is measuring the responsible metabolic factors which initiate and promote CE chemical carcinogenesis, then a mode of action can only be speculation. A nonlinear kinetic model would allow direct estimation of dose-response, or if such a model were not available but there are sufficient kinetic data to determine nonlinearity in cancer-causing metabolic effects, a Margin of Exposure (MOE) method may be possible if proper exposure and kinetic data and rational were to be presented (U.S. EPA, 1996a).

In cases where there are insufficient rationale to determine the shape of the curve, a default model is employed. Currently a linearized multistaged model (LMS) is implemented by Global86 software (U.S. EPA, 1986a; Crump, 1982; Crump 1996). LMS assumes basically one-critical hit followed by a multistaged process. An 95% upper limit of carcinogenicity risk is estimated, reported and used until a suitable biological-based dose response model is derived.

The CE cancer risk determination is particularly problematic in that only one dose was tested by NTP (15,000 ppm), nonetheless that exposure demonstrated a very high tumor incidence (43/50 = 86%) in the uterus of female B6C3F1 mouse (cf. Section 5.3). Adjusted to the time of first tumor, which was a uterine carcinoma on week 67, the incidence is 43/49 = 90%. Because the 1989 NTP bioassay employed a nonstandard protocol with only one point, the LMS derived cancer slope likely is of low dependability. Thus, other approaches to cancer slope estimation are considered in the discussion of confidence (section 5.3.3.) as a check to the default method.

5.3.2.2. *LMS Method*

The default method of cancer quantitative risk estimation in the U.S. EPA is the linearized multistaged model (LMS) (Andersen, 1983; U.S. EPA, 1986). This model assumes functional continuity in the probability-dose function, $P \propto f(d_e)$, where d_e are animal experimental doses in the bioassay and incidence $(P) \geq 0$ for all $d \geq 0$. The probability-dose function is specifically *assumed* to be linear in the human environmental range ($d << d_e$) with incidence $P = (q_{1*}) \times (d)$ where q_{1*} is the unit slope. The cancer potency is found by fitting the test animal incidence data and then finding the upper 95% UCL slope (q_{1*}) of the q_1 term in the multistage equation (Crump, 1996). The LMS procedure uses Global86 software to extrapolate the fit of the high-dose animal data to expected human low-dose incidence (Crump, 1982). The LMS procedure places an upper limit on risk that is considered to be a plausible upper bound on the increased cancer risk from lifetime inhalation of CE. However, the range of true risk extends from the 95%

UCL estimated risk [P = (q_{1*}) × (d)] down to and including zero risk (P = 0). The Agency makes no true risk presumption.

5.3.2.3. *LMS Method Calculation of Cancer Slope*

The cancer response at 15,000 ppm CE (7,070 mg/m^3) is 43/49 uterine cancers and 8/49 liver cancers for a combined response of 44/49 (90%) (Table 13). The denominator in the tumor incidence was corrected to include only those animals alive at the time of the first observed tumor, which was a uterine carcinoma on week 67. This is compared to 3/46 (6.5%) in the concurrent controls, which were all liver cancers, but no uterine cancers, and below normal aging B6C3F1 hepatocellular adenoma or carcinoma incidence. The combined tumor ratio of the CE-treated group to the control group is 90%/6.5% ≈14-fold response.

Table 13. Quantitative cancer responses in the female B6C3F1 mouse liver and uterus

Administered exposure (ppm)	Human equivalent exposure mg/m^3	Uterus incidence	Liver incidence	Combined incidences
0	0	0/46	3/46	3/46
15,000	7,070	43/49	8/49	44/49

The denominators in the tumor incidences were corrected to include only those animals alive at the time of the first observed tumor. First tumor was a uterine carcinoma on week 67. The human equivalent doses were based on the assumptions that are presented in Section 3.2, converting ppm chloroethane exposure to mg/m^3 by a factor 1 ppm = 2.64 mg/m^3, and then adjusting for the specific exposure duration of 6 hr/day (factor: 6 hr/24 hr/day), 5 days/week (factor: 5 days/7days/week).

The cancer risk estimation is based on the responses presented in Section 4.6, p. 38, and Section 5.3.1, p. 50, and the data in Table 13. The shape of the curve of CE carcinogenicity is not knowable from the incidence datum in the NTP bioassay. Therefore, a linear model at all doses is assumed, including the 7070 mg/m^3 dose point. The default LMS method, as applied by Global86, is U.S. EPA policy to determine 95%UCL cancer potency. A Global86 estimate of ED_{10} is estimated to be 100 mg/kg/d or 300 mg/m^3. For the combined incidence of uterine and liver cancers (Table 13), Global86 estimates the inhalation unit risk be 4E-4/mg/m^3. This unit risk is approximately equivalent to an inhalation slope factor of q_1* = 1.14E-4/mg/kg/day, assuming 20 m^3 air breathed/day and body weight of 70 kg. Using the inhalation unit risk of 4E-4/mg/m^3, various CE risk levels may be estimated: at i = 10^{-4}, 300 µg/m^3; at i = 10^{-5}, 30 µg/m^3; and at i = 10^{-6} = 3 µg/m^3. Using the inhalation slope factor q_1* = 1E-4/mg/kg/day, then at 10^{-6} risk, for example, an exposure rate over a lifetime could not exceed 1E-2 mg/kg/day (10 µg/kg/day), i.e., at 10 µg/kg/day lifetime CE exposure one may expect, with 95% confidence, no more than a 10^{-6}

risk in humans based on animal studies, and the risk can be less, considerably less, and even zero (P = 0).

5.3.3. Discussion of Confidence in Cancer Quantitative Assessment in Animals

Any CE cancer potency estimate, such as a cancer unit slope, made is necessarily not certain because of the datum on which the estimate is based. Accordingly, the LMS method is thus not certain because of the one dose-response point and is insufficient for deriving any estimate of the shape of the dose-response curve. In practice Global86 connects this one point to the origin and estimates the 95% UCL on this straight line. Another uncertainty is that the exposed group had nearly 100% tumor incidence (90%), and it is unknown whether such a saturation of effect would have occurred at an even lower dose, in which case, the proposed inhalation slope factor could be an underestimate, the degree of which would be unknown. It is assumed from experience that the plateau on which the response sits is likely $\leq$ threefold wide (Gaylor, 1989). It is also unknown whether there are any sublinearities in the dose-response relationship in the normally observable response range, which could result in the proposed slope factor being an overestimate. Another issue of quantitative uncertainty concerns the fact that the study was terminated early (termination week 100), because there was substantial early mortality in the exposed female mouse group resulting from the tumors (e.g., only 50% of the exposed group were still alive at week 90 compared with 90% in controls). This time component was not taken into account in the risk calculations because animals were dying from uterine carcinogenesis not competing toxicity. Conceivably, a lower dose could have resulted in the same tumor incidence along with later-occurring tumors in life, in which case there would be an underestimation of the proposed slope factor; the time-to-tumor issue may be relatively trivial with respect to the other uncertainties outlined above.

Because of the uncertainties of the LMS method, alternative methods were examined to gain additional perspective on the upper bound of CE cancer potency. One of the first nonparametric methods was a procedure taking the lowest dose (7,070 mg CE/m^3) and incidence (i =0.90), and estimating the upper 95% confidence limit incidence ($\approx$ 1.00), and then define the straight line connecting this point to the 0,0 point. The cancer slope of the line $\approx$ 1/7070 mg/m^3 = 1.4E-4/mg/m^3 (Gaylor and Kodell, 1980).

Another type of estimate can be made which is enabled by the unusually high incidence (i =0.90). In the case of CE, the bioassay produces almost the maximum theoretical response of 100% incidence and can be considered an approximation of the MTD. This assumption is based on the following: 1) many of the female mice died prematurely because of the tumor load (only 2/50 survived until termination at week 100), and 2) the observation of a maximum cancer response

with some toxicity at this dose but not overt noncancer toxicity. As with many chemicals, more CE likely would have been too toxic (i.e., > MTD) from the start of the study and would reduce cancer because sick or morbid animals do not yield tumors beyond certain doses but rather become sickly and/or die before cancer evolution. Just as likely, less CE (just how much less is unknown but assuming in the dose-response range) would have decreased CE carcinogenicity because of less coupling to the reaction sequence (mode of action) causing cancers. Thus, 7070 mg/m^3 can be a crude estimate of the MTD for CE.

It has been found for most chemicals tested so far, that certain dosimetric relationships exist among the parameters MDT, TD_{50}, and the10^{-6} Risk Dose even though the TD_{50} (potency) varies over eight orders of magnitude among the chemicals (Gaylor and Gold, 1995; Krewski et al., 1993; Bernstein et al., 1985; Shlyakhter et al., 1992). The Risk Dose is a "low" dose on the curve presumed to be in the linear range. One of these relationships is that k = (MTD)/(10^{-6} Risk Dose) ≈ 740,000 where "k" a geometric average of 317 diverse structured chemicals with only 14 falling outside a 10-fold interval, i.e, an approximate constant (k) exists among different chemical (for further details see Appendix). Thus, 10^{-6} Risk Dose ≈ MTD/740,000. Because cancer slope = incidence/dose, then slope = (10^{-6} incidence)/(10^{-6} Risk Dose), hence one may estimate an upper limit simulating the LMS q_1* linear cancer slope value (Gaylor, 1989).

. The nonparametric estimation of 10^{-6} Risk Dose involves an *empirical* factor associated with the observed *median* (MDT)/(10^{-6} Risk Dose) ratio (i.e., k ≈ 740,000) (Gaylor and Gold, 1995). Thus, 10^{-6} Risk Dose ≈ 7,070 mg/m^3/740,000 = 9.55E-3 mg/m^3 (3.6E-3 ppm) (Table 14). For inhalation, the CE concentration of the10^{-6} Risk Dose is ≈1E-2 mg/m^3 or ≈ 4 ppb for a lifetime of CE exposure. Next, the unit cancer slope 95%UCL estimation is 10^{-6}/9.55E-3 mg/m^3 = 1.05E-4/mg/m^3. This method compares with the above nonparametric method (1.4E-4/mg/m^3) as well as estimated LMS slope of 4E-4/mg/m^3. These cancer unit slope values appear to be comparable within the limits of error of the methods—see below for reliability. Because the nonparametric methods did not attempt to model the datum, but rather used dosimetric relationships and comparison to 317 previously tested carcinogens, the nonparametric cancer slope estimates lend support to the LMS value 4E-4/mg/m^3.

The slope estimation using the10^{-6} Risk Dose may be compared with historical controls of mice and humans to estimate the Margin of Safety (MOS). Thus, an i = 10^{-6} is less than the NTP historical control incidence of 0.0029 in female B6C3F1 mice, hence i = 10^{-6} is conservative level in mice. However, i = 10^{-6} is somewhat less than the world-wide human incidence of 59 x10^{-6} (5.9 diagnosed cases of uterine cancer per 100,000 females), hence i = 10^{-6} is somewhat conservative in humans. Because the spontaneous frequency of uterine cancer is normally low among female B6C3F1 mice but higher than the assumed 10^{-6} risk, the nonparametric method using k = 740,000 is sufficiently conservative in the mouse by 29,000-fold (2.9E-3/1E-6). Thus, even though there is

uncertainty in the MTD estimation, and hence the 10^{-6} Risk Dose derived from it, there is an ample margin of safety (MOS) of 29,000 that suggests that inhalation exposures yielding $\leq 10^{-6}$ Risk Dose levels of CE will not likely add to, or exceed, the spontaneous levels of uterine cancers in rodents. However, humans apparently have some MOS at $i = 10^{-6}$: $59x10^{-6}/10^{-6} = 59$. The human MOS is less than the rodent MOS because the human cancer rates are normally less than the rodent rates so a smaller numerator in the preceding calculation. Human exposures are concerning too because uterine cancers are relatively common being the 7th highest cancer occuring in females world-wide. So, CE carcinogenicity could add to background.

In conclusion, all three upper-bound cancer slope estimates should be considered uncertain because of the one-point bioassay on which they are based. It is reasoned that a numerical assessment is prudent, however, because of the striking animal response ($i = 0.9$), low spontaneous occurrence of uterine tumors in mice, carcinogenic SAR of BE and 1,2-dichloroethane at the same organ site, and metabolic comparisons to CM. Notwithstanding, the development of a cancer potency estimate does not effect the qualitative assessment of CE carcinogenicity (section 6.3, below).

6. CHARACTERIZATION OF ASSESSMENTS

6.1. ORAL RfD

An acute oral CE palatability study was conducted in F344 rats (Pottenger et al., 1995). F344 rats were administered either 0 or 0.57 g/100 g water for 7- or 14-days. This is at the practical solubility limit of CE in water at room temperature. Toxicology parameters investigated were comparable between treated and control groups. The acute NOEL of CE in water then is 297 mg/kg bw/day for male rats and 361 mg/kg bw/day for female rats.

A necessary chronic oral study was not located to set a RfD for CE, therefore a oral RfD cannot be estimated at this time.

6.2. INHALATION RfC

The CE inhalation RfC of 1E0 mg/m^3 is based on a Dow Chemical Co. teratology study (Scortichini et al., 1986). There is low to medium confidence in this study as this is a fetotoxic effect only in the high-dose group (4,946 ppm CE). Most noncancer effects in the CE database occurred at the higher exposure level tested of 15,000 ppm CE (with none lower than 4,946 ppm or in between). Increases in menstrual periods, decreases in uterine weight, and uterine cancers (see below) are effects (at 15,000 ppm) may support a hormonal mode of action possibly related to fetal development, but changes in blood estrogen and progesterone were examined and were not observed. So the hormonal issue is unresolved. The basis of the single fetotoxic effect in the mouse skull (delayed foramina closure) is not understood.

6.3. CANCER ASSESSMENT

The CE carcinogenic response is highly specific to the female mouse uterus @ 15,000 ppm CE compared to a low spontaneous uterine cancer incidence in concurrent controls (0%) and historical controls (0.29%). The CE mouse uterine response compares the average historical control rate 88%/0.29% = 303-fold, a large increase in incidence that is unlikely due to chance. The significantly increased mouse uterine cancer response to CE seems to be biologically relevant because U.S. uterine historical control rates in humans is relatively common in North America (15.01/100,000) which is about $1/6^{th}$ the breast cancer incidence, the most common, and ½ the incidence of female lung cancer, the 2^{nd} most common, in the same region. Thus, CE exposure

could add to ongoing the uterine cancer rate in the human population. On the other hand, the female rat in the NTP bioassay is not affected with these tumors @ 15,000 ppm CE, only borderline astrocytomas. Bromoethane—a close chemical analogue to CE—supports the mouse CE carcinogenic uterine response in that it too causes uterine tumors in B6C3F1 female mice (i = 28/48 = 0.58 @ 400 ppm). The CE-treated mice present an exceptionally large (i = 43/49 = 0.88 @ 15,000 ppm) uterine cancer response, or any other organ site response for that matter, compared to other chemical carcinogens that the Agency has reviewed to date. The degree of carcinogenicity is exceptional too in that the primary tumors are very aggressive, metastasizing to 16 diverse organ sites in female B6C3Fi mice and killing them early due to tumor load.

By comparison to mouse historical controls, then, chemical carcinogenesis from CE may be inferred in humans, but in comparison to rats it may not. It is Agency policy to assume the worst case until a sufficient mode of action is known that may delineate between the test species. Thus, the human applicability is not assured but a concordant cancer hazard in humans is inferred by the Agency from the powerful mouse responses to CE and BE in the uterus.

Therefore, outlining the elements of CE's carcinogenicity:

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(1) Exceptionally strong cancer incidence in the female mouse uterus (and some liver cancers). Uterine cancers progressed from the endometrium to the adjacent myometrium and from there to 16 secondary malignant organ sites, and female B6C3F1 mice were killed early due to tumor load,

(2) Structural analogues BE and 1,2-dichloroethane cause similar uterine cancer responses,

(3) CE metabolizes similarly to CM: saturation of reductive GSH metabolism and induction of excessive P-450 oxidative metabolism at high CM. Because CM causes renal cortex cystadenomas and adenomas and papillary cystadenocarcinomas in male mice and because CE causes uterine and liver cancers in female mice, the carcinogenicity of both may be linked to their metabolic similarity.

(4) CE's mutagenicity evidence and the prospect that CE can be an alkylating agent under the correct activating conditions.

Thus, because of the striking mouse cancer response, similarity to BE and EDC chemical carcinogenesis, uncommon occurrence of the tumor type, mutagenic and potential alkylating properties, CE exceeds a C Category weight of evidence usually reserved for one-species responses. The weight-of-evidence supports the choice of B2 carcinogenicity classification for CE,

i.e., *a probable human carcinogen* based on no evidence in human beings and sufficient evidence for carcinogenicity in animals (U.S. EPA, 1986). CE is a *likely carcinogen by the inhalation route of exposure* using the *Proposed Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 1996).

Confidence in the carcinogenic categorization is medium based on: (1) the high incidence of uterine tumors in female B6C3F1 mice but none in F344 female rats, (2) the aggressive nature of the cancer proliferation from the endometrium to the myometrium then to many secondary cancers, (3) comparably low historical control rates in mice, (4) the consistency in tumorigenic responses between CE and its structural analogues, BE and EDC, and (5) the metabolic comparison to CM and CE that relates to GSH conjugation and P-450 oxidation, which could relate to CE and CM toxicity and "coupled" carcinogenicity. The mechanistic coupling is shown in the parentheses:

[CE or CM →→toxicity (biochemical and cellular steps in time) →→ carcinogenicity]

The coupling relates exposure and toxicity to a mode of action via a kinetics model. In time, a loss of cellular growth control results. The coupling reactions for CE are not certain at this time. Remaining data gaps include: (1) the lack of dose-response data sufficient to determine the tumor incidence rate at intermediate CE exposure levels (100–4,500 ppm CE), (2) the absence of any detailed information on the triggering site or the target organ-specific biochemical processes that link CE exposure, or intermediate, and response, and (3) any hormonal link that may explain the mouse uterine tumors from CE and BE and whether this applies to humans, (4) specific comparisons of mouse and human metabolic patterns and kinetic for CE.

6.4. CHARACTERIZATION OF HAZARD EXPECTED UPON HUMAN EXPOSURE TO CHLOROETHANE

The most robust toxic effect of CE is the inhalation malignant cancer effect observed in female B6C3F1 mice @ 15,000 ppm, not the noncancer fetotoxic effect @ 4,900 ppm or the 10% weight loss or 35% uterine weight loss @ 15,000 ppm (Table 10). Chronic CE oral toxicity studies are lacking,

Table 14. Comparison of noncancer and cancer hazard evaluations

Critical qualitative effect		Dose [a] (ppm)	Qualitative assessment factors	Cancer or Non-cancer Potency Estimation	
				Method of Estimation [b]	Reference doses [c]
NON-CANCER	Delayed fetal foramina closure (DFFC) in CF-1 mice skulls	4,946	Weak to mild fetotoxic effect; 1-point response; threshold; medium confidence	RfC = NOAEL/(10 × 10 × 3 x 10)] RfC = 3,970/3,000 = 1.32 mg/m^3 (0.50 ppm CE); RfC = 1E0 mg/m^3	RfC = 1.32 mg/m^3 RfC = 1E0 mg/m^3 (500 ppb)
				BMC = 4,028 ppm @ 10%; BMLD = 1,606 ppm RfC = BMLD/3,000 = 1.4 mg/m^3 = 1E0 mg/m^3	RfC = 1.4 mg/m^3 RfC ≈ 1E0 mg/m^3 (535 ppb)
CANCER	Uterine cancer production with subsequent aggressive metastasis, and finally, death due to tumor load	15,000	Strong effect in B2C3F1 mice; malignancy; death due to cancer burden; BE, an analogue of CE, causes similar cancer pattern; category B2; high confidence	*LMS method* **q_1* = inhalation cancer slope = 1E-4/ mg/kg/day** (using Global86 & assuming linearity in the low dose range)	10^{-6} Risk Dose = 1E-4 mg/kg/day (≈ 1 ppb)
				LMS method **cancer unit risk = 4E-4/ mg/m^3** (using Global86 & assuming linearity in the low dose range)	10^{-6} Risk Dose = 2.5E-3 mg/m^3 (≈ 1 ppb)
				Nonparametric method 1 95%UCL incidence on lowest significant point above controls is ≈ 1.0: slope ≈ 1/7070 mg/m^3 [d] **cancer slope = 1.4e-4**/mg/m^3	10^{-6} Risk Dose = 0.7E-2 mg/m^3 (≈ 3 ppb)
				Nonparametric method 2 10^{-6} Risk Dose = MDT [d] divided by k, where k 7.4E+5. Thus, 10^{-6} Risk Dose = 9.95E-3 mg/m^3 cancer slope ≈ 10^{-6}/9.95E-3 mg/m^3 =1.05E-4/mg/m^3 **cancer slope = 1E-4/mg/m**	10^{-6} Risk Dose = 1E-2 mg/m^3 (≈ 4 ppb)

[a] Lowest effect dose; however, in both noncancer and cancer experiments only one dose group produced a critical response.

[b] Details and assumptions of the calculations should be referred to in the text of the document. Conversion: 2.64 mg/m^3/1 ppm CE. Cancer slopes for the LMS and nonparametric methods are given with the cancer calculations and are presented here in bold.

[c] These are reference doses and not implied to be safe in the case of carcinogenic effects; it has been U.S.EPA policy to assume no safe dose exists for carcinogens. For the noncancer effects a threshold may be assumed so an RfC is presented.

[d] Mouse exposure = 15,000 ppm chloroethane gas for 6 hrs/day and 5 days/wk. for 100 wks. adjusted to 7,070 mg/m^3 (human) see text for assumptions.

but acute studies suggest a lack of toxicological activity of oral CE dissolved in water. Firstly, hazard by CE inhalation is caused by GSH pool depletions: this may not only cause more CE exposure to be metabolized improperly but also other impending or extant carcinogens in the body at the time of CE exposure. This GSH depletion is followed by excessive production of oxidants, such as acetaldehyde, which often can not be eliminated fast enough to prevent initiation and promotion of cancer events. This decreased protection by reductive conjugation and unresolved oxidation is the likely mode of action. Specific kinetics of these elimination reactions is lacking in the CE database. The carcinogenic process is likely indirect, however, because CE itself does not seem to accumulate in the mouse uterus.

The absorption and distribution of CE appears to be nonlinear in the female B6C3F1 mouse at high doses (Dow Chemical Co., 1992). The system seems metabolically saturated for GSH conjugation at 15,000 ppm and oxidation, a lesser used pathway for halohydrocarbons, is likely expressed in addition to reductive conjugation. The net catabolism via reduction plus oxidation is likely not linear in CE exposure, but the true net curvature is unknown. There is a decrease in noncancer effects below 15,000 ppm to quantitatively weak effects, like the fetotoxic effect and body weight loss, and little other remarkable toxic effects. All the toxicity evidence for CE suggests that the slope from an anesthetic dose (19,000 ppm), to a carcinogenic dose (15,000 ppm), to mild fetotoxicity (5,000 ppm), is a steep slope of biological interactions. This sharp declination suggests a lack of toxicological activity at lower CE exposures, but because of a paucity of information this cannot be demonstrated. The historic use of short-term human gaseous anesthetic doses, up to 40,000 ppm, as well as dermal topical applications for temporary pain relief has not produced evidence of chronic toxicity, though a systematic study has not been done either.

It has not been demonstrated if CE actually causes human uterine cancer, or any human cancers for that matter. It is notable that CE spray has been used as a human topical anaesthetic. For example, in contact sports in the United States, CE has been used in considerable amounts to temporarily alleviate pain. Also veternarians have until recently used CE sprays for topical animal surgeries. None of these uses have produced reports of adverse effects. This does not mean there are no topical carcinogenic responses, merely that none have been reported. Nonetheless, a human cancer hazard is thought likely from CE chronic inhalation exposure on the basis of rodent studies.

The cancer unit risk for CE is $q_1^* = 4E\text{-}4/mg/m^3$. This cancer unit risk is based on an upper bound estimate but the true unit risk could be less, even down to zero. The use of this unit risk has uncertainty based on limited cancer and mode-of-action data and not having a CE PBPK model. Given these limitations, an upper limit cancer risk to a population with chronic exposure may be approximated from $P(d) = q_1^*$ d which can be restated as risk = 4E-4 d where “d”is exposure in mg/m^3 over a 70-year lifetime. Limited or intermittent CE exposures can be evaluated on a case-by-case basis.

7. REFERENCES

Adams, E; Rowe, V; Spencer, H. (1939) Experimental investigation of the toxicology of ethyl chloride. Dow Chemical Co. as cited by Landry: Fundam Appl Toxicol 2:230-234.

Allen, BC; Crump, KS; Shipp, A. (1988) Correlation between carcinogenic potency of chemicals in animals and humans. Risk Anal 8(4):531-544.

Allen, BC; Kavlock RJ; Kimmel, CA; et al. (1994a) Dose response assessments for developmental toxicity: II. Comparison of generic benchmark dose estimates with NOAELs. Fundam Appl Toxicol 23:487-495.

Allen, BC; Kavlock RJ; Kimmel, CA; et al. (1994b) Dose response assessments for developmental toxicity: III. Statistical models. Fundam Appl Toxicol 23:496-509.

Andersen, EL. (1983) Quantitative approaches in use to assess cancer risk. Risk Anal. 3:277-295.

Araki, A; Noguchi, T; Kato, F; et al. (1994) Improved method for mutagenicity testing of gaseous compounds by using a gas sampling bag. Mutat Res 307:335-344.

ATSDR (Agency for Toxic Substances and Disease Registry). (1997) Toxicological Profile for Chloroethane. Public Comment Draft. U.S. Department of Health and Human Services. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

Behrens, UJ; Hoerner, M; Lasker, JM; et al. (1988). Formation of acetaldehyde adducts with ethanol-inducible P-450IIE1 in vivo. Biochem Biophys Res Comm 29:584-590.

Bernstein, L; Gold, LS; Ames, BN; et al. (1985) Some tautologous aspects of the comparison of carcinogenic potency in rats and mice. Fundam Appl Toxicol 5(1):79-86.

Bircher, AJ; Hampl, K; Hirsbrunner, P; et al. (1994) Allergic contact dermatitis from ethyl chloride and sensitization to dichlorofluoromethane. Contact Dermatitis 31:41-44.

Bolt, HM; Gansewendt, B. (1993) Mechanisms of carcinogenicity of methyl halides. Crit Revs Toxicol 23:237-253.

Breslin, WJ; Berdasco, NM; Phillips, JE; et al. (1988) Ethyl chloride (EtCl): effects on estrous cycling in B6C3F1 mice. Final report with cover letter dated 11/21/88. Dow Chemical Co. EPA Document #86-890000040.

Bucher, JR; Morgan, DL; Adkins, BJR; et al. (1995) Early changes in sex hormones are not evident in mice exposed to the uterine carcinogens chloroethane and bromoethane. Toxicol Appl Pharmacol 130:169-173.

Bush, OF; Bittenbender, G; Adriani, J. (1952) Electrocardiographic changes during ethyl chloride and vinyl ether anesthesia in the dog and man. Anesthesiology 13:197–202.

Chellman, GJ; White, RD; Norton, RM; et al. (1986) Inhibition of the acute toxicity of methyl chloride in male B6C3F1 mice by glutathione depletion. Toxicol Appl Pharmacol 86:93-104.

Cole, WH. (1967) A re-evaluation of the pharmacology of ethyl chloride. Med J. Aust 1:853-855.

Commandeur, J; Stijntjes, G; Vermeulen, PE. (1995) Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Pharmacol Rev 47(2):271-330.

Crump, K. (1982) An improved procedure for low-dose carcinogenic risk assessment from animal data. J Environ Pathol Toxicol 5(4/5):339-348.

Crump, KS. (1996) The linearized multistage model and the future of quantitative risk assessment. Hum Exper Toxicol 15:787-798.

Dobkin, AB; Byles, PH. (1971) Pharmacodynamics of divinyl ether, ethyl chloride, fluroxene, nitrous oxide and trichloroethylene. Textbook Vet Anaesth 94-104.

Dodd, DE; Bus, JS; Barrow, CS. (1982) Nonprotein sulfhydryl alterations in F-344 rats following acute methyl chloride inhalation. Toxicol Appl Pharmacol 62:228-236.

Dow Chemical Co. (1985) Ethyl chloride: inhalation teratology probe in CF-1 mice (final report) with cover letter dated 102591 (sanitized). EPA Document #88-920000184S.

Dow Chemical Co. (1992) Disposition and metabolism of female F344 rats and B6C3F12 mice following inhalation exposure with cover letter dated 012893. EPA document #86-930000120.

Ebert, R; Fedtke, N; Certa, H; et al. (1994) Genotoxicity studies with chloroethane. Mutat Res 322:33-44.

E.I. Dupont de Nemours and Co. (1971) Initial submission: Cardiac sensitization by inhalation exposure in dogs with cover letter dated 101592. Document #88-920009728.

Fedtke, N; Certa, H; Ebert, R; et al. (1994a) Species differences in the biotransformation of ethyl chloride. I. Cytochrome P450-dependent metabolism. Arch Toxicol 68:158-166.

Fedtke, N; Certa, H; Ebert, R; et al. (1994b) Species differences in the biotransformation of ethyl chloride. II. GSH-dependent metabolism. Arch Toxicol 68:217-223.

Gargas, ML; Seybold, PG; Andersen, ME. (1988) Modeling the tissue solubilities and metabolic rate constant (Vmax) of halogenated methanes, ethanes and ethylenes. Toxicol Lett 43:235-256.

Gargas, ML; Burgess, RJ; Voisard, DE; et al. (1989) Partition coefficients of low-molecular weight volatile chemicals in various liquids and tissues. Toxicol Appl Pharmacol 98:87-99.

Gargas, ML; Clewell, HJ; Andersen, ME. (1990) Gas uptake inhalation techniques and the rates of metabolism of chloromethanes, chloroethanes, and chloroethylenes in the rat. Inhal Toxicol 2:295-319.

Gaylor, DW. (1989) Preliminary estimates of the virtually safe dose for tumors obtained from the maximum tolerated dose. Reg Toxicol Pharmacol 9:101-108.

Gaylor, DW; Kodel, RL. (1980) Linear interpolation algorithm for low dose risk assessment of toxic substances. J Environ Path Toxicol 4:305-312.

Gaylor, DW; Gold, LS. (1995) Quick estimate of the regulatory virtually safe dose based on the maximum tolerated dose for rodent bioassays. Regul Toxicol Pharmacol 22:57-63.

Gohlke, JM; Scmidt, P. (1972) Subacute action of low concentrations of chlorinated ethanes on rats with and without additional ethanol-treatment. II. Histological, histochemical and morphometrical studies. Int Arch Arbeitsmed 30:298-312.

Gold, LS; Slone, TH; Bernstein, L. (1989) Summary of carcinogenic potency and positivity for 492 rodent carcinogens in the Carcinogenic Potency Database. Environ Health Perspect 79:259-272, cf. Table 3.

Goodman, G; Wilson, R. (1991) Predicting the carcinogenicity of chemicals in humans from rodent bioassay data. Environ Health Perspect 94:195-218.

Griesemer, RA; Eustis, SL. (1994) Gender differences in animal bioassays for carcinogenicity. J Occup Med 36:855-859.

Haid, B; White, JM; Morris, LE. (1954) Observations of cardiac rhythm during ethyl chloride anesthesia in the dog. Curr Res Anesth 33:318–325.

Hattori, L. (1957) Effects of inhalation of anesthetics on the gaseous metabolism in rats. Nippon Yakwigaku Zasshi 53:136–144.

Heller, S; et al. (1966) Changes in the histochemical pattern of rat liver in prolonged narcosis. Folia Morphol 25:9–20 (1966).

Henderson, VE. (1930) Aesthetic toxicology. Arch Int Pharmacodyn Ther 38:150-165.

Hes, JP; Cohn, DF; Streifler, M. (1979) Ethyl chloride sniffing and cerebellar dysfunction (case report). Isr Ann Psychiatr Relat Discip 17:122-125.

Hinko, M. (1934) The effect of some narcotics upon the sedimentation of red blood cells. Tohoku J Exp Med 23:279-297 (Japanese).

Hiraiwa, K. (1952) Effect of narcotics on the flexion reflex on rats. Osaka Daigaku lgaku Zasshi 5:95–100 (Japanese).

Holder, JW. (1998) Chloroethane carcinogenicity (CAS No. 75-00-3). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, D.C. EPA/600/R-95/099. Available from : http://www.epa.gov/ncea and the National Technical Information Service, Springfield, VA, PB-98-143274.

International Agency for Research on Cancer. (IARC) (1992) Cancer incidence in five continents. Volume VI. IARC Scientific Publication No. 120, Lyon, France.

Ivanetich, KM; Van Der Honert, LH. (1981) Chloroethanes: their metabolism by hepatic cytochrome P-450 in vitro. Carcinogenesis 2:697-702.

Khan, S; O'Brien, PJ. (1991) 1-Bromoalkanes as new potent nontoxic glutathione depletors in isolated rat hepatocytes. Biochem Biophys Res Commun 179(1):436-441.

Kornbrust, DJ; Bus, JS. (1982) Metabolism of methyl chloride to formate in rats. Toxicol Appl Pharmacol 65:135-143.

Kornbrust, DJ; Bus, JS; Doerjer, G; et al. (1982) Association of inhaled [^{14}C]methyl chloride with macromolecules from various rat tissues. Toxicol Appl Pharmacol 65:122-134.

Krewski, D; Gaylor, D; Soms, A; et al. (1993) An overview of the report: Correlation between carcinogenic potency and the maximum tolerated dose: implications for risk assessment. Risk Anal 13 (4):383-398.

Landry, TD; Ayres, JA; Johnson, KA; et al. (1982) Ethyl chloride: a two-week inhalation toxicity study and effects on liver non-protein sulfhydryl concentrations. Fundam Appl Toxicol 2:230-234.

Landry, TD; Gushow, TS; Langvardt, PW; et al. (1983) Pharmacokinetics and metabolism of inhaled methyl chloride in the rat and dog. Toxicol Appl Pharmacol 68:473-486.

Landry, TD; Johnson, KA; Phillips, JE; et al. (1989) Ethyl chloride - 11-day continuous exposure inhalation toxicity study in B6C3F1 mice. Fundam Appl Toxicol 13:516-522.

Lawson, JI. (1965) Ethyl chloride. Br J Anaesth 37:667-670.

Lazarew, NW. (1929) Über die narkotische Wirkungskraft der Dämple der Chlorderivate des Methans, des Äthans und des Äthylens. Naunyn-Schmiedeberg's Arch Exp Pharmacol 141:19-24.

Monticello, T. M. and Morgan, K. T. (1997). Chemically-induced nasal carcinogenesis and epithelial cell proliferation: a brief review. Mutation Res. Fundamental and Molecular Mechanisms of Mutagenesis 380: 33-41.

Morgan, A; Black, A; Belcher, DR. (1970) The excretion in breath of some aliphatic halogenated hydrocarbons following administration by inhalation. Ann Occup Hyg 13:219-233.

Morgan, K. T. (1997). A brief review of formaldehyde carcinogenesis in relation to rat nasal pathology and human health risk assessment. Toxicologic Pathology 25: 291-307.

Morris, JB. (1997) Dosimetry, toxicity and carcinogenicity of inspired acetaldehyde in the rat. Mutation Res. Fundamental and Molecular Mechanisms of Mutagenesis 380:113-124.

Morris, L; Noltensmeyer, MH; White, JM. (1953) Epinephrine induced cardiac irregularities in the dog during anesthesia with trichloroethylene, cyclopropane, ethyl chloride, and chloroform. Anesthesiology 14:153–158.

Neal, MJ; Rodson, JM. (1964) The analgesic properties of subanesthetic doses of anesthetics in the mouse. Br J Pharmacol Chem 22:596–603.

NTP (National Toxicology Program). (1978) Bioassay of 1,2-dichloroethane for possible carcinogenicity. NTP TR No. 55. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

NTP. (1989a) Toxicology and carcinogenesis studies of chloroethane (ethyl chloride) (CAS No. 73-00-3) in F344/N rats and B6C3F1 mice (Inhalation Studies). NTP TR No. 346. U.S. Department of Health and Human Services, National Institutes of Health, Research Triangle Park, NC.

NTP. (1989b) Toxicology and carcinogenesis studies of bromoethane in F344/N rats and B6C3F1 mice. NTP TR No. 363. U.S. Department of Health and Human Services, National Institutes of Health, Research Triangle Park, NC.

Nordin, C; Rosenqvist, M; Hollstedt C. (1988) Sniffing of ethyl chloride — an uncommon form of abuse with serious mental and neurological symptoms. Int J Addict 23:623-627.

Parkin, D.M; Pisani, P.; Ferlay, J. (1999) Global cancer statistics. CA 49 (1): 33-64.

Pottenger, LH; Nieusma, JL; Landry, TD; et al. (1992) Ethyl chloride: disposition and metabolism in female Fischer 344 rats and B6C3F1 mice following inhalation exposure. Part A. Dow Chemical Co. R & D Report. EPA document #86-930000120.

Pottenger, LH; Cosse, P; Quast, J; et al. (1995) Ethyl chloride: palatability and 14-day drinking water toxicity study in Fischer 344 rats. Dow Chemical Co. R & D Report. EPA Document Number: 86-99000022.

Reinhardt, CF; Azar, A; Maxfield, ME; et al. (1971) Cardiac arrhythmias and aerosol “sniffing.” Arch Environ Health 22:265–279.

Sayers, RR; et al. (1929) Physiological response attending exposure to vapors of methyl bromide, methylene chloride, ethyl bromide, and ethyl chloride. U.S. Public Health Bulletin No. 185. U.S. Government Printing Office, Washington, DC.

Schmidt, P; Binneweis, S; Gohlike, R. (1972) Subacute action of low concentrations of chlorinated ethanes on rats with and without additional ethanol treatment. I. Subacute and chronic toxicity studies with 1, 1,2,2-tetrachloroethane. Int Arch Arbeitsmed 38:283-298.

Scortichini, BH; Johnson, KA; Momany-Pfruender, JJ; et al. (1986) Ethyl chloride: inhalation study in CF-mice. Dow Chemical Co. EPA Document #86-870002248.

Seller, C. (1938) Action of hypnotics. Arch Exp Pathol Pharmakol 188:699–713.

Shlyakhter, A.; Goodman G.; Wilson, R. (1992). Monte Carlo Simulation of Rodent Carcinogenicity Bioassays, Risk Analysis 12: 73-82.

Tokita, N. (1953) Influence of various narcotics on cerebral circulation. Tohoku J Exp Med 59:149–158 (Japanese).

Troshina, MN. (1966) Determination of maximum permissible concentration of ethyl chloride in the atmosphere of work premises. Gig Truda i Prof Zabolegania 10:37-42. [Russian with English abstract].

U.S. EPA. (1986a) Guidelines for carcinogenic risk assessment; notice. Federal Register 51:33992-34003.

U.S. EPA. (1986b) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014-34025.

U.S. EPA. (1986c) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006-34012.

U.S. EPA. (1988a) Recommendations for and documentation of biological values for use in risk assessment. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, for the Office of Solid Waste and Emergency Response, Washington, DC. EPA/600/6-87/008.

U.S. EPA. (1988b) Summary review of health effects associated with monochloroethane: health issue assessment. Environmental Criteria and Assessment Office. Office of Health and Environmental Assessment. Research Triangle Park, NC. EPA/600/8-88/080.

U.S. EPA. (1991) Guidelines for developmental toxicity risk assessment. Federal Register 56:63798-63826.

U.S. EPA. (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity: notice of availability. Federal Register 59(206):53799.

U.S. EPA. (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Prepared by the Office of Health and Environmental Assessment, Research Triangle Park, NC. EPA/600/8-90/066F.

U.S. EPA. (1994c) Peer review and peer involvement at the U.S. Environmental Protection Agency. Signed by the U.S. EPA Administrator, Carol M. Browner, dated June 7, 1994.

U.S. EPA. (1995a) Proposed guidelines for neurotoxicity risk assessment. Federal Register 60:52032-52056.

U.S. EPA. (1995b) The use of the benchmark dose approach in health risk assessment. Prepared by the Risk Assessment Forum, Washington, DC. EPA/630/R-94/007.

U.S. EPA. (1996a) Proposed guidelines for carcinogenic risk assessment. Federal Register 61:17960-18011.

U.S. EPA. (1996b) Reproductive toxicity risk assessment guidelines. Federal Register 61(212):56274-56322.

U.S. EPA. (1997) Risk characterization: a practical guidance for NCEA-Washington risk assessors. (draft). Prepared by the Office of Research and Development, Washington, DC.

U.S. EPA. (1998a) Bench mark dose software. Beta version 1.1b. Developed under the direction of Dr. Jeff Gift, U.S. EPA/HERL Research Triangle Park, NC., (919)541-4828.

U.S. EPA. (1998b) Science Policy Council Handbook: Peer Review. Office of Science Policy, Office of Research and Development, Washington, DC; EPA/100/B-98-001. Available from http://www.epa.gov/ncepihom/catalog/EPA100B98001.html.

U.S. EPA. (1998c) Integrated Risk Information System. Online. Office of Health and Environmental Assessment, National Center for Environmental Assessment, Cincinnati, OH.

Van Dyke, RA; Wineman, CG. (1971) Enzymatic dechlorination: dechlorination of chloroethanes and propanes *in vitro*. Biochem Pharmacol 20:463-470.

Van Liere, EJ; Mazzocco, TR; Northrup, DW. (1966) The effect of cyclopropane, trichloroethane, and ethyl chloride on the uterus of the dog. Am J Obstet Gynecol 94(6):861-874.

Zeiger, E; Anderson, B; Haworth, S; et al. (1992) Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Mol Mutagen 19(Suppl. 21):2-141.

8. APPENDIX

Nonparametric Maximum Tolerated Dose Method of Cancer Potency Estimation

An alternative method to fitting parametrically a number of dose response points was sought because of the uncertainty inherent in a one dose point bioassay. Although dose-response incidence points of a carcinogen usually can be parametrically fitted, it is problematic to extrapolate very far from the actual experimental points down to environmental exposures. This has been the subject of a number of model fitting methods and proposals in the last 20 years. It also has been suggested that a nonparametric technique could be used that would nonpresumptively assess cancer potency at environmental exposures "d" that were much less than the experimental exposures d_e (Gaylor and Kodel, 1980). A nonparametric method of interpolation may be used where the lowest experimental dose (d_e) that is significantly increased, statistically and biologically, may be determined. A 95%UCL of the incidence point may be estimated at d_e. This 95%UCL point is connected to the origin to create a straight line which has been interpreted as an estimate of the upper bound limit on risk (Gaylor and Kodell, 1980). Because no threshold is assumed in $P \propto f(d_e)$, this method allows risk estimates even at low doses. This interpolation method of Gaylor and Kodell was found to agree with a multistaged Armitage-Doll model estimation of upper bound potency for a number of chemicals (Gaylor and Kodell, 1980). It was one of the first demonstrations of a nonparametric extrapolation method to assess risk at environmental doses.

Most continuous response curves can be parametrically fit. The Agency fits the experimental points with a polynomial function [$P(d) = b_0 + q_1d + q_2d^2 + q_3d^3\ q_4d^4$ ...] employed by Global86 software. In the low dose region linearity of dose and response is assumed and thus the 95% UCL of the q_1 coefficient is determined (the latter terms are so small that they can be ignored) and is called the q_1*. P(d) in the low range is estimated by q_1* • (exposure). There is one exception to being able to "fit' the curve, and that is when the bioassay has only one dose point (Gaylor and Kodel, 1980). Gaylor and Kodel (1980) have stated: "In the special case where only one dosage level of a chemical is administered to animals, obviously no mathematical model can be obtained." It seems prudent, then, to seek a nonpresumptive method to estimate risk that differs from the LMS method in methodology.

Considering the current CE one dose case (inhalation study at 7,070 mg/m^3 and a concurrent control), the degrees of freedom are n –1 = 2 – 1 = 1, a straight line. The more the degrees of freedom for a data set, the more power or sensitivity it possesses. A two-point set, concurrent control and one experimental point, has low power and low sensitivity to accurately detect a specific response. Of course, if the one point of the bioassay is not duplicated or

replicated, the true variance is not known and the precision is unknown. In such a case where there is just one d_e and one d_0 (concurrent control), a low-dose interpolation method has been suggested (Gaylor and Kodel, 1980). For example, at the 90% response point for CE, if one assumes ~100% as the 95% UCL on a 90% response rate, then cancer unit slope ≃ 1.0/7,070 mg/m^3 = 1.4E-4/mg/m^3. It is notable this agrees with LMS and this is expected since the LMS is essentially doing the same type of calculation.

Some time ago it was suggested that (1) not only may MDTs (maximum dose tested in a bioassay) correlate between rats and mice, but also (2) that the MDTs for a given chemical seem to correlate (in a 30-fold range) with the respective cancer potencies (Bernstein et al., 1985). The cancer potency is abbreviated as TD_{50} and is used as a "midpoint" to characterize a cancer dose-response curve. The TD_{50} is defined as the average daily dose (mg/kg bw/day) *rate* that is estimated to halve the probability of remaining tumor free at a specified organ site in a 2 yr. study. The TD_{50} varies over a10^8 range of dose for the various chemicals bioassayed so far.
The first finding suggests that MDT ≃ MTD = maximum dose tolerated in a 90-day study for both rodent species. These rodent correlations suggest human parameters may also relate in a parallel manner (Allen et al., 1988). The second finding suggests that knowing the MDT for a chemical may allow an estimation of TD_{50} (cancer potency).

Further, for a given chemical, the TD_{50} seems to correlate with TD_{10}, TD_{01}, and even in the low range the $TD_{0.0001}$ (Gaylor, 1989). The latter is the dose at 10^{-4} % incidence or $1:10^6$ which is sometimes referred to as the virtually safe dose (VSD). The U.S. EPA makes no value judgement at 10^{-6} incidence (risk) as being virtually safe or not. Here, we make use of that point as a reference point on the continuous dose-response curve in the low-dose and linear range. It has been suggested, additionally, that the q_1* (cancer slope) varies inversely as the log (TD_{50}) (Gaylor and Gold, 1995). These relationships are summarized in Table 15 and developed further below.

Table 15. Chemical-specific dose parameters

MTD from 90-day study	MDT (chronic) ≃ MTD	MDT $\propto TD_{50}$
$TD_{50} \propto 10^{-6}$ Risk Dose	MDT $\propto 10^{-6}$ Risk Dose	check: 10^{-6} Risk Dose $\propto 1/q_1$*

Note: These relationships are characterized in Gaylor and Gold, 1995; Krewski et al., 1993; Bernstein et al., 1985. Specific constants relating these parameters may found in these references. A log-normal distribution is assumed.

In an expanded study including 69 tumor sites and 38 chemicals for rats and mice (138 cases) chosen for their varied chemical structures, empirical extrapolation from MDT to TD_{50} and then to 10^{-6} Risk Dose correlated with the LMS model estimates of 10^{-6} Risk Dose at 10^{-6} risk

(Gaylor, 1989). The Gaylor empirical method found (MDT)/(10^{-6} Risk Dose) = k where the empirical constant k was first estimated to be 340,000 (geometric mean of the 138 cases). Only 3 of 138 cases were extreme, i.e., over 10-fold different from the geometric mean of the ratio. Because cancer potencies are known to vary over eight orders of magnitude, the relative constancy of (MTD)/(10^{-6} Risk Dose) ratio for a number of chemicals suggests that once the MTD is estimated in a 90-day study, the 10^{-6} Risk Dose may be approximated also.

Further analytical work examined and confirmed the properties of the empirical inverse function of the upper bound on the low dose slope (b) and the MDT (Krewski et al., 1993). The cancer slope "b" varies inversely with MTD: log (b) = (0.01 ± 0.05) – (1.05 ± 0.03) log (MDT). These authors also showed the TD_{50} is also related to the MDT: log (TD_{50}) = (1.04 ± 0.02) log (MDT) – (0.10 ± 0.04). It is clear, then, because TD_{50} and b are related to MDT, that TD_{50} and b are related to each other: log (MDT) = 0.961 log (TD_{50}) + 0.0673. A comprehensive study compiling 139 carcinogens from the NCI database showed the geometric mean of the MDT/TD_{50} ratio is 0.919 for mutagens and 0.764 for nonmutagens (Gaylor and Gold, 1995). That is, the TD_{50} values tend to be greater than the MDTs. Variance for 78% of the 139 chemicals is within a factor of 4-fold and for 98% of them variance is within a factor of 10-fold. From the open literature, representing a more diverse set of chemicals, the MDT/TD_{50} ratios are 1.46 and 0.951 (Gaylor and Gold, 1995). These ratios suggest, in these approximate measures, that MDT/TD_{50} ratios are constant for most dose-response curves. Also suggested is that mutagens and nonmutagens do not differ significantly. The variation of the MDT/TD_{50} ratio is similar to the variation in cancer potency (1/TD_{50}), thus the MTD (90-day study) is a reasonable surrogate for the TD_{50} (chronic study) (Gaylor and Gold, 1995). It follows that at 10^{-6} risk with a corresponding dose of 10^{-6} Risk Dose, "k" may be estimated by the ratio (MDT)/(10^{-6} Risk Dose) for a given chemical. When a larger number of chemicals (317) are considered, the geometric mean constant "k" is 740,000, which happens to be larger (in fact two fold) than the above empirical constant. The change in "k" is likely because more chemicals were considered in this study, therefore 740,000 probably better represents the "geometric average k" (Gaylor and Gold, 1995). The geometric range of 10^{-6} Risk Doses may be estimated in the range of MDT/7,400,000 – MDT/74,000 from the most potent carcinogens ever assayed to the least potent carcinogens, i.e., the 10^{-6} Risk Dose range is a geometric variation of 10-fold around the mean value. Obviously, division by 7,400,000 yields the more conservative 10^{-6} Risk Dose estimate ($\leq 10^{-6}$ risk) and would be at the low end of 10^{-6} Risk Doses of chemicals previously tested. Human exposures ≤ MTD/10^7 have been proposed as negligible risk because it would be assumed the carcinogen is similar to the most potent measured (Gaylor, 1989).

The LMS method uses a polynomial fit to cancer dose-response data, and the coefficients and power depend on the data set. Because the NTP study has one experimental point (15,000

ppm), there are not enough degrees of freedom to find a proper fit (NTP, 1989a). Gaylor and Kodel (1980) have stated: "In the special case where only one dosage level of a chemical is administered to animals, obviously no mathematical model can be obtained." It seems prudent to use a another type of method to estimate risk; at least this approach backs up the default LMS method. It is estimated (see section 5.3.2.) by nonparametric method 2 that the 10^{-6} Risk Dose = 1E-2 mg/m^3 or 4 ppb (above) and the cancer slope estimated as a check is 1.05E-4/mg/m^3 (= 10^{-6}/1E-2 mg/m^3) for a lifetime of CE inhalation exposure. In Table 15 (last cell) it is indicated that 10^{-6} Risk Dose $\propto 1/q_1^*$, which indicates that the nonparametric method provides estimates similar to those made by the LMS method: 1.05E-4/mg/m^3 (*nonparametric #2*) $\approx$4E-4/mg/m^3 (*LMS*). Therefore the Gaylor method is as conservative as the LMS method estimation of chemical carcinogen risk. For further discussion see Gaylor and Gold (1995).

Doses calculated by the cancer slopes in Table 14 (LMS, nonparametric methods 1 and 2) at 10^{-6} risk (a arbitrary low level in the linear range) are1, 3, and 4 ppb CE. It is not implied that at lower environmental doses there is no risk from CE because a linear, no-threshold presumption is made for genotoxic carcinogens. The hazard of the 1–4 ppb range of CE does imply that the additive risk may be no more, and perhaps less, than 10^{-6} risk, and thus exposures up to 1–4 ppb are less than rodent uterine spontaneous cancer, which occurs at a rate of 2.9‰ incidence rate, by a factor of 29,000 (0.0029/10^{-6}). A human approximation of the margin of safety (MOS) may be made by dividing the 1997 frequency of U.S. uterine incidence of 15.01/100,000 North American females by 10^{-6}, which indicates a MOS of 150 in the U.S. Thus, continuous CE inhalation exposures $\leq$ 1–4 ppb likely do not add a significant risk to ongoing human uterine cancer from all causes.

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