



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.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

Chemical Manager/Author

James W. Holder, Ph.D.
National Center for Environmental Assessment–Washington Office
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC 20460

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Internal Reviewers¹

Jane Caldwell, Ph.D.
U.S. EPA
OAQPS
Research Triangle Park, NC 27709

Dennis Lynch, Ph.D.
Experimental Toxicology
NIOSH
Cincinnati, OH

Daniel L. Morgan, Ph.D.
Respiratory Toxicology
NIEHS
P.O. Box 12233
Research Triangle Park, NC 27709

Alberto Protzel, Ph.D.
OPPTS/OPP/HED
U.S. EPA
401 M St., S.W.
Washington, DC 20460

G. Daniel Todd, Ph.D.
Environmental Health Scientist
Toxicology Information Branch
ATSDR
Atlanta, GA

Collegial Reviewers¹

Femi Adeshina
NCEA-CIN/ORD/U.S. EPA
Cincinnati, OH 45268

Gary Foureman, Ph.D.
HPAG/NCEA-RTP/U.S. EPA
Research Triangle Park, NC 27711

Jennifer Jinot
NCEA-W/ORD/U.S. EPA
401 M St., S.W.
Washington, DC 20460

Gary Kimmel, Ph.D.
NCEA-W/ORD/U.S. EPA
401 M Street, S.W.
Washington, DC 20460

Jennifer Seed, Ph.D.
RAD/OPPT/U.S. EPA
401 M St., S.W.
Washington, DC 20460

¹The contributions and criticisms of all the reviewers are appreciated. Peer review of the IRIS support document (ToxR) was performed by Internal (U.S. Government) Reviewers listed on the left. These reviewers were selected without knowledge of the author of this document, whereas the collegial reviewers in the right column were invited because of their expertise on issues that were particularly of concern in characterizing chloroethane toxicology. Comments of all 10 reviewers were reconciled with no major outstanding issues.

LIST OF ABBREVIATIONS

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

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.

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/m³).

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 [(µg/L)⁻¹] drinking water or risk per microgram per cubic meter [(µg/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.

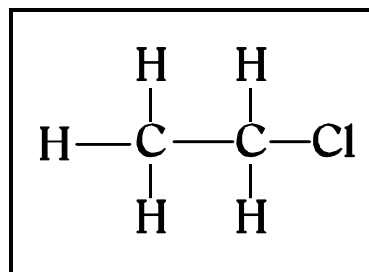


Figure 1. Chloroethane chemical structure.

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.

1 Hazard identification and dose-response assessment for CE follow the general risk
2 assessment principles for established by the National Research Council (1983).

3 EPA guidelines used in the development of this assessment include the following:

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

21
22

23
24 The literature search strategy for CE is based on the CASRN and at least one common
25 name, and includes the following databases: HEAST, RTECS, HSDB, TSCATS, CCRIS,
26 GENETOX, EMIC, EMICBACK, DART, TOXLINE, CANCERLINE, MEDLINE, and
27 MEDLINE backfiles. The current IRIS file for this chemical (U.S. EPA, 1998a) and the ATSDR
28 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	Vapor density: 2.22 (air = 1.0)
Empirical formula: C ₂ H ₅ Cl	water solubility: 5,710 mg/L
Structural formula: CH ₃ CH ₂ Cl	Melting point: -138.7°C
Molecular weight: 64.5	Boiling point: 12.3 °C at 760 mm Hg
Specific gravity: 0.897 (at 20°C)	Log K _{ow} : 1.43
Vapor pressure: 1,000 mm Hg (at 20°C)	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 (COCl₂), 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 ¹⁴C-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 ¹⁴C-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 ¹⁴CO₂ 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 ³⁸Cl-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 ³⁸Cl-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 ¹⁴C-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

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

5 At 150 ppm, female mice and rats, respectively, exhaled 42% and 54% of the counts as
 6 CO₂, 35% and 32% in the tissues and carcass, 16% and 10% in the urine, 6% and 3% in the feces,
 7 and < 2% in the breath as unchanged CE. So, at the low dose, a substantial portion of the inhaled
 8 CE input was metabolized by both species, and there were comparable counts in the various
 9 compartments in both species. These data support those of Morgan et al. (1970) by indicating
 10 that CE can be readily absorbed at the alveolar membrane.

11 However, with exposure at the highest dose treated (HDT), 15,000 ppm, the relative
 12 distributions shifted (Table 1). In both mice and rats, respectively, expired CO₂ decreased from
 13 “150 ppm levels” to 32% and 19%, tissues and carcass decreased to 16% and 8%, urine increased
 14 to 38% in the female mouse but showed no change at 9% in the female rat, feces remained
 15 unchanged at 7% in mice but decreased to 2% in the rat, and expired unchanged CE in the breath
 16

**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

^aValues 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

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

6 3.2. DISTRIBUTION, METABOLISM, AND EXCRETION

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

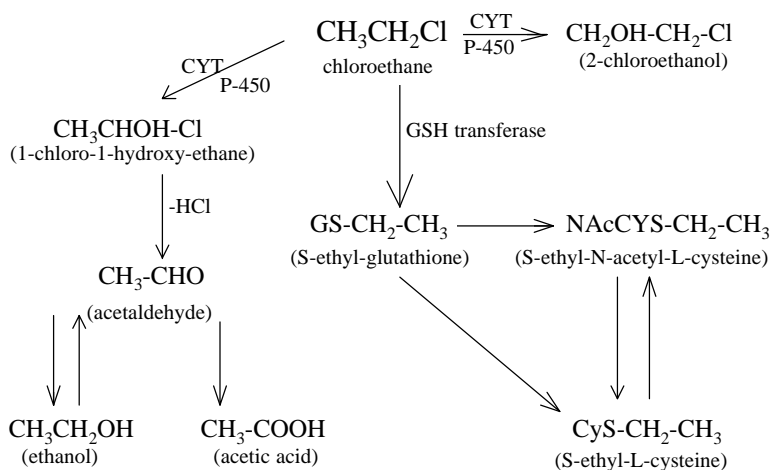


Figure 2. Proposed Metabolic Pathways for Chloroethane. Scheme presented in Fedtke et al., 1994b).

25 Dow Chemical Co. (1992) drew a contrast between the disposition of products of CE
 26 metabolism after inhalation of low (150 ppm) versus high (15,000 ppm) concentrations of the
 27 radiolabeled compound by female F344 rats or female B6C3F1 mice (Table 1). The higher
 28 concentration of CE appeared to saturate the metabolic processes, resulting in an increase in the
 29 proportion of unchanged compound that was exhaled. At the HDT, this CE exhalation was
 30 especially marked in rats (62.81% of recovered radioactivity) versus mice (6.96% of recovered
 31 radioactivity). These data in Table 1 suggest that female B6C3F1 mice may have a greater
 32 capacity to metabolize CE at 15,000 ppm than female F344 rats. Whether as CO₂ or as CE, most
 33 of the exhaled counts and those collected in urine were recovered in the first 24 hrs, thereby
 34 showing rapid CE metabolism. After 48 hr, the primary target tissues appeared to be ovary,
 35 adrenals, and skin (Dow, 1992). By contrast, the Dow research team noted a lack of selective

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

3 **Reduction.** Dow Chemical Co. (1992) explored the effect of inhalation CE exposure on
4 tissue glutathione (GSH) content in female F344 rats and female B6C3F1 mice. GSH is a
5 reducing agent often employed in cells to metabolize xenobiotics (Figure 4). GSH content was
6 measured by analyzing the non-protein bound free sulfhydryls (NPSH). Dow researchers exposed
7 female rats and mice to 150, 3,000, 6,000 (mice only), or 15,000 ppm unlabeled CE for 3 or 6 hr.
8 Effects were seen only at 15,000 ppm CE, which suggests a threshold for GSH depletion
9 (Table 2). During this exposure period (at 15,000 ppm CE), the GSH decreased in mice and rats
10 below normal levels. Mice, for example, showed GSH depletions in the following tissues: liver
11 (21%), kidney (56%), lung (32%), and uterus (55%). Mouse brain and adrenals did not show
12 GSH decreases. Blood showed the largest absolute decrease, 870 → 618 nmol GSH/mg blood.
13 Blood can account for significant amounts of [GSH] changes in tissues, or could reflect systemic
14 GSH changes, or both. The above were the only tissues sampled for GSH. Recovery to control
15 levels and overshooting to excessive GSH tissue concentrations occurred 18 hr after CE exposure
16 in mice (Table 2). The rat data pointed to GSH decreases in the liver (65%), ovaries (57%) and
17 adrenals (32%) (Table 3). The authors discussed that a relationship between CE-induced GSH
18 depletion and the induced toxicity is plausible and a suggested pathway is presented in Figure 3 .

19 Fedtke et al. (1994a,b) sought to explain
20 the biochemical mechanism(s) by which CE is
21 catabolized and the processes by which CE
22 induces metastatic endometrial uterine tumors in
23 B6C3F1 mice but not in F344 rats. Using an
24 analogous protocol to that employed by the NTP
25 (1989a), these workers exposed groups of male
26 and female F344 rats and B6C3F1 mice to 0 or
27 15,000 ppm CE via inhalation, 6 hr/day for 5
28 days.

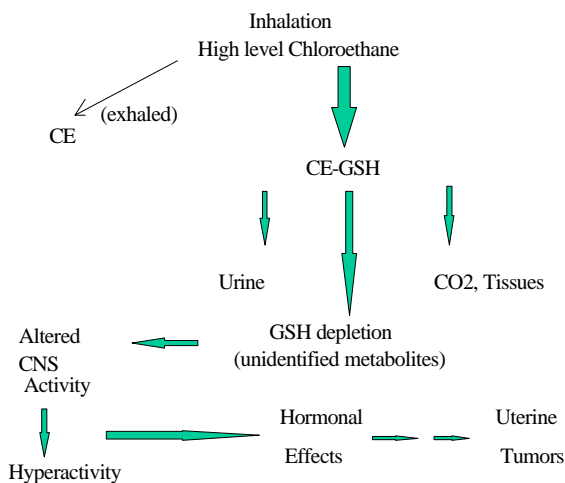


Figure 3. Proposed metabolic scheme for chloroethane disposition and toxicity in mice and rats following a high-level inhalation exposure. Source: This scheme is adapted from a scheme proposed by the Dow Chemical Co., 1992.

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

^aExposure 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.

^bStatistical 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).

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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

^aExposure 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).

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

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

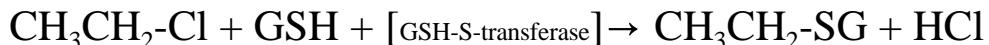


Figure 4. Reductive conjugation of chloroethane with glutathione (GSH).

The same reaction is thought to take place with other methyl and ethyl halides.

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

17 Table 4 summarizes GSH S-transferase enzyme activities in various tissues in mice and
18 rats that were treated with 15,000 ppm CE or were air controls. The GSH S-transferase is
19 measured by use of the nonspecific substrate CDNB, which measures total GSH S-transferase
20 activity in tissue preparations; the use of CDNB may obscure any specific isozymic GSH S-
21 transferase changes. The comparison of S-transferase total activities showed that rats had
22 consistently higher activities than mice (Fedtke et al., 1994b). This does not agree with an earlier
23 Dow study finding that depletions in mice were -80% and in rats were -35% after CE treatment,
24 suggesting higher transferase activity in mice (Pottenger et al., 1992). When the activities were
25 compared within species pre- and posttreatment, the only biologically significant changes were in
26 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
		($\mu\text{mol}/\text{min}\cdot\text{mg}$)	($\mu\text{mol}/\text{min}\cdot\text{mg}$)
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.

1
2
3 metabolites, SENACys and SECys, and demonstrated elevated specific amounts (in $\mu\text{mol}/\text{kg}$ body
4 weight) of SENACys in the urine of mice compared to rats (Table 5). SECys was undetected in
5 the urine of exposed rats, though the compound was present in the urine of both exposed and
6 control mice (Table 6).

7 Taken together, the data presented by Fedtke et al. (1994b) and Pottenger et al. (1992)
8 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.

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

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

13 In the in vivo phase of the study, all animals lost weight after exposure to 15,000 ppm CE
14 6 hr/day for 5 days, though differences between exposed and control animals were not statistically
15 significant. Absolute and relative weights of the major internal organs were likewise unchanged
16 as a result of exposure to CE, except for the uterine weights of CE-exposed female B6C3F1 mice,
17 which averaged about 65% of controls. The 35% loss in uterine weight is considered
18 toxicologically significant. The appearance of acetaldehyde in the urine of exposed animals
19 reflected species-specific differences. In male mice, the urine acetaldehyde concentration ranged
20 from 15.4 – 70.1 $\mu\text{mol/L}$ urine CE treated versus 7.6–20.3 in air male controls. In female mice,
21 there was less of an effect: 11.6–17.0 $\mu\text{mol/L}$ for CE-treated versus 0–18.1 in air-treated controls.
22 In rats, acetaldehyde concentrations in urine were at or below the limit of detection (2 $\mu\text{mol/L}$).
23 These results suggested that at 15,000 ppm CE the P450 oxidative is not normally a major CE
24 pathway in the rat but is employed in the mouse responses.

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

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

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

19 In conclusion, how the CE metabolic pathways are linked to the observed toxicity
20 (fetotoxicity and uterine cancers) is unknown. The suggestion of metabolic saturation and implied
21 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 (N₂O) 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.

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

17 18 **4.1.3. Dermal Exposure**

19
20 CE has been used as a pain-killing spray for such conditions as fibrositis, dysmenorrhoea,
21 causalgia, and renal colic because it can cause a local rapid lowering of temperature, thereby
22 acting as a surface analgesic (Lawson, 1965). CE has been used in sports such as American
23 football to relieve local traumatic pain. In a recent report, Bircher et al. (1994) described a
24 patient with an allergic contact reaction to CE, with sensitization to dichlorodifluoromethane
25 (Freon 12). Immunohistochemical analysis identified responses that were consistent with a T-cell-
26 mediated allergic reaction.

27 28 **4.2. ACUTE, SUBCHRONIC, AND CANCER BIOASSAYS IN ANIMALS—ORAL AND** 29 **INHALATION**

30 31 **4.2.1. Oral Exposure**

32
33 A 7- or 14-day oral CE palatability study was conducted in F344 rats; it investigated acute
34 toxicology of aqueous CE (Pottenger et al., 1995). The F344 rats were administered at either 0
35 or 0.57 g CE/100 g water (570,000 ppm), which is at the practical solubility limit of CE in water
36 at room temperature. Toxicology parameters investigated were body weights, body weight gain,

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

11 12 **4.2.2. Inhalation Exposure**

13 14 **4.2.2.1. Landry Inhalation Studies**

15
16 A report by Landry et al. (1982) described the acute inhalation exposure of six F344
17 rats/sex/group and two male beagle dogs/group to CE for 6 hr/day, 5 days/week for 2 weeks.
18 Concentrations applied were 0, 1,590, 3,980, or 9,980 ppm and duration-adjusted values are 0,
19 800, 1,900, and 4,700 mg/m³. Landry et al. (1982) observed daily clinical signs and measured
20 serial body weights before, during, and after the 2-week exposure period. Initial and terminal
21 blood and serum samples were measured for routine hematological and clinical chemistry
22 parameters in rats. Dogs were measured before and after CE exposure for hematology. All
23 animals received a complete gross pathological examination at necropsy, with a full range of
24 tissues and organs processed for histopathological evaluation.

25 Other than transient behavioral excitement, there were few compound-related effects in
26 the dogs due to CE at these exposures. Similarly, except for a slight lethargy in the high-dose
27 rats, there were no clinical signs, body weight changes, gross necropsy, or histopathological
28 effects due to treatment. Hematology, urinalysis, and clinical chemistry fluctuations were
29 unremarkable in male rats. There was, however, a statistically significant decrease in BUN in
30 female rats at the two highest exposures, but it is not interpretable to any toxic effect because of
31 the lack of any associated histopathological changes in the kidneys. There were increases in the
32 relative liver weights of male rats at the two highest concentrations. The authors considered the
33 observed changes to be minor, and to probably represent adaptive rather than toxicological
34 changes. The subchronic study identified the highest level of exposure (9,890 ppm) as a free-
35 standing NOAEL, equivalent to a NOAEL(ADJ) for extrarrespiratory effects of 4,700 mg/m³.

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

8 In an unusual protocol, Landry et al. (1989) exposed seven B6C3F1 mice/sex/group to
9 actual concentrations of 0, 250, 1,247, or 4,843 ppm CE for 23 hr/day for 11 days (duration-
10 adjusted exposure values are 0, 630, 3,200, and 12,200 mg/m³). Animals were observed daily for
11 clinical signs; on day 12 a blinded neurobehavioral observation battery was conducted. Terminal
12 body weights were measured, then blood samples were collected to measure hematological and
13 clinical chemistry parameters. At sacrifice, animals were subjected to a gross pathological
14 examination. Slides of sections of brain, heart, liver, kidney, thymus, and testes from the control
15 and high-dose groups were examined for histopathological lesions.

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

27 28 **4.2.2.2. *Principal Study Performed by the U.S. National Toxicology Program***

29
30 The most comprehensive study on the inhalation toxicology of CE in mice and rats is that
31 sponsored by the U.S. National Toxicology Program (NTP, 1989a). Groups of F344 rats and
32 B6C3F1 mice of both sexes were exposed to CE vapor (whole body) for periods of 2 weeks
33 (acute), 13 weeks (subchronic), or 2 years (chronic). Other acute studies are described that
34 usually explored the anesthetic properties of CE.
35

1 **4.2.2.2.1. NTP acute study.** A single exposure experiment (19,000 ppm for 4 hr) was part of the
2 range-finding exercise that resulted in concentrations of 0 and 15,000 ppm being chosen for the
3 chronic portion of the study. In this acute study, all rats and mice (5/sex/group) survived the
4 single exposure for 4 hr at 19,000 ppm CE with no concurrent or subsequent clinical signs.
5 Similarly, those animals (5/sex/group) exposed for 10 days at 19,000 ppm CE and held 2 weeks
6 survived for the duration of the study. Among the rats, there were no compound-related effects
7 of weight gain, whereas for the mice, body weights of exposed animals were greater than those of
8 controls. Overall, no mice or rats in this portion of the study displayed clinical signs, and gross
9 necropsy and histopathological findings indicated an absence of CE-related effects. Hence, in the
10 NTP study, CE produced no apparent acute effects at a high dose of 19,000 ppm.

11
12 **4.2.2.2.2. Subchronic study.** The subchronic portion of the NTP study featured the
13 administration of 0, 2,500, 5,000, 10,000, or 19,000 ppm CE to 10 F344 rats and B6C3F1
14 mice/sex/group, 6 hr/day, 5 days/week for 13 weeks (NTP, 1989a). Duration-adjusted exposures
15 in units of mg/m³ were 0, 1,180, 2,360, 4,710, and 8,950 mg/m³, respectively. No exposure-
16 related clinical signs or gross or histopathological lesions were evident in either rats or mice in this
17 study.

18 Possible compound-related consequences of exposure were limited to comparatively
19 minor fluctuations in body and liver weights. Thus, for both males and females in 13 weeks, slight
20 decreases in body weights were noted at HDT, i.e., 19,000 ppm CE. Statistically significant
21 increases in relative liver organ weights were observed in male rats (+14%) and female mice
22 (+18%) exposed at the HDT (8,950 mg/m³), however, male mice exposed to 4,710 mg/m³ CE
23 displayed a significant decrease in liver weight. Based on the increases in relative liver weight in
24 male rats and female mice, this study identified a NOAEL(HEC) of 4,710 mg/m³ and a
25 LOAEL(HEC) of 8,950 mg/m³ (HDT). It is notable that 2,500 ppm produces no significant
26 effect. Benchmark concentration modeling was not conducted on liver weight because it was not
27 excessive and monotonic increases with concentration were not observed.

28
29 **4.2.2.2.3. Chronic study.** Male and female F344 rats and B6C3F1 mice (50/sex/group) were
30 exposed to 0 or 15,000 ppm CE (39,570 mg/m³) for 6 hr/day, 5 days/week for 103 weeks (rats)
31 or 100 weeks (mice). The time-adjusted dosage is $39,570 \text{ mg/m}^3 \times 6/24 \times 5/7 = 7,070 \text{ mg/m}^3$.
32 The particular concentration of 15,000 ppm was chosen and was based on an apparent lack of
33 toxicity in the subchronic portion of the study, and on concerns for potential flammability and
34 explosion at higher concentrations. Clinical signs were observed daily, while body weights were
35 recorded weekly for the first 12 weeks, then monthly. A complete histological examination was
36 carried out on all animals dying prematurely and on those animals surviving to term.

1 **4.2.2.3.1. F344 Rat Toxicological Results in the NTP Study**

2 Male F344 rat survivals in control (16/50) and CE-exposed (8/50) groups were low after
3 103 weeks, with no statistically significant difference between control and treated group. The
4 NTP authors suggested that an unusually high incidence of mononuclear cell leukemia in both
5 groups likely contributed to poor male rat survivals (NTP, 1989a). In contrast, female rats
6 showed good survival in control (31/50) and CE-treated groups (22/50) at study termination; and
7 there was no statistical difference between the groups. A slight decrease in mean body weight gain
8 (4–8%) in the male rats compared to controls was observed after wk. 33 of chronic exposure, and
9 the mean body weights of female rats were 5–13% lower than controls from wk. 11 to the end of
10 the study (NTP, 1989a). At termination, the mean body weight of exposed female rats was
11 reduced by 10% compared with concurrent controls. No remarkable clinical signs were observed
12 in the exposed animals, and no CE-induced nonneoplastic lesions were observed even at this high
13 dose. This level of weight loss is not considered to be a critical toxicological effect.

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

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

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

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

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

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

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

29 Further analysis shows, however, that Battelle Pacific Northwest Laboratories had a
30 singular prior incidence of 3/50 (6%) astrocytomas in a female concurrent control group of
31 F344/N rats. This singular control brain tumor incidence happens to be commensurate with the
32 brain response in the 15,000 ppm CE group (Table 7). Thus, if a past concurrent control
33 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 <i>p</i> value^a
Males	Keratoacanthoma = 4/49 (8%) Fibroma = 1/49 (2%) <i>Total = 5/49 (10%)</i>	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%) <i>Total = 8/46 (17%)</i>	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

^aThe *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).

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

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

9 10 **4.2.2.2.3.2. B6C3F1 Mouse Toxicological Results in the NTP Study.**

11 In male B6C3F1 mice, survivals were significantly reduced compared to controls after
12 wk.42. The same was true for female mice after week 81. Thus, because of the low survival
13 rates the NTP mouse study was terminated earlier than protocol called for. The mice were
14 terminated at the 100th week, at which time survivals were 28/50 control versus 11/50 CE in
15 exposed males and 32/50 control versus 2/50 CE in exposed females. The high mortality in the
16 male mice was attributed to a greater than normal incidence of nonneoplastic urogenital lesions
17 observed in both the control and exposed males, although the exposed mice were more severely
18 affected. In female mice, the majority died as a result of CE-induced carcinomas of the uterus,
19 endometrium, myometrium, and complications of metastasis, as further discussed in Section
20 4.2.2.2.3.4, p. 23.

21 Female mice exhibited a characteristic hyperactivity during the daily periods of exposure,
22 a transient response to treatment because activity returned to normal at the end of each exposure
23 period (NTP, 1989a). There was no effect on body weight in either sex, and no other exposure-
24 related clinical signs or nonneoplastic lesions were observed. Based on this absence of
25 noncarcinogenic toxic effects, the single concentration tested (7,070 mg/m³) was a NOAEL for
26 female mice. Benchmark concentration modeling could not be conducted because only one
27 exposure level was tested.

28 Because of poor survivals in mice, the murine portion of the study was terminated at week
29 100. Many of the male mice died prematurely from urogenital infections, thereby reducing the
30 power of the male group results to detect late-developing neoplasms (NTP, 1989a). Survival
31 until termination was 28/50 in male controls and 11/50 in 15,000 ppm males. Notwithstanding
32 male mouse results, there were no significant cancer increases—except possibly an increase in
33 lung adenomas and/or carcinomas. Lung cancer incidence was 5/50 in controls versus 10/48 at
34 15,000 ppm ($p = 0.11$). The poor survivals in the male B6C3F1 mice force the conclusion that
35 the male mouse is inadequate to determine carcinogenicity.

1 Survivals in female mice were 32/50 in controls and 2/50 in 15,000 ppm. The study
 2 diagnosis was that female mice died early because of aggressive carcinogenicity (NTP, 1989a).
 3 Life-shortening is a primary element in assessing carcinogenicity. Treated female mice had a high
 4 incidence of primary tumors in the uterine endometrium (Table 8). High incidence is another
 5 primary element in assessing carcinogenicity. These lesions occurred in almost all females tested:
 6 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		<i>p</i> ^a
	Controls	CE-exposed	
Uterine hyperplasia and cystic 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

^aAs determined using Fischer's exact test.

^bNegative 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.

^cCorrected for time to first tumor at 67 weeks which as a uterine tumor.

Source: NTP (1989a).

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

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

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

6 7 8 **4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION**

9 10 **4.3.1. Oral Exposure**

11
12 No reports of studies were identified that addressed the reproductive/developmental
13 toxicity of CE when administered via the oral route.

14 15 **4.3.2. Inhalation Exposure**

16 17 **4.3.2.1. *Principal Study***

18
19 Scortichini et al. (1986) reported the findings of an inhalation reproductive
20 toxicity/teratological study on 120 pregnant female CF-1 mice exposed to nominal exposure
21 concentrations of 0, 500, 1,500, or 5,000 ppm CE for 6 hr/day on gestation days (GD) 6–15.
22 This is 10 days of CE exposure for the dams. All animals were sacrificed on GD 18. Mean
23 concentrations were found to be $0, 491 \pm 37$ ppm, $1,504 \pm 84$ ppm, or $4,946 \pm 159$ ppm which
24 convert to 0, 1,300, 4,000, and 13,000 mg/m³, respectively. These values were not duration
25 adjusted, in accordance with current EPA practice. As indicators of the compound's potential
26 maternal toxicity, the dams were observed for clinical signs: body weights and food and water
27 consumption were measured every 3 days, and at necropsy, dam liver weights and gravid uterine
28 weights were recorded. As indicators of possible developmental toxicities and teratological
29 effects of CE, fetal observations included the number and position of fetuses in utero, the number
30 of live and dead fetuses and the number of resorption sites, the weight and sex of each fetus, and
31 the incidence of any gross external alterations or cardiac abnormalities. Half of each fetal litter
32 were necropsied to look for visceral abnormalities and skeletal alterations. Serial sections of the
33 head were made in a subset of fetuses.

34 Observations showed no maternal toxicity from CE inhalation exposure as measured by
35 clinical signs, food and water consumption, body weight, and liver weight. Nor were there any
36 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 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 have been followed by a similar study in the 2,000–12,000 ppm CE range to see if there was a DFFC dose response.

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

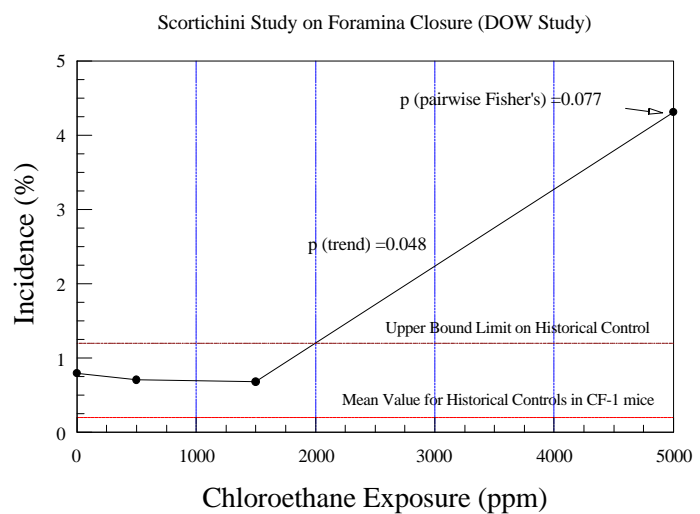


Figure 5. Occurrence of delayed foramina closure in skulls of CF-1 mice. Dams were treated with 0, 491, 1,504, or 4,946 ppm CE by inhalation for 6 hrs/day on days 6–15 of gestation. Similar statistical results were obtained when incidence on a litter basis was considered rather than on an individual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

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)					
<u>External observations</u>					
Cleft palate	<i>Fetuses</i>	0.4 (1)	1 (3)	0.3 (1)	0.4 (1)
	<i>Litters</i>	5 (1)	12 (3)	4 (1)	5 (1)
Exencephaly	<i>F</i>	0	0	1 (2)	0
	<i>L</i>	0	0	8 (2)	0
Micrognathia	<i>F</i>	0	0	0	0.4 (1)
	<i>L</i>	0	0	0	5 (1)
Chloroethane concentration (ppm)					
		0	500	1,500	5,000
Microphthalmia	<i>F</i>	0.4 (1)	0	0	0
	<i>L</i>	5 (1)	0	0	0
<u>Soft tissue observations</u>					
Dilated renal pelvis and ureter	<i>F</i>	0	1 (1)	0	0
	<i>L</i>	0	4 (1)	0	0
Pale spleen	<i>F</i>	0	0	1 (1)	0
	<i>L</i>	0	0	4 (1)	0
Pale foci on liver	<i>F</i>	1 (1)	0	0	0
	<i>L</i>	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	<i>F</i>	0	1 (1)	0	0
	<i>L</i>	0	5 (1)	0	0
Intraventricular hemorrhage	<i>F</i>	1 (1)	0	0	0
	<i>L</i>	5 (1)	0	0	0
<u>Skeletal observations</u>					
Skull -delayed ossification	<i>F</i>	2 (3)	6 (9)	4 (6)	2 (2)
	<i>L</i>	9 (2)	29 (7)	20 (5)	9 (2)
Foramina	<i>F</i>	1 (1)	1 (1)	1 (1)	4 (5) ^a
	<i>L</i>	5 (1)	4 (1)	4 (1)	23 (5)
Irregular pattern of ossification	<i>F</i>	0	0	0	1 (1)
	<i>L</i>	0	0	0	5 (1)
<u>Vertebrae</u>					
Delayed ossification	<i>F</i>	1 (3)	0.3 (1)	1 (2)	0
	<i>L</i>	14 (3)	4 (1)	8 (2)	0
Centra delayed ossification	<i>F</i>	0	0.3 (1)	0.3 (1)	0
	<i>L</i>	0	4 (1)	4 (1)	0
Atlas forked	<i>F</i>	1 (2)	1 (2)	1 (2)	1 (2)
	<i>L</i>	9 (2)	8 (2)	8 (2)	5 (1)
Fused	<i>F</i>	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
	<i>L</i>	0	0	4 (1)	0
<u>Ribs</u>					
Delayed ossification	<i>F</i>	0	0.3 (1)	0	0
	<i>L</i>	0	4 (1)	0	0
Forked	<i>F</i>	0	0.3 (1)	0	0
	<i>L</i>	0	4 (1)	0	0
Fused	<i>F</i>	0	0.3 (1)	0	0
	<i>L</i>	0	4 (1)	0	0
Cervical	<i>F</i>	1 (2)	0.3 (1)	2 (6)	2 (4)
	<i>L</i>	9 (2)	4 (1)	19 (5)	18 (4)
<u>Sternebrae</u>					
Delayed ossification	<i>F</i>	4 (11)	6 (18)	4 (12)	2 (5)
	<i>L</i>	27 (6)	48 (12)	23 (6)	14 (3)
Fused	<i>F</i>	5 (12)	6 (18)	7 (23)	5 (13)
	<i>L</i>	46 (10)	28 (7)	54 (14)	36 (8)
Staggered	<i>F</i>	0	0.3 (1)	0.3 (1)	0.4 (1)
	<i>L</i>	0	4 (1)	4 (1)	5 (1)
Irregular pattern of ossification	<i>F</i>	1 (2)	0	0	0
	<i>L</i>	9 (2)	0	0	0
Misshapen	<i>F</i>	0.4 (1)	0	0	0
	<i>L</i>	5 (1)	0	0	0

^a $p < 0.05$ using a censored Wilcoxon test.

Source: Scortichini et al. (1986).

1 mg/m³ (1,504 ppm) is characterized as a NOAEL for the fetotoxic foramina effect, with the HDT
2 = 13,000 mg/m³ (4,946 ppm), which is the LOAEL.

3 4 **4.3.2.2. Supporting Reproductive or Teratological Studies**

5 In an earlier reproductive/teratological study 8–10 pregnant female CF-1 mice were
6 exposed to 0, 5,000, 10,000, or 15,000 ppm CE for 6 hr/day on GDs 6–15 (Dow Chemical Co.,
7 1985). Among the responses investigated were the number of litters, number of implantation
8 sites/dam, number of live fetuses/litter, resorptions/litter, percentage implantations resorbed, and
9 the ratio of resorptions to litters with resorptions. Most exposed mice displayed stereotypical
10 behavior characterized by repetitive running, and significant decreases in body weight on GD 16
11 and decreased body weight gains on GDs 10–16 were observed at all CE doses. However, there
12 were no compound-related reproductive, developmental, or teratological effects in any treatment
13 group.

14 Breslin et al. (1988) reported an estrous cycling study in B6C3F1 mice. A vaginal lavage
15 technique measured estrous cyclicity, following CE exposure of two groups of 10 female mice to
16 0 or 15,000 ppm for a minimum of 14 consecutive days (three estrous cycles). Before exposure,
17 the groups had been acclimated to the inhalation chambers until their estrous cycles stabilized to a
18 regular estrus periodicity. All animals were also monitored for clinical signs, body weight
19 changes, and reproductive pathology and histopathology at termination. No effects on behavior,
20 gross pathology, or histopathology were observed in the 15,000 ppm group, but mean body
21 weight gain was significantly increased ($p < 0.05$). The mean length of the estrous cycle in exposed
22 mice was 5.6 days, significantly longer than the pre-exposure duration for the same group (5.0
23 days) and for the corresponding control group (4.5 days). The authors noted that, in some
24 animals, the estrous phase was lengthened, while in others it was the diestrous phase that was
25 affected. Consequently, they attributed the observed effects to a generalized stress reaction rather
26 than to any specific reproductive CE effect, but a direct exposure-related effect of CE on
27 neuroendocrine function cannot be ruled out. Thus, assuming that CE does have the ability to
28 disrupt the estrous cycle of mice, these data would point to a duration-adjusted free-standing
29 LOAEL of $\approx 7,071 \text{ mg/m}^3 = \text{LOAEL(HEC)}$.

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

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

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

20 **4.4. OTHER TOXICITY STUDIES**

22 **4.4.1. Acute Toxicity Studies**

24 **4.4.1.1. Neurotoxicity**

26 In many older acute or subchronic inhalation experiments, narcotic or anesthetic doses of
27 CE gas were administered and the doses were often uncertain. In such experiments, use was
28 often made of a saturated substrate (e.g., cotton) that generates a high, but unknown, flow to the
29 nose. In one such CE inhalation study, the rat cerebral cortex demonstrated decreased
30 respiration, but the thalamus and white matter did not appear affected upon gross examination
31 (Seller, 1938). Rats, mice, and rabbits were each anesthetized with CE; acetylcholine was then
32 extracted from the respective frozen brain tissues. Each showed increased acetylcholine levels as
33 a result of CE anesthesia (Sayers et al., 1929). Mice were administered 30,000 or 60,000 ppm
34 CE for up to 1 hr via inhalation (Neal et al., 1964). After 25 minutes, 17% of the mice in the
35 60,000 ppm group had become anesthetized, but no anesthesia occurred in the 30,000 ppm group.

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

3 CE anesthesia for 60 min caused a decreased sedimentation rate of RBCs from rabbits,
4 followed by a period of accelerated sedimentation rate, reaching its maximum 3 hr after anesthesia
5 was initiated and then normalizing in 12–14 hr (Hinko, 1934). Rats were anesthetized with
6 54,000 ppm CE for 5 min, and subsequently O₂ consumption and CO₂ excretion were decreased
7 significantly and the body temperature fell by 2.5 °C (Hattori, 1957). Rats were anesthetized for
8 2 hr with CE, after which occurred a disappearance of liver glycogen, a decrease in acid
9 phosphatase levels, and increases in alkaline phosphatase and succinic dehydrogenase levels
10 (Heller et al., 1966). A two-hr inhalation LC₅₀ value of 60,632 ppm for rats and mice has been
11 reported (Troshina, 1966).

12 Guinea pigs were exposed to CE via inhalation at concentrations ranging from 10,000 to
13 240,000 ppm for times ranging from 5 to 810 min (Sayers et al., 1929). An unsteady gait
14 appeared after 25 min at 20,000 ppm. Some deaths occurred at exposures of 15,300 ppm and
15 higher. At exposures ≥20,000 ppm, pulmonary congestion, hemorrhage, and edema were
16 observed in gross pathology. At 87,000 ppm for 130 minutes, violent shaking occurred in one
17 pig, and after 270 minutes, rales were heard in several guinea pigs. At 127,000, 142,000, and
18 153,000 ppm for 1 minute, there was complete loss of equilibrium, a running movement, and
19 scratching. Abdominal walls seemed distended and convulsion of the intestines was observed.
20 After 15 to 20 min, struggling became less violent; respiration became shallow, rapid, and
21 convulsive; and death occurred in 30 to 40 min. At 232,000 and 240,000 ppm, there was loss of
22 equilibrium in 30–60 seconds, and in 5 min. animals lost consciousness.

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

27 Dogs and rats were examined for neurological behavior at 0, 1,600, 4,000, and 10,000
28 ppm CE inhaled 6 hr/day, 4 days/wk, for 2 wk (Landry et al., 1982). Dog examinations were
29 performed 2 days prior to exposure and at exposure end. Dogs were examined for gait, posture,
30 mental status, cranial nerve reflexes, postural reactions, spinal cord reflexes, muscle tone, and pain
31 perception. An ophthalmoscopic exam was also performed. No reactions were seen in dogs
32 except for some hyperactivity. Only hyperactivity in exposed rats was observed by Landry et al.
33 (1982). Although these were acute observations, longer exposure to CE than 2 weeks may have
34 produced different results.

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

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

15 **4.4.1.2. Immunotoxicity**

17 There have been a number of studies in animals that do not produce immunotoxicological
18 results. For example, 438 ppm for 10 days of inhalation was negative (Schmidt et al., 1972).
19 Likewise, 10,000 ppm for 2 weeks' murine and canine exposures was immunotoxicologically
20 negative (Landry et al., 1982). Moreover, the 1988 NTP studies of 19,000 ppm for 2 weeks or
21 13 weeks, and 15,000 ppm for 104 weeks were all negative immunotoxicologically. There is one
22 study where 5,305 ppm CE for 8.5 weeks, as well as a lower exposure of 216 ppm for 24 weeks,
23 caused reduced leukocytic phagocytes in rats (Troshina, 1966). This study did not report
24 sufficient experimental details and has not been validated.

26 **4.4.1.3. Cardiac Sensitization**

28 CE-anesthetized dogs showed increased cardiac sensitivity to epinephrine, as
29 demonstrated by ventricular tachycardia (Morris et al., 1953). In CE-anesthetized dogs, cardiac
30 irregularities observed are asystole, ventricular standstill, and ventricular tachycardia (Haid et al.,
31 1954). Dogs were either anesthetized with CE only or in combination with atropine, an
32 anticholinergic drug (Bush et al., 1952). They observed electrocardiographic changes that
33 suggested two mechanistic CE effects on the heart: (1) a direct depression of cardiac tissues and
34 (2) a cardiac inhibition resulting from vagus nerve stimulation. Beagle dogs were exposed while
35 conscious to high concentrations of CE 5 minutes after an intravenous injection of 0.008 mg/kg
36 epinephrine. The treatment resulted in an exacerbated incidence of epinephrine-induced

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

7 8 **4.4.1.4. Dermal Effects**

9
10 Eye irritation has been reported by exposing human volunteers to short exposures of
11 40,000 ppm CE but not to 20,000 ppm (Sayers et al., 1929). The eye may be the only surface
12 tissue that responds adversely to CE. Histopathological effects of the dermis (canine and murine,
13 10,000 ppm or 19,000 ppm for 2 weeks) were negative (Landry et al., 1982; NTP, 1989a). It has
14 been noted: 1) after 48 hr inhalation exposure to 15,000 ppm CE (saturating metabolic
15 conditions), the B6C3F1 mice show more metabolism than F344 rats, and 2) the primary target
16 rat tissues appeared to be ovary, adrenals, and skin (Dow, 1992). If there are acute dermal
17 toxicological effects in the rat, these may build up during chronic exposure. NTP F344 rats
18 treated with CE by inhalation for 2 years did have more skin tumors: basal cell carcinomas = 3/46
19 (7%), keratoacanthoma = 2/46 (4%), squamous cell carcinoma = 1/46 (2%), trichoepithelioma =
20 1/46 (2%), lip and squamous cell carcinoma = 1/46 (2%) (NTP, 1989a). This total is 8 skin
21 cancers in 46 rats (17%) in the CE inhalation group versus 5 in 42 (12%) in concurrent control
22 versus 2/300 in historical controls.

23 24 **4.4.1.5. Kidney Effects**

25
26 Kidney responses in the rat show no effects at low doses (438 ppm for 10 days) (Gohlke
27 and Schmidt, 1972). Other than a decreased BUN there were no renal effects at 4,000 ppm and
28 10,000 ppm by inhalation for 11 days (Landry et al., 1982). NTP showed no adverse kidney
29 effects at 19,000 ppm for 13 weeks (1989a). Moreover, exposure at 9,625 ppm for 6½ months
30 showed no kidney histological effects (unrefereed study by Adams et al., 1939).

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

1 4.4.2. Genotoxicity

2
3 As described in NTP (1989a), a methodological variation is necessary to quantitatively
4 examine the effects of volatile chemicals such as CE in the Ames test. NTP solved this problem
5 by introducing CE into sealed desiccators through the vacuum valves, thereby gassing the plates
6 of *S. typhimurium* bacteria tester strains. Another innovation involved the use of a gas sampling
7 bag as an exposure vessel (Araki et al., 1994). Using these techniques NTP reported CE-induced
8 gene reversion in the *S. typhimurium* base substitution strain TA1535, with or without S-9
9 metabolic activation. Negative results were obtained in strains TA100 or TA98 (NTP, 1989a).
10 The positive result was confirmed for TA1535 as well as a related strain, TA1537 (Araki et al.,
11 1994). These authors also observed CE-induced gene reversion by CE in *E. coli* WP2 uvrA.

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

21 Therefore, based on the totality of the genotoxicity/mutagenicity evidence, CE may be
22 considered to be a positive mutagen based on its strong gene reversion effects in certain strains of
23 *S. typhimurium* and *E. coli*. However, the absence of positive genotoxic effects of CE in vivo
24 leaves open the question of the compound's carcinogenic mechanism in animal studies.
25
26

27 4.5. SYNTHESIS AND EVALUATION OF MAJOR NON-CANCER EFFECTS AND 28 MODE OF ACTION—ORAL AND INHALATION

29
30 Data gaps limit the toxicological (and carcinogenic) evaluations of CE. For example, first,
31 there is no published information on the toxicity of CE when chronically administered via the oral
32 route. Second, there is no two-generation CE exposure reproduction study. Thirdly, most of the
33 well-documented toxicological effects of CE, that have been described, have resulted from
34 frequent exposures to comparatively high concentrations, i.e., 15,000 ppm CE, but no inhalation
35 studies identified effects at concentrations lower than 250 ppm (660 mg/m³) (Landry et al., 1989).
36 It is plausible to infer that CE is not very toxic at low exposure levels because the noncancerous

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

7 8 **4.5.1. Primary Effect**

9 10 **4.5.1.1. Reproductive and Developmental Toxicity**

11
12 Reproductive organ and fetal developmental CE effects have been shown in various
13 experiments. For example, at higher CE concentrations in the dog, the uterus has been noted to
14 respond with decreased muscle tone and lessened contraction force (Van Liere et al., 1966).
15 Moreover, in an acute study Fedtke et al. (1994a) reported a 35% decrease in relative uterine
16 weight in B6C3F1 mice, but not in F344 rats, exposed to 15,000 ppm (39,570 mg/m³) CE 6
17 hr/day for 5 days. These workers also found decreased GSH levels in the uterus of CE-exposed,
18 mice and rats. An NTP chronic cancer bioassay has demonstrated quite clearly in mice that the
19 uterus is the primary organ site for carcinogenesis at 15,000 ppm CE; male rats had skin tumors
20 and female rats had brain tumors, both marginal (NTP, 1989a). The above findings are consistent
21 with the hypothesis that the uterus is a primary CE target tissue in rats and mice.

22 Breslin et al. (1988) observed a statistically significant lengthening of the estrous cycle
23 (+0.6 days) in B6C3F1 mice exposed to 15,000 ppm (39,600 mg/m³) for 6 hr/day for a minimum
24 of 14 consecutive days (3 estrous cycles), although no single phase of the cycle appeared to be
25 uniquely affected. Bucher et al. (1995) also found a statistically significant increase (+0.4 days) in
26 the mean duration of the estrous cycle in B6C3F1 mice exposed to 15,000 ppm (39,600 mg/m³) 6
27 hr/day for 3 weeks.

28 Evidence points to a perturbation of fetal skeletal development in pregnant CF-1 mice
29 (Table 9, p. 26; Scortichini et al., 1986). These authors reported an apparent statistically
30 significant (by trend only, $p = 0.048$) increased incidence of delayed foramina ossification closure
31 in the skulls of fetal CF-1 mice, but only at the highest exposure of 4,946 ppm CE (13,000
32 mg/m³) and not at lower doses of 0, 491, or 1,504 ppm CE (Figure 5, p. 25). This HDT effect
33 likely represents a weak but true fetotoxic response to CE exposure because it was manifested in
34 the absence of maternal toxicity by all measures. It is the lowest dose (4,946 ppm) and shortest
35 time representing a critical toxicological effect for CE. Therefore, the fetuses of CF-1 dams
36 exposed by inhalation during organogenesis to 4,946 ppm CE (13,200 mg/m³) represent a

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

3 4 **4.5.2. Secondary Effects**

5 6 **4.5.2.1. *Weight Loss***

7
8 As noted in Section 4.2.2.1.3.1, a slight (4–8%) decrease in mean body weight gain in
9 male rats treated at 15,000 ppm CE compared with controls was observed after week 33 of
10 chronic exposure, with the mean body weights of female rats 5–13% lower than controls from
11 week 11 to the end of the study (NTP, 1989a). At bioassay termination, the mean body weight of
12 exposed female rats was reduced by 10% compared with controls. No food consumption data
13 were described in the study report. Neither clinical signs nor other CE-induced nonneoplastic
14 lesions were observed. This suggests that the observed weight loss may have been compound-
15 related and not simply a consequence of food aversion. Because the extent of the weight loss
16 (10%) is at the threshold that EPA considers to be toxicologically significant, the response is thus
17 considered a secondary noncancer effect. On the basis of decreased body weight in female rats at
18 the single exposure level tested, this study identified a LOAEL(ADJ) for CE of 7,070 mg/m³, a
19 concentration that would represent a NOAEL in males.

20 21 **4.5.2.2. *Hepatotoxicity***

22
23 Increased relative liver weight in response to CE exposure at 19,000 ppm for 13 weeks
24 was observed in both sexes of B6C3F1 mice (NTP, 1989a). In addition, slight increases in mean
25 relative liver weights with a possibly related increase in the degree of hepatocellular vacuolization
26 were reported by Landry et al. (1989) in mice exposed to 5,000 ppm for 23 hr/day for 11
27 consecutive days. Similarly, statistically significant increases in relative liver weights were also
28 observed in male rats exposed to 4,000 or 10,000 ppm for 6 hr/day, 5 days/week for 2 weeks
29 (Landry et al., 1982). However, these liver changes appeared to be unaccompanied by any
30 evidence of compound-related histopathology.

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

1 CE effects were alluded to in a Russian report in which rats were exposed daily (4 hr/day)
2 to 570 mg/m³ (220 ppm) for 6 months (Troshina, 1966). This exposure was reported to result in
3 perturbed hepatic function and lipid degenerative changes, along with decreased arterial blood
4 pressure and some dystrophic changes to the lungs. However, these findings remain
5 uncorroborated by other workers and have generally been discounted because of inadequate
6 reporting (ATSDR, 1997; U.S. EPA, 1998c).

7 8 **4.5.2.3. Neurotoxicity**

9
10 A range of neurotoxicological responses to CE have been reported in human beings and in
11 laboratory animals, with a wide range of doses. Thus, CE can induce anesthesia in humans at
12 higher doses (Cole, 1967; Dobkin and Byles, 1971; Lawson, 1965), and hyperactivity in mice
13 (NTP, 1989a), even as low as 5,000 ppm (Dow Chemical Co., 1985). However, these transient
14 effects may not be appropriate as the basis for developing an RfC because of the acute reversible
15 nature of the responses. More sensitive measures of central nervous system effects have not been
16 observed, although Landry et al. (1989) conducted a neurobehavioral observation battery for mice
17 exposed to concentrations of CE up to 5,000 ppm for 23 hr/day for 11 consecutive days. In
18 general, mechanisms of anesthesia are not well understood, though it is likely that the observed
19 effects are due to the direct action of high concentrations of the parent compound on nervous
20 tissue.

21 22 **4.5.3. Mode of Action of Toxic Effects**

23
24 It is difficult to determine the exact nature of CE toxicity in each responding tissue. It also
25 is difficult to know if there is a interconnecting mode of action among tissues. Section 3.2
26 discusses what is known on CE metabolic issues, that may underlie the mode of toxic action.

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

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

4
5
6 **4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER**
7 **CHARACTERIZATION—SYNTHESIS OF HUMAN, ANIMAL AND OTHER**
8 **SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY,**
9 **AND LIKELY MODE OF ACTION**

10
11 Although no data exist that document a tumorigenic effect of CE in human beings, there
12 are consistent lines of evidence indicating that CE is a carcinogen in animal test systems. These
13 include:

- 14
- 15 (1) **Chemical carcinogenesis.** The clear-cut demonstration of CE's causality in uterine
16 carcinogenicity in female B6C3F1 mice (uterine incidence: 43/50 = 86%), which is
17 relevant because uterine cancer is rare to uncommon in B6C3F1 mice occurring at an
18 uncommon rate of 4/1,371 (0.29%) in a limited population (NTP, 1989a; IARC, 1992).
19
 - 20 (2) **Mutagenesis.** CE is mutagenic in it's capacity to induce gene reversion in certain strains
21 of *S. typhimurium*.
22
 - 23 (3) **Structural Activity Relationship.** BE, a structural CE analogue, induces similar
24 tumorigenic effects in the uterus of B6C3F1 mice as does dichloroethane.
25
 - 26 (4) **Metabolism.** CE's ability to lower GSH pools, similar to methyl halohydrocarbons
27 CH₃Cl and CH₃CH₂Br, and oxidative metabolism proceeding via acetaldehyde. CM may
28 be a carcinogen too forming the renal cortex and papillary cystadenocarcinomas in male
29 mice exposed. BE causes uterine tumors and marginally other tumors: lung,
30 pheochromocytomas of the adrenals, and brain.
31

32 Section 4.2.2.1.3 gives a detailed summary of the principal carcinogenesis study (NTP,
33 1989a). As noted, the primary effect was the high incidence of uterine carcinomas in female
34 B6C3F1 mice (43/50 at 15,000 ppm vs. 0/49 in controls). The tumors were of endometrial origin
35 and showed a profound capacity for metastasizing. First the cancers moved to the neighboring
36 myometrial tissue and from there disseminated to such secondary tissue sites as lung, ovary,
37 lymph nodes, kidney, adrenal gland, pancreas, mesentery, urinary bladder, spleen, heart and, to a
38 lesser extent, colon, stomach, gall bladder, liver, small intestine, and ureter. The complications
39 arising from these CE-induced tumors are considered to be the cause of the poor survival in these
40 female B6C3F1 mice (NTP, 1989a). Thus, in addition to metastasis, life-shortening tumor effects

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

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

7 As noted in Section 4.2.2.1.3.3, marginal increases in some uncommon skin tumors in
8 male F344 rats were not persuasive enough to unequivocally designate the compound as a
9 carcinogen in the rat animal model, but the rat skin response is suggestive of tumorigenesis. The
10 female F344 rat brain astrocytoma response, an uncommonly occurring response, was also
11 equivocal because of its low incidence (3/49) versus controls (0/46), but the rat brain response is
12 suggestive. Table 11 summarizes all the relevant tumor-forming effects of CE from the NTP
13 (1989a) study in F344 rats and B6C3F1 mice. It is concluded: CE is clearly carcinogenic in female
14 B6C3F1 mice, but the evidence for CE carcinogenicity in male and female F344 rats is equivocal.

15 Taken as a whole, the mutagenicity and metabolic data that have been amassed for CE are
16 consistent with the findings of carcinogenicity but some information is lacking (Figure 2). For
17 example, well-documented positive point mutations have been obtained in Ames tests (NTP,
18 1989a; Zeiger et al., 1992; Araki et al., 1994), but CE does not induce in vivo clastogenic
19 responses in the same strain of mice (B6C3F1) in which the uterine carcinomas were described
20 (Ebert et al., 1994). GSH links with the ethyl group of CE in an elimination reductive conjugation
21 pathway. If CE is dosed high enough, GSH pool levels likely become limiting for further
22 ethylation of CE. GSH pools likely become rate-limiting for other detoxification reactions too
23 (Figure 2, p. 6). In this way the excess CE then would be forced to flow through oxidative
24 metabolism (oxidation in mice is twice that of rats) with acetaldehyde being an intermediate
25 (Figure 1, p. 1). This can cause cancer at high internal intermediate mutagenic doses; whether
26 excessive systemic SEG or acetylaldehyde are involved in the mode of carcinogenic action, or
27 both, is not known. CE induces its own metabolism, likely p-450 enzyme P450IIE1, which
28 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
<i>Carcinogenicity</i>						
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	
<i>Noncancer toxicity</i>						
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)
<i>Table 10 is continued on the following page</i>						

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)

1
2 **Table 11. Summary and Conclusions of Tumorigenesis in Rats and Mice**

3

Sex	F344 rats	B6C3F1 mice
Males	<i>Marginal evidence</i> ! Skin tumors	<i>Inadequate evidence</i>
Females	<i>Equivocal evidence</i> ! Brain tumors	<i>Clear evidence</i> ! 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)

4
5

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

7
8

9 Evidence in support of the carcinogenicity of CE is also provided by similar long-term
10 experimental studies that were carried out on its structural analogue, BE (NTP, 1989b). When
11 challenged with concentrations of BE at 100, 200, and 400 ppm, female B6C3F1 mice responded
12 with the formation of uterine squamous cell carcinomas, adenomas, and carcinomas, in direct
13 analogy to CE. The uterine responses were 4/50, 5/47, and 27/48 for 100, 200, and 400 ppm BE
14 exposure groups, respectively, versus an incidence in controls of 0/50. Although not statistically
15 significant, 1,2-dichloroethane administered by gavage produced adenocarcinomas of the uterus in
16 3/49 mice at 148 mg/kg and 4/47 mice at 229 mg/kg in 78 weeks (NTP, 1978). Related
17 chlorohydrocarbons that do not cause increased uterine tumors are 1,1-dichloroethane,
18 1,1,1-trichloromethane, 1,1,2-trichloroethane, 1,1,1,2-tetrachloroethane,
19 1,1,2,2-tetrachloroethane, pentachloroethane, and hexachloroethane (all referenced in NTP,
20 1989a). Other analogues such as methylchloride cause only cystadenomas and adenomas of male
21 mice. Methylbromide seems not to be carcinogenic; dibromoethane does produce uterine cancers
22 and also produces alveolar/bronchiolar carcinomas in male and female mice, as well as
23 hemangiosarcomas, fibrosarcomas in the subcutaneous tissue, nasal carcinomas, and mammary
24 adenocarcinomas in female mice.

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

1 Based on the criteria set forth in the current *Guidelines for Carcinogenic Risk Assessment*
2 (U.S. EPA, 1986), a weight-of-evidence classification of B2 is indicated. That is, CE is a
3 *probable human carcinogen* based on no evidence in human beings and adequate evidence in
4 animals with medium confidence. Categorizing CE according to the weight of evidence approach
5 proposed by the *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 1996) would derive a
6 *likely human carcinogen by the inhalation route* classification for CE.
7
8

9 **4.7. OTHER HAZARD IDENTIFICATION ISSUES**

10 **4.7.1. Possible Structural-Activity Relationships**

11
12
13 As discussed in Section 4.6, BE and chloromethane (CM) are structural analogues of CE.
14 Bromoethane can deplete GSH, much like CE and CM, and therefore is normally detoxified
15 primarily by reductive conjugation to GSH (Khan and O'Brien, 1991). Excessive amounts of
16 deplete GSH and become oxidized to unresolved acetaldehyde (from BE and CE) and
17 formaldehyde (from BM and CM). "Unresolved" refers to the lack of metabolic steady state and
18 a buildup of the toxicant intermediate. The toxicity of these analogues may be used to explore
19 and illuminate the toxicity and mechanism of action of CE.

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

31 Though BE displays greater toxicity than CE, especially in the nasal passages and lungs, a
32 comparison of the carcinogenic endpoints of the two halogenated hydrocarbons implies a domain
33 of commonality in toxicity mechanisms. That is, the most sensitive carcinogenic response to BE
34 was the incidence of uterine cancers of female B6C3F1 mice: 0/50 at 0 ppm, 4/50 at 100 ppm (p
35 = 0.06), 5/50 at 200 ppm (p = 0.03), and 28/48 ppm (p < 10^{-8}) at 400 ppm BE. This same uterine
36 carcinogenic response was also reported for mice exposed to CE: 0/50 at 0 ppm versus 43/50 (p

1 < 10⁻⁸) at 15,000 ppm (NTP, 1989a). Other notable cancer sites for BE were lung,
2 pheochromocytomas, bronchiolar, nasal, and low-level granular-type tumors and gliomas in the
3 brain; other cancer sites for CE included the liver. The tumor spectra share the uterus, but the
4 other carcinogenic sites are different. The conclusion of carcinogenicity drawn for BE supports
5 the chemical carcinogenesis hypothesis for CE.

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

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

22 There appear to be striking analogies between the metabolic fates of CM and CE. For
23 example, following rapid uptake via the lungs, important metabolic products of CM were
24 identified as formaldehyde, formic acid, and carbon dioxide, with part of the material being
25 incorporated into the C₁-pool (tetrahydrofolic acid) of intermediate metabolism (Kornbrust and
26 Bus, 1982; Landry et al., 1983). An important outcome of these experiments was the
27 demonstration that the incorporation of ¹⁴C from CM into major structural macromolecules such
28 as DNA occurred as a consequence of normal protein synthesis rather than as a result of
29 methylation (Kornbrust et al., 1982).

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

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

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

1 (3) the demonstration of both P450-mediated oxidative and GSH-mediated
 2 reductive pathways for the metabolism of CM (Kornbrust and Bus, 1982)
 3 (4) the inhibition of the acute toxicity of CM in male B6C3F1 mice by GSH depletion
 4 (Chellman et al., 1986).

5 Bolt and Gansewendt (1993) used the latter inhibition data to develop a cancer
 6 mechanistic hypothesis for CM. They assumed a relationship existed between GSH depletion and
 7 cancer because the exposure CM levels for the onset of kidney tumors in male B6C3F1 mice and
 8 the depletion of kidney GSH levels are comparable. Decrementing GSH can switch CM from the
 9 reductive pathway, which is used principally for CM metabolism, to also include oxidative
 10 pathway, i.e., catabolism by P450 of CM to formaldehyde, formate, and CO₂. Excessive
 11 formaldehyde can cause cancer (Morgan, 1997; Morris, 1997; Monticello and Morgan, 1997).
 12 GSH depletion can likewise cause a paucity of the cofactor GSH for formate dehydrogenase, the
 13 enzyme inactivating formaldehyde. This effect can promote the formation of DNA-protein
 14 crosslinks in susceptible target organs.

15 As shown in Table 12, lines of evidence that draw parallels between the metabolism of CE
 16 and CM include (1) the duality of CE metabolism, in which reductive metabolism through binding
 17 to GSH and oxidative metabolism mediated by cytochrome P450IIE1 are featured,
 18

**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

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

3 If CE and CM share similar mechanisms for tumorigenesis, the occupational exposure
 4 information that has accumulated for CM in humans may also be of relevance to the potential
 5 carcinogenicity of CE. In general, CM data show a marked diversity in the ability of persons to
 6

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

6 7 **4.7.2. Possible Gender Differences**

8
9 A report by Griesemer and Eustis (1994) summarized the findings of NTP with regard to
10 the sex- and tissue-specific onset of carcinogenicity observed throughout their series of 2-year
11 toxicity/carcinogenicity studies. A total of 1,760 untreated control groups from 440 studies using
12 F344 rats and B6C3F1 mice (approximately 88,000 animals, from which 3.5 million tissues were
13 examined microscopically) have contributed data on the gender-specific background rates of
14 tumor formation. These data apply both to major organs such as lung, liver, and kidney, and to
15 gender-specific organs, such as uterus, ovary, and testis. Of particular interest to the potential
16 carcinogenicity of CE (and BE) is the markedly low background frequency of uterine tumor
17 formation in B6C3F1 mice (0.3%). Based on the high incidence of uterine tumors at the single
18 CE concentration tested (15,000 ppm), and on the dose-dependent increase in response to
19 challenge with BE, the conclusion may be reasonably drawn that the occurrence of these uterine
20 tumors has direct etiological association with the target compounds. Because CE and BE cause
21 female uterine cancers to the greatest extent of their carcinogenicity, the mode of action of CE
22 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/m³) is the noncancer critical effect LOAEL for CE, and 1,500 ppm CE (3,970 mg/m³) 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 extrapulmonary 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, $LOAEL(HEC) = LOAEL(ADJ) \times (\lambda_A/\lambda_H) = 13,057 \text{ mg/m}^3 \times (1) = 13,057 \text{ mg/m}^3$. Thus, for the fetotoxic effect, the $LOAEL(HEC) = 13,057 \text{ mg/m}^3$, and $NOAEL(HEC)$ is $3,970 \times 1 = 3,970 \text{ mg/m}^3$.

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 $17,832 \text{ mg/m}^3$ (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 $13,421 \text{ mg/m}^3$ (5,084 ppm CE).

1 The litter quantal model reduces all fetal incidence data to a question of whether any
2 fetuses in a litter are affected, while the above fetal model allows the use of fetal data grouped by
3 dose. Because there were no interlitter biases reported and the dams are the units that are treated,
4 the litters are considered the representative biological units of CE-induced fetotoxicity. The litter
5 data 1/22, 1/24, 1/25, and 5/22 (Table 9) were also modeled using the probability of a litter being
6 affected. Good fits were obtained at 0, 491, 1,504, and 4,946 ppm CE (*p*-values 0.88). The
7 BMC for 10% extra risk was chosen for litters and is based on the results for generic quantal
8 models in Allen et al. (1994b). The BMC for litters is estimated to be 10,634 mg/m³ (4,028 ppm)
9 using the Weibull model; the lower 95% confidence limit (BMLD) is 4,240 mg/m³ (1,606 ppm
10 CE). The polynomial model [$P(d) = 1 - \exp\{-q_0 - q_1*(d) - q_2*(d)^2 \dots - q_k*(d)^k\}$] was little different in
11 results. These litter results are more conservative than the above per-skull basis results. The
12 BMLD for litters (4,261 mg/m³) may be used as a substitute for a NOAEL when a NOAEL
13 cannot be estimated. There is a NOAEL of 3,970 mg/m³ but the BMLD will be used to estimate
14 the RfC, as will the NOAEL. Both results will be compared below.

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

21 An uncertainty factor of 10 is considered for variations in sensitive subpopulations within
22 populations, and a further factor of 10 is used for interspecies extrapolation. An uncertainty
23 factor of 3 is used for extrapolating from a LOAEL to a NOAEL. A full factor of 10 is used for
24 database deficiencies to account for the lack of a multigeneration reproductive study, and because
25 no evaluation of reproductive function following long-term exposure is available. These
26 uncertainty components combine ($10 \times 10 \times 3 \times 10$) to an overall uncertainty factor (UF) of
27 3,000.

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

- 30 • Method I RfC = BMLD ÷ 3,000

31 RfC = 4,261 mg/m³ ÷ 3,000 = 1.4 mg/m³ (0.54 ppm CE)

32 RfC = 1.4E0 mg/m³

- 33 • Method II RfC = NOAEL/3,000

34 RfC = 3,970/3,000 = 1.32 mg/m³ (0.50 ppm CE)

35 RfC = 1E0 mg/m³

1 The traditional method of estimating a dose to which one may reference as relatively safe
2 (RfC) is to factor down the noncancer NOAEL by the UF. With the fetotoxic effect, the RfC =
3 $1E0 \text{ mg/m}^3$ (rounded off) and will be the recommended value. The RfC in Method I employing a
4 fit to the data, establishment of the 95% LCL on dose, and factoring down by the UF yields
5 essentially the same answer (which is a good check).
6

7 **5.3. CANCER ASSESSMENT**

8 **5.3.1. Qualitative Cancer Assessment in Animals**

9
10
11 No human cancer data exists on CE. Indirect evidence for the carcinogenicity of CE was
12 observed in laboratory animal studies in a single NTP study (NTP, 1989a). In the bioassay 50
13 B6C3F1 mice and 50 F344 rats/sex/group were exposed via inhalation to only one dose: 15,000
14 ppm CE, 6 hr/day, 5 days/week for 2 years. This is a nonstandard protocol because normally
15 there are 2 or 3 doses. Tumorigenic responses in male B6C3F1 mice were compromised by poor
16 survival and the onset of urogenital infections. The B6C3F1 female mice responses were quite
17 remarkable because of the strong carcinogenic response: uterine carcinogenicity in 43 female mice
18 of 50 put on test (86%). This incidence is relevant because uterine cancer is an uncommon cancer
19 site in B6C3F1 mice. The human historical incidence for uterine cancer is approximately 0.006
20 %, making it the seventh most common female human cancer (Parkin et al., 1999). The historical
21 rate for B6C3F1 mice is 0.29%, an uncommon but not rare cancer in mice bioassayed so far
22 (NTP, 1989a; IARC, 1992). The uterine incidence ratio of the CE-treated B6C3F1 group to the
23 historical group is $86\%/0.29\% = 297$ -fold response.

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

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

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

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

10 **5.3.2. Quantitative Cancer Assessment in Animals**

11 **5.3.2.1. Considerations in Quantitative Cancer Assessment**

12
13 The absorption and distribution of metabolized CE seems to be nonlinear at high doses of
14 CE (cf. p. 6). The metabolism of the halohydrocarbons CE, BE, dichloroethane, and EDB likely
15 proceeds under normal circumstances by a reductive conjugation pathway mediated by GSH and
16 various specific glutathionetransferases (Fedtke, 1994a; Commandeur et al., 1995). Methyl
17 chloride and methyl bromide are metabolized similarly by GSH (Bolt and Gansewendt, 1993).
18 When exposures of these halohydrocarbons exceed the capacity of the reduction pathway
19 enzymes, a lesser used pathway, oxidation, becomes more predominant. Specifically, CE is
20 oxidized via a P450 pathway through acetaldehyde (a toxic compound itself when in excess),
21 acetic acid, and finally to CO₂ and H₂O as terminal oxidation products (Fedtke, 1994b). It is
22 difficult to hypothesize that this change in metabolism, in any combination, occurs by a linear
23 dose-response process, yet the present database is not informative enough to discern
24 nonlinearity. The shape of the total metabolic curve, and perhaps the coupled carcinogenicity, is
25 unknown. Experiments studying CE cellular binding sites, GSH depletion kinetics, and
26 acetaldehyde kinetics could be useful.

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

1 More kinetic data points are needed to establish this issue. Further, not enough is known about
2 the relationship of CE metabolism, whatever it's true kinetic response with dose, and the apparent
3 CE cancer outcome in the uterus. Unless it is known that current metabolic evidence is measuring
4 the responsible metabolic factors which initiate and promote CE chemical carcinogenesis, then a
5 mode of action can only be speculation. A nonlinear kinetic model would allow direct estimation
6 of dose-response, or if such a model were not available but there are sufficient kinetic data to
7 determine nonlinearity in cancer-causing metabolic effects, a Margin of Exposure (MOE) method
8 may be possible if proper exposure and kinetic data and rational were to be presented (U.S. EPA,
9 1996a).

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

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

23 24 **5.3.2.2. LMS Method**

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

UCL estimated risk [$P = (q_1^*) \times (d)$] 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/m³) 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% \approx 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/m³ by a factor 1 ppm = 2.64 mg/m³, 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/m³ 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 ED₁₀ is estimated to be 100 mg/kg/d or 300 mg/m³. For the combined incidence of uterine and liver cancers (Table 13), Global86 estimates the inhalation unit risk be 4E-4/mg/m³. This unit risk is approximately equivalent to an inhalation slope factor of $q_1^* = 1.14E-4$ /mg/kg/day, assuming 20 m³ air breathed/day and body weight of 70 kg. Using the inhalation unit risk of 4E-4/mg/m³, various CE risk levels may be estimated: at $i = 10^{-4}$, 300 μ g/m³; at $i = 10^{-5}$, 30 μ g/m³; and at $i = 10^{-6} = 3 \mu$ g/m³. 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 μ g/kg/day), i.e., at 10 μ g/kg/day lifetime CE exposure one may expect, with 95% confidence, no more than a 10^{-6}

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

4 **5.3.3. Discussion of Confidence in Cancer Quantitative Assessment in Animals**

5
6 Any CE cancer potency estimate, such as a cancer unit slope, made is necessarily not
7 certain because of the datum on which the estimate is based. Accordingly, the LMS method is
8 thus not certain because of the one dose-response point and is insufficient for deriving any
9 estimate of the shape of the dose-response curve. In practice Global86 connects this one point to
10 the origin and estimates the 95% UCL on this straight line. Another uncertainty is that the
11 exposed group had nearly 100% tumor incidence (90%), and it is unknown whether such a
12 saturation of effect would have occurred at an even lower dose, in which case, the proposed
13 inhalation slope factor could be an underestimate, the degree of which would be unknown. It is
14 assumed from experience that the plateau on which the response sits is likely \leq threefold wide
15 (Gaylor, 1989). It is also unknown whether there are any sublinearities in the dose-response
16 relationship in the normally observable response range, which could result in the proposed slope
17 factor being an overestimate. Another issue of quantitative uncertainty concerns the fact that the
18 study was terminated early (termination week 100), because there was substantial early mortality
19 in the exposed female mouse group resulting from the tumors (e.g., only 50% of the exposed
20 group were still alive at week 90 compared with 90% in controls). This time component was not
21 taken into account in the risk calculations because animals were dying from uterine carcinogenesis
22 not competing toxicity. Conceivably, a lower dose could have resulted in the same tumor
23 incidence along with later-occurring tumors in life, in which case there would be an
24 underestimation of the proposed slope factor; the time-to-tumor issue may be relatively trivial
25 with respect to the other uncertainties outlined above.

26 Because of the uncertainties of the LMS method, alternative methods were examined to
27 gain additional perspective on the upper bound of CE cancer potency. One of the first
28 nonparametric methods was a procedure taking the lowest dose (7,070 mg CE/m³) and incidence
29 ($i = 0.90$), and estimating the upper 95% confidence limit incidence (≈ 1.00), and then define the
30 straight line connecting this point to the 0,0 point. The cancer slope of the line $\approx 1/7070 \text{ mg/m}^3 =$
31 $1.4\text{E-}4/\text{mg/m}^3$ (Gaylor and Kodell, 1980).

32 Another type of estimate can be made which is enabled by the unusually high incidence
33 ($i = 0.90$). In the case of CE, the bioassay produces almost the maximum theoretical response of
34 100% incidence and can be considered an approximation of the MTD. This assumption is based on
35 the following: 1) many of the female mice died prematurely because of the tumor load (only 2/50
36 survived until termination at week 100), and 2) the observation of a maximum cancer response

1 with some toxicity at this dose but not overt noncancer toxicity. As with many chemicals, more CE
2 likely would have been too toxic (i.e., > MTD) from the start of the study and would reduce
3 cancer because sick or morbid animals do not yield tumors beyond certain doses but rather become
4 sickly and/or die before cancer evolution. Just as likely, less CE (just how much less is unknown
5 but assuming in the dose-response range) would have decreased CE carcinogenicity because of less
6 coupling to the reaction sequence (mode of action) causing cancers. Thus, 7070 mg/m³ can be a
7 crude estimate of the MTD for CE.

8 It has been found for most chemicals tested so far, that certain dosimetric relationships
9 exist among the parameters MDT, TD₅₀, and the 10⁻⁶ Risk Dose even though the TD₅₀ (potency)
10 varies over eight orders of magnitude among the chemicals (Gaylor and Gold, 1995; Krewski et
11 al., 1993; Bernstein et al., 1985; Shlyakhter et al., 1992). The Risk Dose is a “low” dose on the
12 curve presumed to be in the linear range. One of these relationships is that $k = (\text{MTD})/(\text{10}^{-6} \text{ Risk Dose}) \approx 740,000$ where “k” a geometric average of 317 diverse structured chemicals with only 14
13 falling outside a 10-fold interval, i.e, an approximate constant (k) exists among different chemical
14 (for further details see Appendix). Thus, $10^{-6} \text{ Risk Dose} \approx \text{MTD}/740,000$. Because cancer slope
15 = incidence/dose, then slope = $(10^{-6} \text{ incidence})/(\text{10}^{-6} \text{ Risk Dose})$, hence one may estimate an upper
16 limit simulating the LMS q₁* linear cancer slope value (Gaylor, 1989).

17 . The nonparametric estimation of 10⁻⁶ Risk Dose involves an *empirical* factor associated
18 with the observed *median* (MDT)/(10⁻⁶ Risk Dose) ratio (i.e., $k \approx 740,000$) (Gaylor and Gold,
19 1995). Thus, $10^{-6} \text{ Risk Dose} \approx 7,070 \text{ mg/m}^3/740,000 = 9.55\text{E-}3 \text{ mg/m}^3$ (3.6E-3 ppm) (Table 14).
20 For inhalation, the CE concentration of the 10⁻⁶ Risk Dose is $\approx 1\text{E-}2 \text{ mg/m}^3$ or $\approx 4 \text{ ppb}$ for a lifetime
21 of CE exposure. Next, the unit cancer slope 95%UCL estimation is $10^{-6}/9.55\text{E-}3 \text{ mg/m}^3 = 1.05\text{E-}$
22 $4/\text{mg/m}^3$. This method compares with the above nonparametric method ($1.4\text{E-}4/\text{mg/m}^3$) as well as
23 estimated LMS slope of $4\text{E-}4/\text{mg/m}^3$. These cancer unit slope values appear to be comparable
24 within the limits of error of the methods—see below for reliability. Because the nonparametric
25 methods did not attempt to model the datum, but rather used dosimetric relationships and
26 comparison to 317 previously tested carcinogens, the nonparametric cancer slope estimates lend
27 support to the LMS value $4\text{E-}4/\text{mg/m}^3$.

28 The slope estimation using the 10⁻⁶ Risk Dose may be compared with historical controls of
29 mice and humans to estimate the Margin of Safety (MOS). Thus, an $i = 10^{-6}$ is less than the NTP
30 historical control incidence of 0.0029 in female B6C3F1 mice, hence $i = 10^{-6}$ is conservative level
31 in mice. However, $i = 10^{-6}$ is somewhat less than the world-wide human incidence of 59×10^{-6} (5.9
32 diagnosed cases of uterine cancer per 100,000 females), hence $i = 10^{-6}$ is somewhat conservative in
33 humans. Because the spontaneous frequency of uterine cancer is normally low among female
34 B6C3F1 mice but higher than the assumed 10⁻⁶ risk, the nonparametric method using $k = 740,000$
35 is sufficiently conservative in the mouse by 29,000-fold ($2.9\text{E-}3/1\text{E-}6$). Thus, even though there is
36

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

9 In conclusion, all three upper-bound cancer slope estimates should be considered uncertain
10 because of the one-point bioassay on which they are based. It is reasoned that a numerical
11 assessment is prudent, however, because of the striking animal response ($i = 0.9$), low spontaneous
12 occurrence of uterine tumors in mice, carcinogenic SAR of BE and 1,2-dichloroethane at the same
13 organ site, and metabolic comparisons to CM. Notwithstanding, the development of a cancer
14 potency estimate does not effect the qualitative assessment of CE carcinogenicity (section 6.3,
15 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/m³ 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 1/6th the breast cancer incidence, the most common, and 1/2 the incidence of female lung cancer, the 2nd most common, in the same region. Thus, CE exposure

1 *could* add to ongoing the uterine cancer rate in the human population. On the other hand, the
2 female rat in the NTP bioassay is not affected with these tumors @ 15,000 ppm CE, only
3 borderline astrocytomas. Bromoethane—a close chemical analogue to CE—supports the mouse
4 CE carcinogenic uterine response in that it too causes uterine tumors in B6C3F1 female mice
5 ($i = 28/48 = 0.58$ @ 400 ppm). The CE-treated mice present an exceptionally large ($i = 43/49 =$
6 0.88 @ 15,000 ppm) uterine cancer response, or any other organ site response for that matter,
7 compared to other chemical carcinogens that the Agency has reviewed to date. The degree of
8 carcinogenicity is exceptional too in that the primary tumors are very aggressive, metastasizing to
9 16 diverse organ sites in female B6C3Fi mice and killing them early due to tumor load.

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

15
16 Therefore, outlining the elements of CE's carcinogenicity:
17
18

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

23
24 **(2) Structural analogues BE and 1,2-dichloroethane cause similar uterine cancer**
25 **responses,**

26
27 **(3) CE metabolizes similarly to CM: saturation of reductive GSH metabolism and induction**
28 **of excessive P-450 oxidative metabolism at high CM. Because CM causes renal**
29 **cortex cystadenomas and adenomas and papillary cystadenocarcinomas in male**
30 **mice and because CE causes uterine and liver cancers in female mice, the**
31 **carcinogenicity of both may be linked to their metabolic similarity.**

32
33 **(4) CE's mutagenicity evidence and the prospect that CE can be an alkylating agent under**
34 **the correct activating conditions.**

35
36
37
38 Thus, because of the striking mouse cancer response, similarity to BE and EDC chemical
39 carcinogenesis, uncommon occurrence of the tumor type, mutagenic and potential alkylating
40 properties, CE exceeds a C Category weight of evidence usually reserved for one-species
41 responses. The weight-of-evidence supports the choice of B2 carcinogenicity classification for CE,

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

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

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

13 The coupling relates exposure and toxicity to a mode of action via a kinetics model. In time, a loss
14 of cellular growth control results. The coupling reactions for CE are not certain at this time.

15 Remaining data gaps include: (1) the lack of dose-response data sufficient to determine the tumor
16 incidence rate at intermediate CE exposure levels (100–4,500 ppm CE), (2) the absence of any
17 detailed information on the triggering site or the target organ-specific biochemical processes that
18 link CE exposure, or intermediate, and response, and (3) any hormonal link that may explain the
19 mouse uterine tumors from CE and BE and whether this applies to humans, (4) specific
20 comparisons of mouse and human metabolic patterns and kinetic for CE.

23 **6.4. CHARACTERIZATION OF HAZARD EXPECTED UPON HUMAN EXPOSURE TO** 24 **CHLOROETHANE**

25
26 The most robust toxic effect of CE is the inhalation malignant cancer effect observed in
27 female B6C3F1 mice @ 15,000 ppm, not the noncancer fetotoxic effect @ 4,900 ppm or the 10%
28 weight loss or 35% uterine weight loss @ 15,000 ppm (Table 10). Chronic CE oral toxicity studies
29 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 × 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	<i>LMS method</i> q₁* = 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)
				<i>LMS method</i> cancer unit risk = 4E-4/ mg/m³ (using Global86 & assuming linearity in the low dose range)	10 ⁻⁶ Risk Dose = 2.5E-3 mg/m ³ (≈ 1 ppb)
				<i>Nonparametric method 1</i> 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/m³	10 ⁻⁶ Risk Dose = 0.7E-2 mg/m ³ (≈ 3 ppb)
				<i>Nonparametric method 2</i> 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)
<p>^a Lowest effect dose; however, in both noncancer and cancer experiments only one dose group produced a critical response.</p> <p>^b Details and assumptions of the calculations should be referred to in the text of the document. Conversion: 2.64 mg/m³/1 ppm CE. Cancer slopes for the LMS and nonparametric methods are given with the cancer calculations and are presented here in bold.</p> <p>^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.</p> <p>^d Mouse exposure = 15,000 ppm chloroethane gas for 6 hrs/day and 5 days/wk. for 100 wks. adjusted to 7,070 mg/m³ (human) see text for assumptions.</p>					

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

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

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

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

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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 Kodell, 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 [$P(d) = b_0 + q_1d + q_2d^2 + q_3d^3 + q_4d^4 \dots$] 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 $q_1^* \cdot (\text{exposure})$. There is one exception to being able to “fit” the curve, and that is when the bioassay has only one dose point (Gaylor and Kodell, 1980). Gaylor and Kodell (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 $7,070 \text{ mg/m}^3$ 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

1 replicated, the true variance is not known and the precision is unknown. In such a case where
 2 there is just one d_c and one d_0 (concurrent control), a low-dose interpolation method has been
 3 suggested (Gaylor and Kodel, 1980). For example, at the 90% response point for CE, if one
 4 assumes $\sim 100\%$ as the 95% UCL on a 90% response rate, then cancer unit slope $\approx 1.0/7,070$
 5 $\text{mg}/\text{m}^3 = 1.4\text{E-}4/\text{mg}/\text{m}^3$. It is notable this agrees with LMS and this is expected since the LMS is
 6 essentially doing the same type of calculation.

7 Some time ago it was suggested that (1) not only may MDTs (maximum dose tested in a
 8 bioassay) correlate between rats and mice, but also (2) that the MDTs for a given chemical seem to
 9 correlate (in a 30-fold range) with the respective cancer potencies (Bernstein et al., 1985). The
 10 cancer potency is abbreviated as TD_{50} and is used as a “midpoint” to characterize a cancer
 11 dose-response curve. The TD_{50} is defined as the average daily dose (mg/kg bw/day) *rate* that is
 12 estimated to halve the probability of remaining tumor free at a specified organ site in a 2 yr. study.
 13 The TD_{50} varies over a 10^8 range of dose for the various chemicals bioassayed so far.
 14 The first finding suggests that $\text{MDT} \approx \text{MTD} =$ maximum dose tolerated in a 90-day study for both
 15 rodent species. These rodent correlations suggest human parameters may also relate in a parallel
 16 manner (Allen et al., 1988). The second finding suggests that knowing the MDT for a chemical
 17 may allow an estimation of TD_{50} (cancer potency).

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

25
 26 **Table 15. Chemical-specific dose parameters**

27 MTD from 90-day study	MDT (chronic) \approx MTD	MDT \propto TD_{50}
28 $\text{TD}_{50} \propto 10^{-6}$ Risk Dose	MDT $\propto 10^{-6}$ Risk Dose	check: 10^{-6} Risk Dose $\propto 1/q_1^*$
29 Note: These relationships are characterized in Gaylor and Gold, 1995; Krewski et al., 1993; Bernstein et al., 30 1985. Specific constants relating these parameters may found in these references. A log-normal distribution is 31 assumed.		

32
 33 In an expanded study including 69 tumor sites and 38 chemicals for rats and mice (138
 34 cases) chosen for their varied chemical structures, empirical extrapolation from MDT to TD_{50} and
 35 then to 10^{-6} Risk Dose correlated with the LMS model estimates of 10^{-6} Risk Dose at 10^{-6} risk

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

7 Further analytical work examined and confirmed the properties of the empirical inverse
8 function of the upper bound on the low dose slope (b) and the MDT (Krewski et al., 1993). The
9 cancer slope “ b ” varies inversely with MDT: $\log(b) = (0.01 \pm 0.05) - (1.05 \pm 0.03) \log(MDT)$.
10 These authors also showed the TD_{50} is also related to the MDT: $\log(TD_{50}) = (1.04 \pm 0.02) \log$
11 $(MDT) - (0.10 \pm 0.04)$. It is clear, then, because TD_{50} and b are related to MDT, that TD_{50} and b
12 are related to each other: $\log(MDT) = 0.961 \log(TD_{50}) + 0.0673$. A comprehensive study
13 compiling 139 carcinogens from the NCI database showed the geometric mean of the MDT/TD_{50}
14 ratio is 0.919 for mutagens and 0.764 for nonmutagens (Gaylor and Gold, 1995). That is, the
15 TD_{50} values tend to be greater than the MDTs. Variance for 78% of the 139 chemicals is within a
16 factor of 4-fold and for 98% of them variance is within a factor of 10-fold. From the open
17 literature, representing a more diverse set of chemicals, the MDT/TD_{50} ratios are 1.46 and 0.951
18 (Gaylor and Gold, 1995). These ratios suggest, in these approximate measures, that MDT/TD_{50}
19 ratios are constant for most dose-response curves. Also suggested is that mutagens and
20 nonmutagens do not differ significantly. The variation of the MDT/TD_{50} ratio is similar to the
21 variation in cancer potency ($1/TD_{50}$), thus the MTD (90-day study) is a reasonable surrogate for
22 the TD_{50} (chronic study) (Gaylor and Gold, 1995). It follows that at 10^{-6} risk with a
23 corresponding dose of 10^{-6} Risk Dose, “ k ” may be estimated by the ratio $(MDT)/(10^{-6} \text{ Risk Dose})$
24 for a given chemical. When a larger number of chemicals (317) are considered, the geometric
25 mean constant “ k ” is 740,000, which happens to be larger (in fact two fold) than the above
26 empirical constant. The change in “ k ” is likely because more chemicals were considered in this
27 study, therefore 740,000 probably better represents the “geometric average k ” (Gaylor and Gold,
28 1995). The geometric range of 10^{-6} Risk Doses may be estimated in the range of $MDT/7,400,000$
29 $- MDT/74,000$ from the most potent carcinogens ever assayed to the least potent carcinogens, i.e.,
30 the 10^{-6} Risk Dose range is a geometric variation of 10-fold around the mean value. Obviously,
31 division by 7,400,000 yields the more conservative 10^{-6} Risk Dose estimate ($\leq 10^{-6}$ risk) and
32 would be at the low end of 10^{-6} Risk Doses of chemicals previously tested. Human exposures \leq
33 $MTD/10^7$ have been proposed as negligible risk because it would be assumed the carcinogen is
34 similar to the most potent measured (Gaylor, 1989).

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

1 ppm), there are not enough degrees of freedom to find a proper fit (NTP, 1989a). Gaylor and
2 Kodel (1980) have stated: “In the special case where only one dosage level of a chemical is
3 administered to animals, obviously no mathematical model can be obtained.” It seems prudent to
4 use a another type of method to estimate risk; at least this approach backs up the default LMS
5 method. It is estimated (see section 5.3.2.) by nonparametric method 2 that the 10^{-6} Risk Dose =
6 $1E-2 \text{ mg/m}^3$ or 4 ppb (above) and the cancer slope estimated as a check is $1.05E-4/\text{mg/m}^3$
7 ($= 10^{-6}/1E-2 \text{ mg/m}^3$) for a lifetime of CE inhalation exposure. In Table 15 (last cell) it is indicated
8 that 10^{-6} Risk Dose $\propto 1/q_1^*$, which indicates that the nonparametric method provides estimates
9 similar to those made by the LMS method: $1.05E-4/\text{mg/m}^3$ (*nonparametric #2*) $\approx 4E-4/\text{mg/m}^3$
10 (*LMS*). Therefore the Gaylor method is as conservative as the LMS method estimation of chemical
11 carcinogen risk. For further discussion see Gaylor and Gold (1995).

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

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24
25 ***