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Chloroethane Carcinogenicity (CAS No. 75-00-3)

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ABSTRACT

Chloroethane (CE; CAS No. 75-00-3) can alkylate cellular constituents but binding studies do not exist. CE is mutagenic in four tester strains of *Salmonella typhimurium,* plus and minus S9 activating fraction. A National Toxicology Program study tested CE carcinogenicity at one dose 15,000 ppm in F344/N rats and B6C3F$_1$ mice: 6 hrs/day, 5 days/wk for 102 wks. Male rats responded with basal cell carcinomas, keratoacanthomas, squamous cell carcinomas, and trichoepitheliomas. The rat skin cancer incidence is 8/46 (17%) *vs* controls at 5/49 (10%) (p=0.23) *vs* historical controls at 2/300 (0.7%) (p=2x10^{-6}). Female rats showed brain astrocytomas at an incidence of 3/50 (6%) *vs* 0/50 (0%) controls *vs* 1/297 (0.3%) in historical controls. Only comparisons with the historical control are significant. Female mice responded with malignant and metastasizing endo- and myo-metrial cancers. They spread to the lung, ovary, lymph nodes, kidney, adrenal gland, pancreas, mesentery, urinary bladder, spleen, and heart. Supporting CE carcinogenicity is that bromoethane (BE), an analogue, produces a similar spectrum of tumors—lung, pheochromocytomas, and brain tumors in F344/N rats and uterine tumors in B6C3F$_1$ female mice. The structure-activity relationship lends weight to CE carcinogenicity. The SAR, positive mutagenicity, and an exceptional degree and severity of carcinogenicity all indicate that CE causes cancer in rodents and *is probably carcinogenic to similarly exposed humans.* CE is classified by the human inhalation route as Category B2 according to the U.S. Environmental Protection Agency's 1986 Guidelines for Carcinogen Risk Assessment, and according to Proposed Guidelines for Carcinogen Risk Assessment, it is classified as *a likely human carcinogen .*

PREFACE

Chloroethane (CE) is a potentially hazardous air pollutant (HAP) that has been listed in the 1990 Clean Air Act Amendment, Section 112b. This report is an assessment of the carcinogenicity of inhaled chloroethane in rodents, F344/N rats and B6C3F$_1$ mice. This report has been prepared by the National Center for Environmental Assessment-Washington Office (NCEA-W). It represents a weight-of-evidence approach, and it represents the summary NCEA-W scientific position on chloroethane carcinogenicity.

This document was developed originally as a draft to assist the Office of Health and Environmental Assessment Air Program Committee to construct a test rule in association with the Office of Prevention, Pesticides, and Toxic Substances. On December 7, 1994, chloroethane draft was presented orally and to the Carcinogen Risk Assessment Verification Endeavor (CRAVE). A final version of the CE support document was sent to CRAVE along with an Information System (IRIS) database IRIS summary on April 24, 1995. The CE support document was reviewed again administratively in February 1997 by NCEA-W, updated, and approved in April 1998.

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1. INTRODUCTION

This report is a characterization of chloroethane (CE, CAS No. 75-00-3) carcinogenicity. CE is a potentially hazardous air pollutant (HAP) listed in the 1990 Clean Air Act Amendment, Section 112b. Alternate chemical names or trade name products are ethyl chloride, monochloroethane, Kelene, Narcotile, muriatic ether, ether chloridum, and chloryl anesthetic. The chemical formula of CE is CH_3CH_2Cl, the molecular weight is 64.51, and the structural formula is presented in figure 1.

CE is produced commercially by the free radical chlorination of ethane or by bubbling ethylene $(CH_2=CH_2)_g$ through $(HCl)_l$. CE production in the United States in 1985 was >460 million pounds (NTP, 1989a). CE can be reacted with lead and a free radical initiator to make tetraethyl lead, an antiknock compound for gasoline. The manufacturing of tetraethyl lead has been the largest source of human exposures to CE, but these exposures have declined precipitously due to reduced use of leaded gasoline. At present, chloroethane is commonly used as an industrial solvent, a chemical intermediate, and a blowing agent such as in styrene plastic manufacture. At a production level of nearly one-half billion pounds in the United States, there is potential for involuntary inhalation exposure.

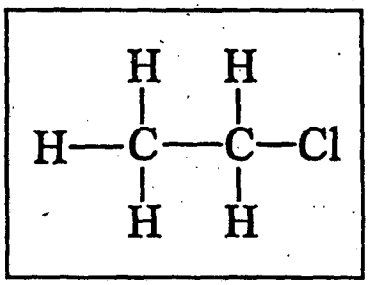

Figure 1. Chloroethane chemical structure. Chloroethane is a small, hydrophobic molecule in which C-1 is susceptible to nucleophilic attack due to the polarity of the C–Cl bond (see also figure 2).

The compound is flammable, especially in the gaseous state, and the flash point is -50°C (closed cup), and the explosive limits are 3.8% to 14.8% (v/v). Other physical properties of CE are: melting point = -136.4°C, boiling point = 12.3°C, density = 0.9214, vapor density = 2.22 (air = 1.00), and vapor pressure = 1,199 mm Hg (at 25°C). CE's volatility at 20°C is presented in table 1 (number 12), compared with the volatility of other reference gaseous compounds. The data in table 1 indicate that CE is quite volatile, and this further suggests that CE can be an inhalation toxicant wherever it is stored, handled, or disposed.

CE is chemically stable under neutral, metal-free conditions. However, CE can chemically react with water, which is bipolar, at the C-1 carbon atom (attached to the chlorine atom) even under slightly basic conditions and prolonged reaction times. A base such as OH^-,

Table 1. Vapor pressure of comparable compounds (mm Hg)

Number	Compound	Vapor pressure
1	Naphthalene	0.53
2	Ethylene dibromide	10.1
3	Water	17.3
4	Dichloroethane	60.6
5	Benzene	74.6
6	Carbon tetrachloride	76.4
7	Hexane	120.0
8	Chloropropane	278.1
9	Ethyl ether	290.8
10	Bromoethane	475.0
11	Acetaldehyde	764.3
12	*Chloroethane*	1,002.3

which is symbolized as B^- in figure 2, acts as an activator or catalyst in an S_N2 reaction, thereby generating an activated ethyl group and releasing a Cl^- ion. For example, ethyl alcohol CH_3CH_2–OH forms from CE hydrolysis where water is the nucleophile and a proton is released. It may be anticipated that, at sufficiently high input of CE in cell water, an ethanolic acid solution could be formed locally in situ that might be toxic.

CE is a colorless gas with a pungent odor that is similar to that of ethyl ether, but at high concentrations, CE gas has a burning nasal sensation and taste. The Occupational Safety and Health Administration has recommended a threshold limit of 1,000 ppm CE (2,600 mg/m^3). CE is a skin and eye irritant. The International Technical Information Institute (ITII) set the TC_{Lo} level equal to 1,300 ppm CE (ITII, 1979). Excessive inhaled CE doses lead to central nervous system suppression, headache, nausea, and lack of coordination (ataxia). Prolonged or high exposures produce feelings of inebriation, cardiac arrhythmias, unconsciousness, and cardiac arrest. The mechanism of cardiac interference is likely to be by vagal nerve stimulation, which can be reversed by atropine administration.

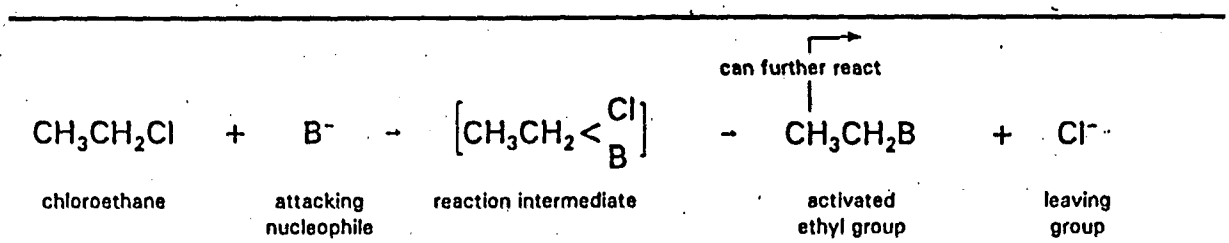

Figure 2. S_N2 mechanism of nucleophilic substitution of the chlorine in the chloroethane

CE has been used as a local dermal anesthetic in humans; however, this use in humans has declined ever since the chemical alkylation (ethylation) property of CE has been recognized (see figure 2). However, CE is still used as a local percutaneous anesthetic in veterinary procedures. The mechanism of topical anesthesia is that heat is rapidly transferred from the skin to the liquid CE raising it to the boiling point with rapid evaporation (expansion) from the skin, leaving the skin frozen.

Metabolism data for CE were not located in the literature, and this is a data gap. Based on its low molecular weight and high volatility, it is expected that inhaled CE would have free access to the compartments of the corpus. CE is expected to deposit (to some degree) in the fat depots but would not be a lingering corporal contaminant. The mixed function oxygenase system should oxidize CE by C-1 oxidation to acetaldehyde, which in turn is further oxidized to acetate. The acetate would then be catabolized from the two-carbon pool by the trichloroacetic acid cycle. Glutathione (GSH) transferases should conjugate GSH and CE to GS-ethyl for elimination. Most metabolic products should be passed through the urine.

2. CARCINOGENICITY OF CHLOROETHANE

A Health Effects Assessment (HEA) document was developed previously on CE (U.S. EPA, 1987). This 1987 document did not cite any cancer bioassays because such assays were not available at that time. Mutagenicity was reviewed, and CE was found to be mutagenic in four test strains of *Salmonella typhimurium* with and without S9 metabolic activation. Thus, using only mutagenicity data, CE was classified in the HEA as an International Agency for Research on Cancer Group 3 carcinogen, which translates to the U.S. Environmental Protection Agency (EPA) cancer classification of Category D, insufficient data to characterize the carcinogenicity of CE.

Carcinogenicity data have become available since the 1987 HEA report. A National Toxicology Program (NTP) study was started on March 17, 1982, and was reported as a final report in 1989 (NTP, 1989a). The NTP study is the only cancer study appearing in the cancer literature since 1989. The results of this NTP study (final report no. 346) provide the bases of the current EPA assessment of CE carcinogenicity by inhalation. The NTP study was designed to determine the cancer effects of inhalation exposure to CE. The lower explosive limit for CE is 38,000 ppm in air, and to be safe, the carcinogenicity testing dose was set below this limit at 15,000 ppm. Both F344/N rats and B6C3F$_1$ mice were exposed to CE at 15,000 ppm (39,000 mg/m^3) only, and no other dose group was employed. CE was introduced into the inhalation chamber as a 99.5% pure gas that was stable during the test and did not degrade. The animals were dosed with CE for 6 hours/day, 5 days/week for 102 weeks. Groups of 50 F344/N rats or B6C3F$_1$ mice (obtained from the Frederick Cancer Research Facility in Maryland) for each sex were used as control or treated animals. The treated group was compared with air-dosed control animals for carcinogenicity. Good animal husbandry and good laboratory practices were apparently observed at Battelle Pacific Northwest Laboratories where the rodent inhalation exposure study was performed.

The tissues that were necropsied were adrenal glands, brain, bronchial lymph nodes, clitoral or preputial gland, cecum, urinary bladder, esophagus, gallbladder, trachea, tissue masses with regional lymph nodes and any gross lesions, heart, thymus, thyroid, ileum, jejunum, rectum, kidneys, spleen, sternebrae, salivary glands, larynx, liver, lungs, bronchi, mammary gland, mandibular lymph nodes, snout, pancreas, parathyroid glands, pituitary, prostate, testes, and epididymis or ovaries. Tumor discovery was either from (1) early adventitial death, (2) early death by other pathologic means, or (3) planned autopsy.

3. CANCER BIOASSAY RESULTS

3.1. F344/N RATS

The male rat lifetime growth curves were assessed by measuring body weights. The male rat group exposed to 15,000 ppm CE had approximately 3% to 10% decreased body weights compared with the concurrent control group from week 40 to termination. The body weights of female rats exposed to 15,000 ppm showed a decrease of 5% to 13% compared with female controls from week 15 to study termination. Although these are not large differences, the female body weight decrement shows that the maximum tolerated dose (MTD) was approximated ($\geq$10%). Male and female survival rates were computed by the Kaplan-Meier method and showed an apparent precipitous decrement in both male control and treated groups (NTP, 1989a, p. 38). Survival at terminus was low for male rats: 32% for controls and 16% for treated group. Female F344/N rats showed better terminal survivals: 62% for controls and 44% for the treated group. However, the differences between the survival rates of controls and the treated groups in either male or female rats were not statistically significant (table 2), so a treatment-related effect on survival was not observed.

A high number of mononuclear cell leukemias were found in a number of tissues in both the control and 15,000 ppm CE groups (table 3). This leukemic condition may account for the lowered survivals in both treated and untreated groups at the end of the 2-year NTP study, but it was reasoned not to have compromised the study (NTP, 1989a).

The male and female F344/N rat tumor occurrences are listed in table 4. CE may be associated with low incidences of total skin tumors in male rats and with brain tumors in female rats. The total tumor response in male rat skin seems to show that skin and certain skin appendages are displaying a cancer response. Because skin is exposed to CE under the fur in the inhalation chamber during the 102 weeks, there is some dermal exposure. When compared with the concurrent control incidence, that is, 5/49 (10%) versus 8/46 (17%), the male rat malignant whole skin response is not statistically increased (p=0.23). The first skin tumor, a subcutaneous fibroma, occurred at 79 weeks in the treated group. Moreover, the rates are not significantly increased in the treatment group when rates are adjusted for animals dying before the first skin tumor. The comparison in this case is 5/42 (12%) versus 8/42 (19%), p=0.27.

When the skin tumors of the treated group are compared with those of the historical inhalation controls from the same testing laboratory, there is a statistically significant increase in

Table 2. Survival of F344/N rats at 2 years[a]

Sex/treatment groups	Controls		15,000 ppm chloroethane		Probability of survival effect (p)
	Survival	$S_{1/2}$	Survival	$S_{1/2}$	
Males	16/50 (32%)	98	8/50 (16%)	92	0.161
Females	31/50 (62%)	—	22/50 (44%)	97	0.083

[a] Survival is defined as the number of animals alive at study termination divided by the starting number of animals in that group. The percentage survival is presented in parentheses. $S_{1/2}$ is the time in weeks that it takes to decrease to 50% survival compared with the start of the study. When survival is >50% at study termination, no $S_{1/2}$ exists by definition, and a dash is indicated.

Table 3. Rat leukemia incidence (2-year bioassay)

Sex	Controls	15,000 ppm chloroethane	Probability of survival effect (p)
Males	33/50 (66%) (87.6% adj.)	36/50 (72%) (96.9% adj.)	0.33
Females	20/50 (40%) (48.1% adj.)	26/50 (52%) (52% adj.)	0.16

epithelial cancers: 2/300 (0.7%) versus 8/46 (17.4%), $p=2\times10^{-6}$. Similarly, when NTP controls from noninhalation historical experiments are compared with the treated group (28/1,936 [1.4%] vs. 8/46 [17.4%], $p=8\times10^{-5}$), there is also a statistically significant increase in epithelial skin tumors.

Historical incidence rates can be characterized. For example, tumor incidences may be subjectively ranked: (1) incidence rates <0.5% are rare, (2) incidences occurring >0.5% but <2% may be considered uncommon, and (3) incidences >2% are generally common to aging test rodents. These definitions are operational, not absolute, and they represent expert judgment. In this bioassay, the historical malignant skin tumor incidence is 0.7%, and NTP incidence is 1.4% where both are designated as uncommon tumor incidences. On the other hand, the above *observed* control skin incidence is 10% (5/49) (table 4). Comparing either the observed or historical control incidences to the treated group incidences lead to different conclusions: there

Table 4. Tumors of F344/N rats at 2 years

Sex	Controls	15,000 ppm chloroethane	Estimate of p value*
Males	keratoacanthoma = 4/49 (8%) fibroma = 1/49 (2%) *total = 5/49 (10%)*	basal cell carcinomas = 3/46 (7%) keratoacanthoma = 2/46 (4%) squamous cell carcinoma = 1/46 (2%) trichoepithelioma = 1/46 (2%) lip, squamous cell carcinoma = 1/46 (2%) *total = 8/46 (17%)*	0.23
	adjusted to first appearance of tumor (79 weeks) (42 males) tumor incidence = 5/42 (12%)	adjusted to first appearance of tumor (79 weeks in treated group) (42 males) tumor incidence = 8/42 (19%)	0.27
	skin historical controls = 2/300 (inhalation) (0.7%)	see above, 8/46	2.0×10^{-6}**
	skin historical controls = 30/1,936 (noninhalation) (2%)	see above, 8/46	1.3×10^{-6}**
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**
	historical astrocytoma controls = 23/1,969 (all studies) (1.1%)	see above, 3/50	0.02**

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

** Designates statistical significance in a Fisher's exact test comparison. Data taken from NTP report no. 346 (NTP, 1989a).

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

In the female rats, brain astrocytomas occurred at a low incidence of 3/50 (6%) (table 4). In analyzing the significance of this low incidence brain tumor, it is known that astrocytomas are not common in most strains of rat or in humans. So low incidences could be a sign of carcinogenicity. There is extra concern when they do occur because such a tumor type in the

brain has fatal implications in rodents and humans. When compared statistically with the concurrent control (0/50 [0%] vs. 3/50 [6%]), the response yields statistical insignificance (p=0.12), which suggests that there *may* be no effect. The same may be stated when the adjusted rates are examined by subtracting the number of animals dying before the first astrocytoma appears (52 weeks): 0/46 versus 3/49, p=0.12.

When rare tumors occur, the tumor rates require special consideration. Uncommon or rare tumor incidences *may* not indicate a statistical increase when compared with their respective concurrent control incidences. This is because the number of trials (i.e., the number at risk in the control and treated groups) is small, ≈50/sex/group, and a larger number of animals (in this case, at the 95% level of confidence, ≈150/sex/group) is needed to statistically score a rare tumorigenic event. Accordingly, when the observed incidence (3/50) is compared with historical pooled control incidence (1/297) from the same testing laboratory (Battelle Pacific Northwest Laboratories), the statistically significant increase in astrocytomas is p=0.01 (table 4). Note that the larger denominator affects the statistical inference in the case of rare tumors. Similarly, when the observed 3/50 astrocytomas in female F344/N rats are compared with the incidence of all experimentally discovered astrocytomas in NTP studies (23/1,969), the statistical significance is p=0.02 (table 4).

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

Further analysis shows, however, that Battelle Pacific Northwest Laboratories had a singular prior incidence of 3/50 (6%) astrocytomas in a female concurrent control group of F344/N rats. This singular control brain tumor incidence happens to be commensurate with the brain response in the 15,000 ppm CE group (table 4). Thus, if a past concurrent control incidence can reach as high as 3/50 (6%), the apparent statistical significance of the dosed group response—also an incidence of 3/50 (6%)—becomes less important. Moreover, in past NTP studies, the average astrocytoma incidence is 0.9% (18/1,969) and the range is 0% to 6% in female F344/N rats. Here, too, it is observed that an incidence level as high as 6% of astrocytoma cancers may be observed in concurrent controls.

It is determined, then, that this female rat astrocytoma effect may be real but is marginal if it is real. Sensitivity analysis indicates that only one more rat with an astrocytoma would have

shifted the concern for a real response. Therefore, the female rat brain response is designated as equivocal evidence for carcinogenicity.

It is concluded that both the male F344/N rats (skin tumors) and female rats (brain tumors) present equivocal sets of evidence for carcinogenicity. This means that the rat data are not negative, but they cannot be used in regulatory decisions as a positive bioassay cancer site. However, both marginal sites of skin and brain may suggest clues to the mechanism of action of CE carcinogenicity.

3.2. $B6C3F_1$ MICE

The growth was comparable between the no-dose control and the 15,000 ppm CE-treated groups, as measured by mean body weights for male and female $B6C3F_1$ mice. Mice survivals are shown in table 5. Survivals in the 15,000 ppm group were significantly lower than survivals in the control mice (NTP, 1989a) for both the males and females. Male mice died earlier than the females: male mice survivals reached the 50% survival rate at 73 weeks, whereas female mice reached the 50% survival rate at 89 weeks.

There were few cancer incidence observations that could be interpreted as carcinogenic responses in the male mice. Generally, the observed male $B6C3F_1$ mice cancer occurrences are considered random and usual for aging mice of this strain (table 6). There was the suggestion of a male lung response, with 10/48 (20.8%) responding versus 5/50 (10%) in control male $B6C3F_1$ mice (p=0.11). The lung tumors were benign and composed of mostly adenomas (8/10 [80%] in

Table 5. Survival of $B6C3F_1$ mice at 2 years[a]

	Controls		15,000 ppm chloroethane		Probability of survival effect (p)
Sex	Survival	$S_{1/2}$	Survival	$S_{1/2}$	
Males	28/50 (56%)	—	11/50 (22%)	73	3.91×10^{-3}
Females	32/50 (64%)	—	2/50 (4%)	89	$<10^{-8}$

[a] Survival is the number of animals alive at study termination divided by the starting number of animals in that group. The percentage survival is presented in parentheses. $S_{1/2}$ is the time in weeks that it takes to decrease to 50% survival compared with the start of the study. When survival is >50% at study termination, no $S_{1/2}$ exists by definition and a dash is indicated; the survival only is presented for this group in the previous column.

Table 6. Tumors of B6C3F$_1$ mice at 2 years

Sex	Controls	15,000 ppm chloroethane	(p)
Males	early deaths, urinary tract infections, no tumors of interest	early deaths, urinary tract infections, no tumors of interest	no male cancer effects
Females	uterine carcinoma = 1/49 (2%) (not endometrial)	uterine carcinomas = 43/50 (86%)	$<10^{-8*}$
	uterine carcinoma = 1/46 (corrected for time to 1st tumor, which was at 67 weeks)	uterine carcinoma = 43/48 (corrected for time to first tumor, which was at 67 weeks)	$<10^{-8*}$
	historical controls = 4/1,371 (inhalation studies)(0.29%)	cf. above, 43/50	$<10^{-8*}$
	historical controls = 3/951 (corn oil)(0.32%)	cf. above, 43/50	$<10^{-8*}$
	uterine lymphomas = 1/49 (2%)	uterine lymphomas = 7/50 (14%)	0.03*

* Designates statistical significance in a Fisher's exact test comparison. Data taken from NTP report no. 346 (NTP, 1989a).

the treated and 3/5 [60%] in the control groups). The corrected lung incidence (adenomas + carcinomas) comparison for animals dying before the first tumor is 5/28 controls versus 10/30 treated, p=0.15. This oncogenic response is a marginal—to not significant—oncogenic lung response. Moreover, many B6C3F$_1$ males died before study termination, thus decreasing the power of the treatment incidence comparison with male control incidence. It is notable that inhaled bromoethane, a structural analogue, causes significant lung cancers (see section 4, Discussion, and NTP, 1989b).

Urogenital infections occurred in the male treated group (observed as suppurative inflammation, and necrosis) (table 6). These infections may have contributed to the early male mouse deaths in the 15,000 ppm CE group. There can be no assurance that tumors may not have been caused by CE *if* the treated male mice had lived long enough to develop tumors. That is, the test has reduced statistical power to the point where any cancer inferences are compromised.

Therefore, the male B6C3F$_1$ mouse lung response is considered inadequate to determine inhalation carcinogenicity.

The female B6C3F$_1$ mouse survival also was reduced significantly ($p<10^{-8}$, table 5). The study diagnosis in female mice is that they died of complications caused by uterine carcinomas (NTP, 1989a). Therefore, reduced survival in female mice was not incidental but rather was due to the onset of cancer. The female B6C3F$_1$ mice responded to inhaled CE with 43 primary endometrial tumors out of a total of 50 female mice (table 6). This is a primary uterine cancer incidence of 86%. The endometrium of the uterus is the mucous and glandular lining that contains columnar epithelial cells and is surrounded by a smooth muscular layer called the myometrium. The endometrial tumors caused by CE were highly malignant because they (1) spread from the endometrium to the surrounding myometrium, and (2) upon tumor progression, then metastasized to many distal organs. Second-site tumors, which had their origin in the uterine primary site, were observed in 34/43 female B6C3F$_1$ mice (79% of responders). That is, 68% of the original 50 treated female B6C3F$_1$ mice responded with frank, malignant, and metastasizing cancers. This uterine carcinogenic response and the subsequent metastases show clear evidence of carcinogenicity caused by CE in female B6C3F$_1$ mice.

There was an additional primary liver carcinogenic (6%) response in female B6C3F$_1$ mice. Control liver rates were 0/49 (0%) adenomas and 3/49 (6%) hepatocellular carcinomas, whereas the 15,000 ppm-treated group had 1/48 (2%) adenomas and 7/48 (15%) carcinomas. The combined liver response is 3/49 (6%) and 8/48 (17%), which is a significant difference from control liver rates (p=0.025). There were increases in hematopoietic cancer involvement with CE treatment, including increases of a number of white cell types in bone marrow, lymph nodes (uterine, iliac, mediastinal, mandibular), spleen, and thymus. These effects are difficult to differentiate from the secondary metastatic effect or second primary site effects by CE. Nevertheless, these responses lend support to the powerful carcinogenic effects of CE in female mice.

The organ types and the number of female B6C3F$_1$ mice affected by metastasized uterine cells were:

- Lung (23)
- Ovary (22)
- Lymph nodes (18)
- Kidney (8)
- Adrenal gland (8)
- Pancreas (7)
- Mesentery (7)
- Urinary bladder (7)
- Spleen (5)
- Heart (4)

Organs that had disseminated uterine cells, but to a lesser extent, were:

- Colon
- Liver
- Stomach
- Small intestine
- Gallbladder
- Ureter

3.3. MUTAGENICITY OF CHLOROETHANE

Genotoxicity is useful in assessing carcinogenicity of a suspected environmental carcinogen. CE has tested positive for mutagenicity in Salmonella in two studies, one by Zeiger et al. (1992) and the other by NTP (1989a). In both cases, the experiments were carried out in desiccators because of the volatility of CE. Zeiger et al. (1992) tested CE and 310 other chemicals under code in the presence or absence of liver S9 from Aroclor-induced male Sprague-Dawley rats and Syrian hamsters. CE was tested at 0.002 to 0.017 moles per desiccator in strains TA 100 and TA 1535 in Ames assays. In strain TA 100, CE produced less than a twofold maximal increase in mutation over background in the absence of exogenous metabolic activation or in the presence of rat liver S9. These were questionable responses. There was nearly a twofold maximal increase with hamster liver +S9, which is a weak response. However, CE was clearly positive in strain TA 535; maximal increases were 4.5-fold in the absence of S9, 6-fold with hamster S9, and 7-fold with rat S9, all with dose-response relationships.

Genetic toxicology studies described in the NTP bioassay report on CE (NTP, 1989a) were carried out in strains TA 98, TA 100, and TA 1535 in the absence or presence of S9 as described above in Zeiger et al. (1992). The doses tested were 10 and 20 μg per plate. Testing in strain TA 100 was negative without S9, equivocal with hamster S9, and positive with rat S9. Testing in strain TA 1535 was clearly positive with and without hamster or rat S9; increases in mutation over background ranged from 7-fold to 34-fold.

In addition to the above two studies, Ricco et al. (1983) published an abstract. CE was tested in strains TA 98, TA 100, TA 1535, and TA 1537 with and without Aroclor 1254-induced S9 derived from male and female Osborne-Mendel rats and B6C3F$_1$ mice. As in the studies described above, this study also was performed in desiccators. The bacteria were exposed to CE vapor over at least three dose levels. The abstract states that CE was mutagenic both with and without metabolic activation. It did not state the dose levels or the Salmonella strains that tested positive. No data were presented.

4. DISCUSSION

CE is a volatile industrial solvent that has narcotic and toxic properties at high inhaled doses (NTP, 1989a). A 2-year cancer bioassay in rodents has been reported and is the sole source of the current surrogate cancer toxicology on CE (NTP, 1989a). This report includes studies in F344/N rats and B6C3F$_1$ mice. The nonstandard NTP protocol (only one-dose group), but with apparently good laboratory practices, was used by Battelle Pacific Northwest Laboratories.

The results suggest that male F344/N rats may not have significantly responded with whole skin tumors (epidermis, dermis, and appendages) (table 4). Concurrent controls are considered the most relevant comparison unless something is known to be experimentally wrong with the concurrent control group. Because nothing was reported to be wrong with the concurrent control, the adjusted concurrent control comparison is being used (p=0.23), which suggests a lack of cancer effect in the skin of male F344/N rats. Whereas historical control comparisons suggest statistically increased skin carcinogenic responses in male rats, the concurrent control comparison indicates no positive carcinogenicity in male rat skin. This historical and concurrent control comparison indicates that the male F344/N rat skin cancer response is a marginal cancer effect, at most.

The female F344/N rat astrocytoma response is also equivocal because a low-level response of 6% was found (0/46 [0%] vs. 3/49 [6%], p=0.12). However, astrocytomas are uncommon cancers in rodents and are rare in humans (personal communication, A. Koppikar, NCEA-W/ORD/U.S. EPA). Astrocytomas are often malignant tumors, sometimes invasive, and contain varying amounts of fibrillar stroma. They are tumors of concern when they occur in test animals. The response was only 3/50, which presented no significance in a Fisher's exact test when compared with concurrent control female rats (0/50), but it did show statistical significance when similarly compared with historical control F344/N rats (table 4). Experience at Battelle Pacific Northwest Laboratories, where the bioassay was conducted, with a single control group with 3/50 astrocytomas (the same as the responding group in the current bioassay) suggests that the historical control comparison may be less important than the concurrent control comparison. Moreover, an upper-range limit of astrocytoma occurrence of 6% in NTP historical control astrocytoma incidences also suggests the current putative brain response is equivocal.

The male F344/N rats showed equivocal skin effects, and the female F344/N rats showed equivocal evidence in the brain. Both the skin and brain cancer responses are suggestive, mainly by comparisons with historical controls, but they are only marginally positive at the most or are false negatives at the least.

Survivals were poor in male $B6C3F_1$ mice, but tumor occurrences are what one would expect in aging 2-year-old male mice. There was the suggestion of a lung response in male mice (mostly adenomas and not carcinomas). However, the lung rates adjusted for mortality were 5/28 in treated versus 9/30 in control groups. These rates suggested no lung response (p=0.22). Because so many male mice died before study termination, there can be no assurance that more lung tumors might not have resulted if all males had lived to the end of the bioassay. That is, the statistical power was sufficiently reduced so as to compromise the male mouse results being used to infer cancer response. Thus, in this study the male $B6C3F_1$ mice results are considered not declarative and are inadequate to determine carcinogenicity in humans.

There was a marginal liver response (first tumor at 81 weeks) in female $B6C3F_1$ mice inhaling 15,000 ppm (3/45 vs. 7/37, p=0.09), but this was not considered a statistically relevant cancer response in this group. However, a strong uterine carcinogenic response was observed in female $B6C3F_1$ mice (0/49 vs. 43/50, $p=<10^{-8}$). These uterine tumors were highly malignant, metastatic, and aggressive. Dissemination occurred in many distal organ sites, that is, 16 sites. The complications from these tumors were reasoned to be the cause of poor survival in these female $B6C3F_1$ mice (NTP, 1989a). These earlier-than-normal cancer-related deaths lend even more credence to the carcinogenic effects of CE in female $B6C3F_1$ mice. The data indicate clear evidence of CE carcinogenicity in female $B6C3F_1$ mice, which is determined to be useful in predicting human cancer.

A summary of the rodent surrogate cancer results is presented in table 7. The information presented indicates equivocal carcinogenicity in the F344/N rat (male and female) and strong evidence for carcinogenicity in the female $B6C3F_1$ mouse.

Structure-activity relationships (SARs) are useful in assessing CE carcinogenicity. Bromoethane (CH_3CH_2Br) is a structural analogue to CE. Bromoethane is a volatile compound but less so than CE (table 1). While bromoethane has not been categorized as to carcinogenicity, it was tested for carcinogenicity at inhaled concentrations of 100 ppm, 200 ppm, and 400 ppm (NTP, 1989b). Female $B6C3F_1$ mice responded to inhaled bromoethane with uterine adenocarcinomas, carcinomas, and squamous cell carcinomas. The uterine responses at 100 ppm, 200 ppm, and 400 ppm doses were 4/50 (8%), 5/47 (11%), and 27/48 (56%) respectively (NTP, 1989b). When the control incidence, 0/50 (0%), is compared with the 400 ppm dose

Table 7. Summary of tumors in F344/N rats and B6C3F$_1$ mice at 2 years[a]

Sex	F344/N rat	B6C3F$_1$ mouse
Males	Marginal evidence ■ skin tumors (∓)	Inadequate for carcinogenicity determination (0)
Females	Equivocal evidence ■ brain tumors (±)	Clear uterine cancer evidence and metastasis to 16 secondary organ sites. Weak liver primary response. Hematopoietic response in a number of tissues and lymph nodes. (strong positive)

[a] Conclusions based on results and data taken from NTP report no. 346 (NTP, 1989a).

incidence, 27/48 (56%), a statistically significant increase in uterine cancer is observed ($p = <10^{-8}$). Bromoethane causes uterine cancer in mice, just as CE causes uterine cancer in mice.

Bromoethane also causes low-level brain tumors in male rats: 0/45 in controls and 3/50 (6%) at 100 ppm, 0/50 at 200 ppm, and 0/50 at 400 ppm. This is not a statistically significant cancer trend. In the low-dose bromoethane group (100 ppm), the response level was the same as the 15,000 ppm CE inhalation level. Male F344/N rats responded to inhaled bromoethane with 5 granular cell brain tumors in 150 rats summed over the 3 dose groups (0/48 in controls and 3/50, 1/50, and 1/50 in the treated groups). Again, this is not a positive trend statistically but rather is a low-dose response. No tumors of this type have been seen at Battelle Pacific Northwest Laboratories, and only 0.2% have been seen in all the NTP studies. Gliomas, including astrocytes, occurred in 3/150 bromoethane-treated rats. This brain tumor response in bromoethane-treated (inhalation) rats also demonstrates organ site concordance to the brain response in chloroethane-exposed (inhalation) rats.

Taken together, the structural analogues CE and bromoethane have in common the following: a uterine and low-incidence-level brain response. The conjunction of these results—both uncommon tumor types— in different bioassays indicates a response pattern that is unlikely to occur by chance alone. Both the uterine *and* brain carcinogenic responses demonstrate (1) organ site concordance in rodents, (2) the involvement of similar cancer mechanisms between the two haloethanes, (3) the replication of these respective bioassays, and

(4) a pattern of carcinogenicity applicable to assessing cancer hazard for these monohalogenated ethanes in humans.

Further structural comparisons show that 1,2-dichloroethane (Category B2) produced a marginal uterine carcinogenic response in female mice gavaged for 78 weeks (NCI, 1978). The doses and uterine adenocarcinoma responses in these female mice were 148 mg/kg (3/49) and 299 mg/kg (4/47). The uterine tumors were not statistically increased. If this experiment had been prolonged to 2 years as in the CE bioassay, more uterine tumors may have resulted from 1,2-dichloroethane. Therefore, the SAR comparison to 1,2-dichloroethane is only suggestive. It is notable that higher halogenated ethane analogues (1,1-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, pentachloroethane, and hexachloroethane) do not appear to cause uterine tumors.

The direct effect on the respiratory tract of halogenated ethanes, including CE, is not clear. There does not appear to be a CE-induced respiratory neoplastic response in the NTP inhalation bioassay. Other portions of the respiratory tract, such as the nasal cavity, do not seem to be responding to CE exposure with a chronic oncogenic response. This is not true for bromoethane and dibromoethane (ethylene dibromide); both of these compounds cause respiratory lesions and neoplasms (NTP, 1989b). It is not known why CE does not affect the respiratory system at 15,000 ppm.

CE is a direct, base-pair substitution mutagen in Salmonella. This direct mutagenicity makes CE like other alkylators a candidate carcinogen by a direct DNA-based mechanism. In vitro and in vivo studies in mammalian mutagenicity systems are needed to further characterize the mutagenic potential of this chemical.

The monosubstituted ethanes, such as CE, *can* form primary alkyl carbonium ions (CH_3CH_2+). This requires a formal charge separation in moving Cl^- away from CH_3CH_2+ ions. Primary alkyl carbonium ions are relatively unstable compared with tertiary carbonium ions, for example, *tert*-butyl carbonium ion. As such, the CE carbonium ion, if it forms at all, would have a relatively short half-life in solution. A short ion half-life means fewer chances to react in the active carbonium ion state. Thus, the primary carbonium ion concentration would have to be higher to be toxic by ethylation. This pathway mechanism is likely to be minor or nonexistent.

Another potential metabolic mechanism is that CH_3CH_2Cl can react by an S_N2 reaction with a cellular intermediate that is basic or is a nucleophile, making it electron-rich. For example, chemical intermediates are normally and purposely activated this way in biosynthesis. Similarly, the CH_3CH_2Cl could react with a basic intermediate *B:* to form the intermediate, and then react with nucleophiles in the cell such as DNA and proteins to form altered cellular

macromolecules (figure 3). These chemical changes may then lead to oncogenic sequelae. Relevant macromolecular conjugation information was not found in the literature, and it exists as a CE data gap.

Under oxidative conditions, CE can react with cellular water: $CH_3CH_2Cl + H_2O \rightarrow CH_3CH_2OH + HCl$. The ethyl alcohol from CE metabolism can proceed to acetaldehyde and then finally to acetic acid. Under other conditions, B^- can be a nucleotide (in DNA or RNA) or a cellular protein, and these macromolecules are ethylated. Glutathione (GSH) would be expected to transfer the ethyl group from GS–ethyl. If these ethylation events are in excess of normal excision and/or repair metabolism, then toxicity can be expected to ensue. Low-level ethylations are likely managed by the cell so that toxic effects, including cancer, do not ensue.

It might be expected that, since CE (15,000 ppm) and bromoethane (400 ppm) both cause a similar pattern of oncogenicity in brain, skin, and mainly uterine cancer, there may be a hormonal mechanism of action causing tumor promotion and progression. Working on this "hormonal" thesis, Bucher et al. (1995) looked for changes in estrous cyclicity in $B6C3F_1$ females and did not find significant differences in the mean estrous cycle length (≈5.1 days) at the cancerous doses of either haloethane. Minor changes in time observed among proestrus, estrus, metestrus, and diestrus, when carefully compared with controls, were judged to be not significant. Neither circulating estradiol nor progesterone levels measured in these studies varied significantly with doses commensurate with oncogenicity (Bucher et al., 1995). Bucher and his colleagues interpreted this to mean that the uterine cancer responses were not based on predisposed changes in uterine hormones or in the estrous cycle. Later occurring hormone effects (i.e., >21 days) still may be involved and are not ruled out by these studies. Bucher and his colleagues speculated, that since there is no reason to suspect specific uterine organ sequestration of CE or its metabolites, there must be other mechanisms such as oncogene

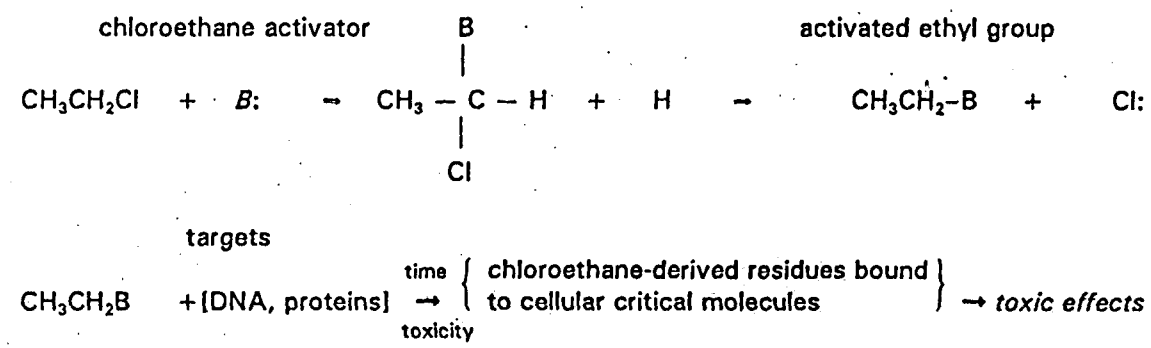

Figure 3. Potential mechanism of carcinogenesis of chloroethane.

activation or mutation spectrum that should be further explored (Bucher et al., 1995). Moreover, we are most curious whether this strong uterine cancer response in mice is site concordant with humans, that is, do they share the same mechanism and site of action?

CE's metabolic mechanisms remain to be directly demonstrated by experiments. The postulated mechanisms presented here should be considered when the cancer response of this xenobiotic is considered. These mechanisms likely become more important at high CE exposure levels, whereas at low exposure levels CE may be accommodated by normal steady-state xenobiotic metabolism. This is not known at this time. Metabolic information is needed on the dosimetry of CE's metabolic intermediates and their cellular effects, especially concerning oncogene activation, suppressor inactivation, DNA alkylation, and mutation spectra.