LANL Ref

1, 2, DCE_ mammel_ 0685

TOXICOLOGY AND APPLIED PHARMACOLOGY 63, 409–421 (1982)

0685

Effects of 1,2-Dichloroethane and 1,1,1-Trichloroethane in Drinking Water on Reproduction and Development in Mice[1]

RICHARD W. LANE,[2] BARRY L. RIDDLE, AND JOSEPH F. BORZELLECA[3]

Division of Toxicology, Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Received August 31, 1981; accepted December 22, 1981

Effects of 1,2-Dichloroethane and 1,1,1-Trichloroethane in Drinking Water on Reproduction and Development in Mice. LANE, R. W., RIDDLE, B. L., AND BORZELLECA, J. F. (1982). *Toxicol. Appl. Pharmacol.* **63**, 409–421. A multigeneration reproduction study was modified to include screening for dominant lethal and teratogenic effects of 1,2-dichloroethane (1,2-DCE) and 1,1,1-trichloroethane (1,1,1-TCE) in drinking solution (Emulphor:deionized water, 1:99, v/v). Male and female ICR Swiss mice received either 1,2-DCE at concentrations of 0, 0.03, 0.09, or 0.29 mg/ml or 1,1,1-TCE at concentrations of 0, 0.58, 1.75, or 5.83 mg/ml. These concentrations were designed to yield daily 1,2-DCE doses of 0, 5, 15, or 50 mg/kg and 1,1,1-TCE doses of 0, 100, 300, or 1,000 mg/kg. No taste aversion was evident for either of the chemicals at any concentration. There appeared to be no dose-dependent effects on fertility, gestation, viability, or lactation indices. Pup survival and weight gain were not adversely affected. 1,2-DCE and 1,1,1-TCE failed to produce significant dominant lethal mutations or terata in either of the two generations tested.

1,2-Dichloroethane (ethylene dichloride; 1,2-DCE) is used industrially as a solvent, chemical intermediate, and gasoline additive; it is used agriculturally as a fumigant of stored grains (IARC, 1979a). 1,1,1-Trichloroethane (methyl chloroform; 1,1,1-TCE) is used as a degreaser and chemical intermediate (IARC, 1979b). Both chemicals are found in drinking water (U.S. EPA, 1975). Because these chlorinated hydrocarbons are ubiquitous in the environment, men and women may be exposed to them during their reproductive years. A review of the literature revealed a paucity of data concerning the effects of these chemicals on reproduction.

Human exposure to 1,2-DCE may result in central nervous system depression, anorexia, nausea, abdominal pain, and dysfunction of the hepatic, renal, and hematic systems (IARC, 1979a). 1,2-DCE is mutagenic in *Salmonella typhimurium* strains TA100, TA1530, and TA1535 and causes sex-linked recessive lethality in *Drosophila melanogaster* (IARC, 1979a; Rannung, 1980). No genetic effects were observed in *Aspergillus nidulans*, in various strains of *Escherichia coli*, or in the mouse micronucleus test (IARC, 1979a; Rannung, 1980). Metabolically activated 1,2-DCE binds to protein and to DNA (Banerjee *et al.*, 1980). 1,2-DCE administered by gavage to B6C3F1 mice (195 mg/kg/day for high-dose males and 299 mg/kg/day for high-dose females) for 78 weeks was tumorigenic (National Cancer Institute, 1978). Male and female Osborne Mendel rats receiving a high dose

[1] This investigation was supported in part by EPA Grant R-806481. Presented in part at the Annual Meeting of the Society of Toxicology, San Diego, California, March 1981.

[2] Supported by NIEHS Training Grant IT32E507087.

[3] To whom requests for reprints should be addressed.

0041-008X/82/060409-13$02.00/0
Copyright © 1982 by Academic Press, Inc.
All rights of reproduction in any form reserved

9332

TABLE 1

PREPARATION OF DOSING SOLUTIONS

Compound	Group	Concentration (mg/ml)	Nominal dose (mg/kg/day)
1,2-Dichloroethane[b] (1,2-DCE)	Naive control	DW[d]	0
	Emulphor vehicle control	0.00 in EDW[e]	0
	Low concentration	0.03 in EDW	5
	Mid concentration	0.09 in EDW	15
	High concentration	0.29 in EDW	50
1,1,1-Trichloroethane[c] (1,1,1-TCE)	Naive control	DW	0
	p-dioxane-Emulphor vehicle control	0.00 in 0.17 mg/ml *p*-dioxane in EDW	0
	Low concentration	0.58 in EDW	100
	Mid concentration	1.75 in EDW	300
	High concentration	5.83 in EDW	1,000

[a] Calculated on the basis of a 6 ml/mouse/day average fluid consumption for a 35-g mouse.
[b] Aldrich Chemical Co., Milwaukee, Wisc.; 99 + % pure.
[c] Aldrich Chemical Co., Milwaukee, Wisc.; 97% pure, inhibited with 3% *p*-dioxane.
[d] Deionized water.
[e] 1% Emulphor in DW.

of 95 mg/kg/day by gavage for 78 weeks also developed an increased incidence of squamous cell carcinomas in males, mammary gland adenocarcinomas and fibroadenomas in females, and hemangiosarcomas in both sexes (National Cancer Institute, 1978). The tumorigenic effects were not seen following lifetime inhalation exposure to 1,2-DCE (Maltoni, 1980, cited in Guengerich *et al.*, 1980). There is no indication of carcinogenicity in humans (IARC, 1979a). One reproduction study (Alumot *et al.*, 1976) concluded that there were no adverse effects on male or female rats that received 12.2 and 24.5 mg 1,2-DCE/kg/day in fumigated food from 6 weeks to 2 years. Another reproduction study (Rao *et al.*, 1980) found that 60 days of exposure (both males and females) to 15, 75, or 150 ppm of 1,2-DCE vapor produced no adverse effects in rats. The same group (Rao *et al.*, 1980) found that 100 and 300 ppm of 1,2-DCE vapor caused no teratogenic effects, even at maternally toxic doses, in rats and rabbits.

1,1,1-TCE causes central nervous system depression; hepatotoxicity has been reported only at doses near the LD_{50} (IARC, 1979b). 1,1,1-TCE is weakly mutagenic in *Salmonella typhimurium* TA100 with and without microsomal activation (Simmon *et al.*, 1977). There is no indication that 1,1,1-TCE is carcinogenic to mice, rats (National Cancer Institute, 1977), or humans (IARC, 1979b). No data on the reproductive effects of 1,1,1-TCE were found in the literature. Fetal development in mice and rats was not affected by 7 hr of exposure to 875 ppm 1,1,1-TCE on Days 6 to 15 or gestation (Schwetz *et al.*, 1975). Abnormal fetal development and fetal death were exhibited by chicks exposed to 5 to 100 μmol 1,1,1-TCE injected into the air space of the egg (Elovaara *et al.*, 1979).

No studies with either compound have been reported in which the drinking water was the method of exposure. The following studies were therefore performed to determine whether 1,2-DCE or 1,1,1-TCE would affect male or female reproductive function or fetal development when administered subchronically in the drinking water.

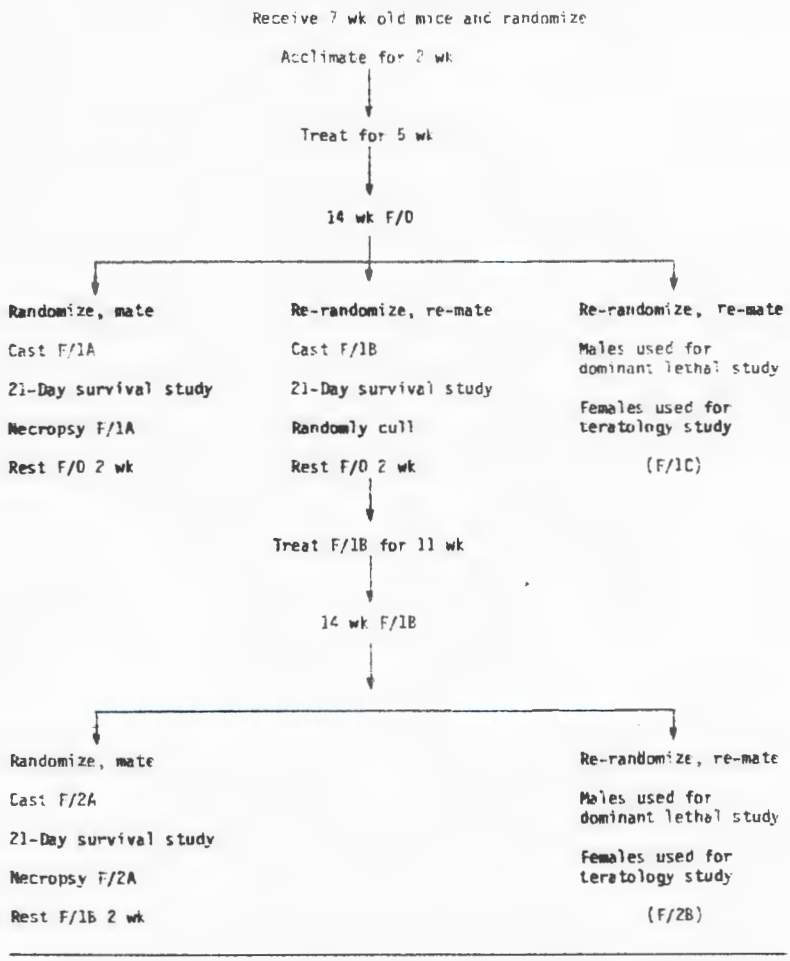

FIG. 1. Flowchart of the modified multigeneration/reproduction protocol used in this study.

METHODS

Animal husbandry. Seven-week-old ICR Swiss mice Flow Laboratories, Dublin, Va.) were acclimated for 2 weeks, randomly assigned to groups, and marked by oe clipping. The mice were housed on sawdust bedding n polycarbonate cages. The environment was mainained at 22 to 23°C with 40 to 60% relative humidity nd 12 hr of light per day. The mice were allowed food Purina Rodent Laboratory Chow 5001) and drinking olution (see below) *ad libitum*. Males were housed singly; females were kept three per cage, except during arturition and lactation, when they were housed one er cage. Males and females were co-housed (1:3, respectively) for 7 days at each mating. Litters were weaned at 21 days of age.

Preparation of drinking solutions. 1,2-DCE (Aldrich Chemical Co., Milwaukee, Wisc.; 99+% pure) and 1,1,1-TCE (Aldrich Chemical Co., 97% pure, inhibited with 3% *p*-dioxane) were dissolved in a 1% solution of Emulphor EL-620 (GAF Corp., Linden, N.J.) in deionized water. This vehicle was necessary due to the limited solubility of the test materials in water. No aversion (decreased fluid consumption) to the vehicle or either haloalkane was observed. Fresh drinking solutions were prepared twice weekly, according to the specifications in Table 1, and were placed in 225-ml amber glass bottles with cork stoppers and stainless steel drinking tubes. Levels of 1,2-DCE and 1,1,1-TCE were based on acute male oral LD_{50} data (unpublished data). The highest concentration was chosen to provide a nominal daily dose (based on a 35-g adult mouse consuming 6 ml

TABLE 2

REPRODUCTIVE PERFORMANCE OF ADULT MICE INGESTING 1,2-DICHLOROETHANE OR 1,1,1-TRICHLOROETHANE

Compound	Concentration (mg/ml)	Litter					
		F/1A		F/1B		F/2A	
		FI[a]	GI[b]	FI	GI	FI	GI
1,2-Dichloroethane (1,2-DCE)	0.00[c]	90.0	92.6	70.0	71.4	76.2	100.0
	0.00[d]	93.3	82.1	76.7	78.2	86.2	96.0
	0.03	89.3	92.0	89.3	84.0	93.1	81.5
	0.09	82.8	83.3	62.1	94.4	82.8	100.0
	0.29	90.0	85.2	70.0	90.5	85.2	78.3
1,1,1-Trichloroethane (1,1,1-TCE)	0.00[c]	90.0	92.6	73.3	90.9	85.2	82.6
	0.00[e]	96.7	92.6	90.0	66.7	70.0	100.0
	0.58	96.7	89.7	82.7	87.5	84.6	90.9
	1.75	90.0	85.2	85.7	83.3	76.7	95.7
	5.83	82.8	75.0	85.2	78.3	94.4	100.0

[a] FI (Fertility Index) = (No. females pregnant/no. females mated) × 100.
[b] GI (Gestation Index) = (No. females with live litters/no. females pregnant) × 100.
[c] Naive control.
[d] 1% Emulphor vehicle control.
[e] 0.17 mg/ml *p*-dioxane in 1% Emulphor.

solution per day) approximately equal to 1/10th the LD_{50}. Mid and low doses, respectively, were one-half and one log unit lower than the high dose.

Experimental design. Figure 1 depicts the multigeneration reproduction study modified to include screening of dominant lethal and teratogenic effects. Test animals were continuously maintained on drinking solutions containing specified concentrations of 1,2-DCE and 1,1,1-TCE or on appropriate naive and vehicle control solutions (see Table 1). The F/0 mice were randomized by computer into test groups of 10 males and 30 females, acclimated for 2 weeks, and then placed on the appropriate test regimen. After 35 days on the test solutions, the 14-week old F/0 mice were randomly mated to produce the F/1A litters. Two weeks postweaning of the F/1A litters, the F/0 adults were rerandomized and remated to produce the F/1B litters. Parental stock for the second generation was drawn randomly from the F/1B litters. F/0 females were rested for 2 weeks, following weaning of the F/1B pups. The F/1C mating was for dominant lethal and teratology screening as described below.

At weaning, the F/1B litters were culled to 30 females and 10 males per group. Matings between siblings were avoided. The F/1B weanling mice were placed on appropriate test solutions, and at 14 weeks of age, were randomly mated to produce the F/2A litters. Two weeks postweaning of the F/2A pups, the F/1B adults were randomly remated (F/2B mating) for dominant lethal and teratology screening.

Adult observations. Weekly body weight and twice-weekly fluid consumption data were collected for the F/0 and F/1B adult mice throughout the study. Using the Statistical Analysis System (Raleigh, N.C.), mean daily fluid consumption per mouse was calculated and analyzed for significant ($p \leq 0.05$) group differences with Duncan's multiple range test. Mean body weights were analyzed similarly.

Adult reproductive performance was evaluated by calculation of fertility and gestation indices (FI and GI, respectively; Collins, 1977).

Adult percentage mortality was calculated at the termination of each generation (25 weeks of dosing for the F/0; 24 for the F/1B). Mice found moribund or sacrificed at the end of the study were necropsied.

Litter observation. Twenty-one-day survival studies were performed on litters from the F/1A, F/1B, and F/2A matings. Litter size was recorded on Days 0, 4, 7, 14, and 21. Litters were randomly culled to 10 pups each on Day 4 (Collins, 1977). Offspring were weighed collectively on Days 7 and 14 and individually on Day 21. Viability and lactation indices (VI and LI, respectively; Collins, 1977; Fitzhugh, 1968) were calculated. The statistical sampling unit for litter survival studies was the litter (Gaylor, 1977). Litter size and viability and lactation indices were tested for group effects by

TABLE 3

MORTALITY AMONG ADULT MALES AND FEMALES INGESTING 1,2-DICHLOROETHANE OR 1,1,1-TRICHLOROETHANE

Compound	Concentration (mg/ml)	F/0 percentage mortality[a]		F/1B percentage mortality[b]	
		Males	Females	Males	Females
1,2-Dichloroethane	0.00[c]	0.0	3.3	20.0	7.4
(1,2-DCE)	0.00[d]	0.0	3.3	0.0	0.0
	0.03	20.0	13.3	0.0	3.3
	0.09	0.0	6.7	0.0	3.3
	0.29	0.0	0.0	0.0	0.0
1,1,1-Trichloroethane	0.00[c]	20.0	20.0	0.0	0.0
(1,1,1-TCE)	0.00[d]	10.0	3.3	0.0	0.0
	0.58	0.0	10.0	0.0	7.4
	1.75	10.0	6.7	0.0	0.0
	5.83	20.0	13.3	0.0	0.0

[a] After 25 weeks of dosing.
[b] After 24 weeks of dosing.
[c] Naive control.
[d] 1% Emulphor vehicle control.
[e] 0.17 mg/ml *p*-dioxane in 1% Emulphor.

the Kruskal–Wallis χ^2 approximation, effects were localized by Dunn's approximation to a distribution-free multiple comparison (Hollander and Wolfe, 1973). Mean pup weights were analyzed in the same manner as the adult body weights.

All pups from each litter were sacrificed and necropsied at the conclusion of each 21-day survival study.

Dominant lethal screening. In the F/1C and F/1B matings, treated males were co-housed 1:3 with 9-week-old naive, nulliparous females for 7 days (Green *et al.*,

TABLE 4

MEAN LITTER SIZE AT BIRTH[a] FOR LITTERS OF MICE INGESTING 1,2-DICHLOROETHANE OR 1,1,1-TRICHLOROETHANE

Compound	Concentration (mg/ml)	Litter		
		F/1A	F/1B	F/2A
1,2-Dichloroethane	0.00[c]	13.1 ± 3.2	13.1 ± 4.5	11.8 ± 2.4
(1,2-DCE)	0.00[d]	12.0 ± 2.3	12.1 ± 3.0	12.2 ± 2.1
	0.03	13.2 ± 3.2	12.5 ± 4.1	11.3 ± 3.8
	0.09	12.9 ± 2.7	10.5 ± 4.4	12.3 ± 2.7
	0.29	11.4 ± 2.7	10.4 ± 4.8	12.6 ± 1.9
1,1,1-Trichloroethane	0.00[c]	12.7 ± 2.4	11.4 ± 4.1	12.8 ± 2.3
(1,1,1-TCE)	0.00[d]	12.1 ± 3.5	13.3 ± 4.3	11.3 ± 3.4
	0.58	11.8 ± 2.4	11.2 ± 4.8	12.2 ± 2.5
	1.75	12.0 ± 3.1	10.3 ± 5.2	12.9 ± 2.2
	5.83	11.8 ± 3.1	10.6 ± 4.1	12.1 ± 2.2

[a] Mean pups per litter ± SD.
[b] Naive control.
[c] 1% Emulphor vehicle control.
[d] 0.17 mg/ml *p*-dioxane in 1% Emulphor.

TABLE 5

MEAN POSTNATAL BODY WEIGHTS[a] OF OFFSPRING OF MICE INGESTING 1,2-DICHLOROETHANE OR 1,1,1-TRICHLOROETHANE

	Litter								
	F/1A			F/1B			F/2A		
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
1,2-DCE concentration (mg/ml)									
0.00[b]	4.8 ± 1.0	7.1 ± 1.3	11.0 ± 2.4	4.8 ± 0.8	7.7 ± 1.5	12.0 ± 1.5	3.7 ± 1.2	5.2 ± 2.2	7.1 ± 3.3
0.00[c]	4.8 ± 0.5	7.5 ± 0.7	11.5 ± 1.5	5.0 ± 0.5	8.0 ± 0.7	12.7 ± 4.1	4.0 ± 0.6	5.7 ± 1.5	7.6 ± 2.9
0.03	4.8 ± 0.5	7.1 ± 0.8	10.5 ± 1.3	5.0 ± 0.4	7.9 ± 0.7	12.2 ± 1.3	4.7 ± 0.9	7.0 ± 1.6	9.7 ± 2.9
0.09	4.7 ± 0.7	7.4 ± 1.1	10.9 ± 1.8	5.0 ± 0.8	7.6 ± 1.0	11.0 ± 2.2	3.7 ± 1.1	5.3 ± 2.0	7.1 ± 2.7
0.29	5.1 ± 0.6	7.1 ± 0.9	10.7 ± 1.7	4.9 ± 0.7	7.8 ± 1.4	11.0 ± 2.3	4.4 ± 0.5	6.6 ± 0.9	8.9 ± 1.3
1,1,1-TCE concentration (mg/ml)									
0.00[b]	4.8 ± 1.0	7.5 ± 1.0	10.9 ± 2.4	5.2 ± 1.9	8.7 ± 2.1	13.1 ± 3.6	3.8 ± 0.7	5.5 ± 1.7	7.5 ± 2.9
0.00[c]	4.8 ± 0.5	7.5 ± 0.7	11.5 ± 1.5	4.8 ± 0.4	7.1 ± 0.8	10.4 ± 1.3	3.6 ± 0.5	5.7 ± 1.1	7.8 ± 2.1
0.58	4.8 ± 0.5	7.1 ± 0.8	10.5 ± 1.8	4.8 ± 1.0	7.3 ± 1.5	11.5 ± 2.7	3.9 ± 0.7	5.5 ± 0.9	7.1 ± 1.4
1.75	4.7 ± 0.7	7.4 ± 1.1	10.9 ± 1.8	5.0 ± 0.8	8.1 ± 1.1	11.6 ± 2.1	3.9 ± 1.1	6.0 ± 1.7	8.0 ± 2.6
5.83	5.1 ± 0.6	7.1 ± 0.9	10.7 ± 1.7	4.7 ± 0.6	7.0 ± 0.6	10.3 ± 1.5	3.9 ± 1.2	5.9 ± 2.0	7.9 ± 3.0

[a] Mean pup body weight (g) ± SD.
[b] Naive control.
[c] 1% Emulphor vehicle control.
[d] 0.17 mg/ml p-dioxane in 1% Emulphor.

1977). Females were sacrificed 14 days from the midweek of co-housing, their uteri were exposed, and the number of fetal implants, early and late resorptions, and viable fetuses were counted. Females without implantations were disregarded except for calculation of the fertility index (FI; Collins, 1977). These data were used to calculate a number of reproductive indices (see Tables 7a and b), as well as the frequency of dominant lethal factors (Ehling *et al.*, 1978). Statistical analyses of the dominant lethal reproductive indices were performed using two by two contingency tables, with a correction for continuity.

Teratology screening. Also in the F/1C and F/1B matings, the treated females were co-housed 3:1 with 9-week-old naive males for 7 days. Females were examined each morning for copulation plugs. The discovery of a vaginal plug marked Day 0 of gestation. Failure to find a vaginal plug during the week of mating disqualified the female from the teratology screening. Females were sacrificed on the 18th day of gestation, their uteri were exposed, and the number of implants, resorptions, and viable and nonviable males and females were counted. Fetuses were individually weighed and examined for gross defects. In the event that no gross malformations were found, every third fetus was fixed in Bouin's fluid for subsequent serial sectioning and examination for visceral anomalies. The remainder of the fetuses were prepared for skeletal visualization by evisceration, removal of soft tissue in 1% KOH, and staining with aqueous alizarin red S (Barrow and Taylor, 1969; Wilson, 1965). The number of visceral and skeletal malformations were tabulated. Appropriate statistical tests were performed (Gaylor, 1977).

RESULTS

Adult Findings

Statistical analysis indicated no significant treatment-related reduction in mean daily fluid consumption by the test animals receiving either haloalkane. Body weights of F/0 male and female animals treated with 1,2-DCE and 1,1,1-TCE were not affected by either compound (data not shown). F/1B body weights for 1,2-DCE- and 1,1,1-TCE-treated mice (data not shown) also demonstrated no compound or dose-related effects. The difference between F/0 high concentration 1,1,1-TCE-treated and control female mean body weights following the F/1A mating appeared to be due to a fluctuation in the fertility index of the high-dose group

TABLE 6

SURVIVAL INDICES FOR LITTERS OF MICE INGESTING 1,2-DICHLOROETHANE OR 1,1,1-TRICHLOROETHANE[a]

Compound	Concentration (mg/ml)	Litter F/1A VI[b]	F/1A LI[c]	F/1B VI	F/1B LI	F/2A VI	F/2A LI
1,2-Dichloroethane (1,2-DCE)	0.00[d]	97.2	94.8	96.9	90.4	88.5	86.3
	0.00[e]	97.5	98.2	94.0	94.4	89.6	81.3
	0.03	98.1	97.8	96.7	96.4	91.8	95.0
	0.09	94.3	97.5	97.0	99.0	89.6	86.8
	0.29	93.0	97.2	93.1	97.7	92.3	89.6
1,1,1-Trichloroethane (1,1,1-TCE)	0.00[d]	92.3	97.0	96.4	97.2	92.3	76.4
	0.00[f]	94.1	97.4	90.2	94.0	92.3	87.5
	0.58	97.4	97.5	91.8	85.5	88.0	88.0
	1.75	85.9	93.2	94.1	94.0	89.6	83.0
	5.83	91.3	95.7	89.6	92.1	83.0	80.0

[a] The F/1C and F/2B pregnancies were interrupted for dominant lethal and teratology studies.

[b] VI (viability index) = $\sum_{i=1}^{N} \left[\frac{(\text{Day 4 litter size})_i}{(\text{Day 0 litter size})_i}\right] / N$; N = No. litters

[c] LI (lactation index) = $\sum_{i=1}^{N} \left[\frac{(\text{Day 21 litter size})_i}{(\text{pups kept at Day 4})_i}\right] / N$; N = No. litters. Pups kept at Day 4 ≤ 10.

[d] Naive control.

[e] 1% Emulphor vehicle control.

[f] 0.17 mg/ml *p*-dioxane in 1% Emulphor.

(Table 2). That is, the smaller percentage of pregnant females in the high concentration group skewed their mean body weight. This gap in mean body weights was narrowed in subsequent weeks. Fertility and gestation indices are presented in Table 2. Adult mortality is summarized in Table 3. The reason for the sporadic incidences of increased mortality could not be discerned at necropsy. At scheduled necropsy (after Week 24 or 25 of dosing), neither chemical- nor dose-related gross pathology was observed in either generation.

Litter Findings

Data from the three 21-day litter survival studies (F/1A, F/1B, and F/2A) are shown in Tables 4 to 6. 1,2-DCE and 1,1,1-TCE appeared to produce no significantly adverse intragenerational or transgenerational effects on mean litter size at birth (Table 4), mean postnatal body weights (Table 5), or birth to Day 4 survival and Days 4 to 21 survival (Table 6). Values of the F/2A postnatal body weights (Table 5) and survival indices (Table 6) were decreased from the F/1A and F/1B values with few exceptions. The reason for this decrease is not known. The decrease occurred among all groups, so it is not believed to be treatment related. Necropsies of weanling male and female pups from each litter yielded no evidence of dose-dependent gross pathology or congenital malformations for either haloalkane.

Dominant Lethal Screening

Findings from the dominant lethal screenings of both chemicals are listed in Tables

TABLE 7a

RESULTS OF DOMINANT LETHAL SCREENING IN FEMALES MATED TO MALES INGESTING 1,2-DICHLOROETHANE

	Concentration (mg/ml)	Number pregnant	Fertility index[a]	Implants[b]	Resorptions[b]	Live fetuses[b]	DF/LF	DF ≥ 1	DF ≥ 2	FL%
F/1C	0.00[c]	17	56.7	14.1	1.4	12.7	23/216	9/8	6/11	
Mating	0.00[d]	19	63.3	14.0	0.7	13.3	13/252*	11/8	2/17	−1.89
	0.03	16	66.6	14.0	1.6	12.4	26/198*	8/8	1/15	2.60
	0.09	23	76.7	14.5	0.9	13.6	21/312	16/7	5/18	−6.77
	0.29	17	56.7	13.2	0.6	12.5	11/213	7/10	2/15	1.42
F/2B	0.00[c]	15	62.5	12.2	1.0	11.2	15/168	3/12	1/14	
Mating	0.00[d]	25	83.3	11.6	0.8	10.8	19/271	9/16	3/22	3.21
	0.03	27	90.0	12.3	0.9	11.4	23/309	14/13	5/22	−2.14
	0.09	24	80.0	12.0	1.7	10.3	40/247*	12/12	5/19	8.13
	0.29	16	63.3	10.9	0.1	10.8	2/172*	2/14	0/16	4.02

TABLE 7b

RESULTS OF DOMINANT LETHAL SCREENING IN FEMALES MATED TO MALES INGESTING 1,1,1-TRICHLOROETHANE

	Concentration (mg/ml)	Number pregnant	Fertility index[a]	Implants[b]	Resorptions[b]	Live fetuses[b]	DF/LF	DF ≥ 1	DF ≥ 2	FL%
F/1C	0.00[c]	14	58.3	13.9	1.0	12.9	14/181	7/7	4/10	
Mating	0.00[e]	22	81.5	13.2	1.9	11.3	41/248*	14/8	8/14	12.77
	0.58	19	63.3	13.2	0.7	12.5	13/238*	9/10	2/17	3.02
	1.75	21	77.8	12.8	0.7	12.1	15/254*	8/13	5/16	6.35
	5.83	15	50.0	13.7	0.9	12.8	13/192*	8/7	5/10	0.93
F/2B	0.00[c]	17	56.7	12.6	1.1	11.5	19/196	11/6	5/12	
Mating	0.00[e]	19	63.3	11.8	0.6	11.3	11/214	10/9	1/18	2.34
	0.58	27	90.0	12.1	1.3	10.8	35/292*	10/17	6/21	6.24
	1.75	28	93.3	11.9	0.8	11.2	21/313	12/16	4/24	3.04
	5.83	25	83.3	12.0	0.7	11.3	17/283	12/13	4/21	1.82

[a] Indices defined:

$$\text{Fertility index} = \frac{\text{number of females pregnant}}{\text{number of females available}} \times 100;$$

$$\text{DF/LF} = \frac{\text{total number of dead fetuses}}{\text{total number of live fetuses}};$$

$$\text{DF} \geq 1 = \frac{\text{total number of females with one or more dead fetuses}}{\text{total number of females with zero dead fetuses}};$$

$$\text{DF} \geq 2 = \frac{\text{total number of females with two or more dead fetuses;}}{\text{total number of females with less than two dead fetuses}};$$

$$\text{FL\% (frequency of dominant lethal factors)} = \left[1 - \left(\frac{\text{mean live fetuses, treatment}}{\text{mean live fetuses, naive}}\right)\right] \times 100 \text{ (Ehling } et\ al.\text{, 1978)}$$

[b] Mean value per dam.

[c] Naive control.

[d] 1% Emulphor vehicle control.

[e] 0.17 mg/ml *p*-dioxane in 1% Emulphor.

* Significantly different from control at $p \leq 0.05$. Vehicle controls were compared to naive controls: treatment groups were compared with their vehicle controls.

TABLE 8a

RESULTS OF TERATOLOGY SCREENING IN FEMALES INGESTING 1,2-DICHLOROETHANE

	Concentration (mg/ml)	No. of litters	Fecundity index[a]	Implants[b]	Resorptions[b]	Live fetuses[b]	DF/LF[c]	DF ≥ 1[c]	DF ≥ 2[c]	M:F[c]
F1/C mating	0.00[e]	9	90.0	12.0	1.8	10.2	16/92	4/5	1/8	49:51
	0.00[d]	8	100.0	12.1	5.6	6.5	47/51*	6/2	6/2*	59:41
	0.03	10	100.0	14.9	2.5	12.4	25/121*	6/4	5/5	48:52
	0.09	6	100.0	13.8	5.3	8.5	32/51	5/1	3/3	48:52
	0.29	8	80.0	13.4	1.0	12.4	8/99*	3/5	2/6	43:57
F/2B mating	0.00[e]	9	100.0	14.1	1.0	13.1	9/118	7/2	2/7	47:53
	0.00[d]	6	100.0	14.5	2.7	11.8	17/71*	3/3	2/4	39:61
	0.03	4	100.0	16.0	0.8	15.2	3/61*	2/2	1/3	57:43
	0.09	9	100.0	13.1	2.7	10.5	24/94	5/4	2/7	46:54
	0.29	6	85.7	13.0	0.7	12.3	5/74*	5/1	0/6	49:51

TABLE 8b

RESULTS OF TERATOLOGY SCREENING IN FEMALES INGESTING 1,1,1-TRICHLOROETHANE

	Concentration (mg/ml)	No. of litters	Fecundity index[a]	Implants[b]	Resorptions[b]	Live fetuses[b]	DF/LF[c]	DF ≥ 1[c]	DF ≥ 2[c]	M:F[c]
F/1C mating	0.00[e]	5	100.0	13.8	0.8	13.0	4/65	2/3	2/3	52:48
	0.00[e]	4	100.0	16.3	0.5	15.8	2/63	1/3	1/3	46:54
	0.58	0	—	—	—	—	—	—	—	—
	1.75	9	100.0	14.3	4.9	9.4	44/85*	7/2	5/4	49:51
	5.83	5	71.4	12.2	2.2	10.0	11/50*	4/1	4/1	62.38
F/2B mating	0.00[e]	4	100.0	14.5	1.5	13.0	6/53	3/1	1/3	46:54
	0.00[e]	5	83.3	15.0	1.0	14.0	5/70	4/1	1/4	54:46
	0.58	11	100.0	14.1	1.5	12.6	16/140	7/4	1/10	41:59
	1.75	6	75.0	15.7	3.0	12.7	18/77*	2/4	1/5	43:57
	5.83	5	100.0	15.2	1.8	13.4	9/67	5/0	2/3	34:66

[a] Indices defined: Fecundity index = percentage of copulation plug-positive females bearing live fetus(es) at sacrifice. For definitions of DF/LF, DF ≥ 1, and DF ≥ 2 see footnotes to Table 7. M:F = ratio of live male to female fetuses expressed as a percentage of the total number of live fetuses.

[b] Mean value per dam.

[c] Naive control.

[d] 1% Emulphor vehicle control.

[e] 0.17 mg/ml *p*-dioxane in 1% Emulphor.

* Significantly different from control at $p \leq 0.05$. Vehicle controls were compared to naive controls; treatment groups were compared with their vehicle controls.

7a and b. Statistically significant effects in the ratio of dead to live fetuses (DF/LF) were observed in both generations for both compounds. However, these effects, which were both increases and decreases compared to controls, do not appear to be dose related. In two cases, greater effects were seen among the vehicle control groups than among the treated groups. The frequency of dominant lethal factors (F_L%; Ehling *et al.*, 1978) for both chloroalkanes in both generations was minimal (−7 to +12) when compared to the results in females mated to males receiving 0.05 mg/ml cyclophosphamide in drinking water for 14 weeks (F_L%, cyclophosphamide = 62, data not shown).

TABLE 9a

DISTRIBUTION OF VISCERAL AND SKELETAL MALFORMATIONS AMONG FETUSES/LITTERS OF FEMALES INGESTING 1,2-DCE

	F/1C litters					F/2B litters				
Conc. (mg/ml):	0.00[a]	0.00[b]	0.03	0.09	0.29	0.00[a]	0.00[b]	0.03	0.09	0.29
Total No. fetuses/total No. litters:	92/9	51/8	121/10	51/6	99/8	118/9	71/6	61/9	94/9	74/6
Visceral malformations										
Total number examined	33/8	19/7	46/9	18/4	29/7	38/9	24/5	20/4	29/8	24/6
Hydrocephalus	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Cleft palate	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Atrial, ventricular, or cardiac hypertrophy	0/0	0/0	1/1	0/0	0/0	0/0	1/1	0/0	1/1	0/0
Malrotation of the heart	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Hydronephrosis	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Dilated renal pelvis	0/0	1/1	0/0	2/1	1/1	1/1	0/0	0/0	0/0	0/0
Dilated bladder	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0
Cryptorchidism/malpositioned testis	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Skeletal malformations										
Total number examined			(c)			80/9	47/5	41/4	65/8	50/6
Dysplastic skull						0/0	0/0	0/0	0/0	1/1
Dysplastic supraoccipital region						3/2	3/2	0/0	2/2	1/1
Microagnathia						0/0	0/0	0/0	0/0	0/0
Asymetric sternebrae						24/6	9/4	2/2	9/5	16/6
Bifid sternebrae						8/3	1/1	7/2	4/3	5/3
Hypoplastic sternebrae						3/1	0/0	0/0	0/0	1/1
Extra ribs						2/2	1/1	0/0	2/2	2/2
Wavy ribs						0/0	0/0	0/0	1/1	0/0

[a] Naive control.
[b] 1% Emulphor vehicle control.
[c] F/1C skeletal specimens were lost due to a preparation error.

Teratology Screening

Maternal ingestion of 1,2-DCE or 1,1,1-TCE produced no apparent adverse reproductive effects (Tables 8a and b) or increased incidence of fetal visceral or skeletal anomalies (Tables 9a and b). No F/1C females consuming the lowest dose of 1,1,1-TCE were found with copulation plugs. Although some females did become pregnant, it was impossible to ascertain when pregnancy began and, hence, no teratological examinations were performed on this group.

DISCUSSION

1,2-DCE and 1,1,1-TCE are industrially important chemicals which also are found in the drinking water. Since people of reproductive age may be exposed to these materials, an evaluation of the effects of 1,2-DCE and 1,1,1-TCE on reproduction and development was indicated. Mice were exposed for a minimum of 6 weeks prior to the initiation of mating. The total duration of exposure for the F/O and F/1B adults, respectively, was 25 and 24 weeks. Adult re-

TABLE 9b

DISTRIBUTION OF VISCERAL AND SKELETAL MALFORMATIONS AMONG FETUSES/LITTERS OF FEMALES INGESTING 1,1,1-TCE

	F/1C litters					F/2B litters				
Conc. (mg/ml):	0.00[a]	0.00[b]	0.58	1.75	5.83	0.00[a]	0.00[b]	0.58	1.75	5.83
Total No. fetuses/total No. litters:	65/5	63/4	0/0[c]	85/9	50/5	53/4	70/5	140/11	77/6	67/5
Visceral malformations										
Total number examined	23/5	22/4	—	29/7	18/5	18/4	23/5	49/10	27/5	23/5
Hydrocephalus	0/0	0/0	—	1/1	0/0	0/0	0/0	0/0	0/0	0/0
Cleft palate	0/0	0/0	—	0/0	1/1	0/0	0/0	0/0	0/0	0/0
Atrial, ventricular, or cardiac hypertrophy	0/0	0/0	—	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Malrotation of the heart	0/0	0/0	—	0/0	0/0	0/0	0/0	1/1	0/0	0/0
Hydronephrosis	0/0	0/0	—	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Dilated renal pelvis	1/1	0/0	—	1/1	0/0	1/1	0/0	1/1	0/0	0/0
Dilated bladder	0/0	0/0	—	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Cryptorchidism/malpositioned testis	0/0	0/0	—	2/2	0/0	0/0	0/0	1/1	0/0	0/0
Skeletal malformations										
Total number examined			(d)			35/4	47/5	91/10	50/5	44/5
Dysplastic skull						1/1	0/0	1/1	0/0	0/0
Dysplastic supraoccipital region						1/1	0/0	0/0	0/0	4/1
Micrognathia						0/0	1/1	1/1	0/0	0/0
Asymetric sternebrae						16/4	13/3	16/9	15/5	8/3
Bifid sternebrae						1/1	2/1	10/5	4/3	2/1
Hypoplastic sternebrae						2/1	1/1	1/1	0/0	1/1
Extra ribs						0/0	0/0	0/0	1/1	0/0
Wavy ribs						0/0	0/0	0/0	1/1	0/0

[a] Naive control.
[b] 0.17 mg/ml *p*-dioxane in 1% Emulphor.
[c] There were no plug-positive females in this group.
[d] F/1C skeletal specimens were lost due to a preparation error.

productive performance, litter survival and growth, dominant lethal effects, teratogenesis, and general pathology were evaluated.

No dose of 1,2-DCE produced deleterious effects on male or female reproductive function or on offspring development. These negative findings contrast with the genetic toxicity demonstrated by 1,2-DCE (see Introduction) and with the severe reproductive toxicity demonstrated by a close structural analog, 1,2-dibromoethane (Rannung, 1980). The results presented for 1,2-DCE support the findings of Alumot *et al.* (1976) and Rao *et al.* (1980) for different routes of administration.

The results for 1,1,1-TCE were also uniformly negative at the concentrations tested. The 1,1,1-TCE teratological findings of this study agree with those obtained by Schwetz *et al.* (1975) in both mice and rats exposed to 875 ppm 1,1,1-TCE for 7 hr per day on Days 6 to 15 of gestation. Calculations indicate that the inhalation dose (mg/kg/day) was in the range of the low to the middle

doses used in the present drinking water study.

EPA-reported mean concentration drinking water levels for 1,2-DCE and 1,1,1-TCE are 4.3 and 1.5 ppb, respectively (U.S. EPA, 1975). For a 70-kg man consuming 2 liters of water per day, these concentrations represent doses of 1×10^{-4} mg 1,2-DCE/kg/day and 4×10^{-5} mg 1,1,1-TCE/kg/day. Using the highest concentration of each haloalkane available to a 35-g mouse consuming 6 ml/day, a mouse would receive 50 mg 1,2-DCE/kg/day or 1000 mg 1,1,1-TCE/kg/day. The highest doses of 1,2-DCE and 1,1,1-TCE administered to mice in the present study are approximately five and seven orders of magnitude, respectively, greater than the potential average human dose (assuming exposure by the drinking water only). These differences between average animal and human exposures are well above the "safety factor" of two orders of magnitude generally used when extrapolating from a highest no-observed-effect level in animals to man.

On the basis of these results and other published reports, it is concluded that the concentrations of 1,2-dichloroethane and 1,1,1-trichloroethane in the drinking water pose little hazard to human reproduction and development.

ACKNOWLEDGMENTS

The authors are grateful to Dr. R. L. Jordan of the MCV Department of Anatomy for his assistance in preparing and examining the teratology specimens and Karen Soderquist for her technical assistance.

REFERENCES

ALUMOT, E., NACHTOMI, E., MANDEL, E., HOLSTEIN, P., BONDI, A., AND HERZBERG, M. (1976). Tolerance and acceptable daily intake of chlorinated fumigants in the rat diet. *Food Cosmet. Toxicol.* **14**, 105–110.

BANERJEE, S., VAN DUUREN, B. L., AND ORUAMBO, F. I. (1980). Microsome-mediated covalent binding of 1,2-dichloroethane to lung microsomal protein and salmon sperm DNA. *Cancer Res.* **40**, 2170–2173.

BARROW, M. V., AND TAYLOR, W. J. (1969). A rapid method for detecting malformations in rat fetuses. *J. Morphol.* **127**, 291–306.

COLLINS, T. F. X. (1977). Multigeneration reproduction studies. In *Handbook of Teratology* (J. G. Wilson and F. C. Frazer, eds.), Vol. 4, pp. 191, 214. Plenum, New York.

EHLING, U. H., MACHEMER, L., BUSELMAUER, W., DYCKA, J., FROHBERG, H., KRATOCHVILORN, J., LANG, R., LORKE, R., MULLER, D., PEH, J., ROHRBON, G., ROLL, R., SCHULZE-SCHENCKING, M., AND WIEMANN, H. (1978). Standard protocol for the dominant lethal test on male mice. *Arch. Toxicol.* **39**, 173–185.

ELOVAARA, E., HEMMINKI, K., AND VAINIO, H. (1979). Effects of methylene chloride, trichloroethane, trichloroethylene, and toluene on the development of chick embryos. *Toxicology* **12**, 111–119.

FITZHUGH, O. G. (1968). Reproduction tests. In *Modern Trends in Toxicology*. (E. Boyland and R. Goulding, eds.), Vol. 1, pp. 75, 85. Butterworth, London.

GAYLOR, D. W. (1977). Methods and concepts of biometrics applied to teratology. In *Handbook of Teratology* (J. G. Wilson and F. C. Frazer, eds.), Vol. 4, pp. 429, 444. Plenum, New York.

GREEN, S., MORELAND, F. M., AND FLAMM, W. G. (1977). A new approach to dominant lethal testing. *Toxicol. Appl. Pharmacol.* **39**, 549–552.

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

HOLLANDER, M., AND WOLFE, D. A. (1973). *Non-Parametric Statistical Methods*. Wiley, New York.

IARC (1979a). *International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans*, Vol. 20, pp. 429, 442. Lyon.

IARC (1979b). *International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans*, Vol. 20, pp. 515, 525. Lyon.

NCI (1977). *National Cancer Institute Bioassay of 1,1,1-Trichloroethane for Possible Carcinogenicity*. DHEW publication No. (NIH) 77-803.

NCI (1978). *National Cancer Institute Bioassay of 1,2-Dichloroethane for Possible Carcinogenicity*. DHEW publication No. (NIH) 78-1361.

RANNUNG, U. (1980). Genotoxic effects of 1,2-dibromoethane and 1,2-dichloroethane. *Mutat. Res.* **76**, 269–295.

RAO, K. S., MURRAY, J. S., DEACON, M. M., JOHN, H. A., CALHOUN, L. L., AND YOUNG, J. T. (1980).

Teratogenicity and reproduction studies in animals inhaling ethylene dichloride. In *Banbury Report 5, Ethylene Dichloride: A Potential Health Risk* (B. Ames, P. Infante, and R. Reitz, eds.). pp. 149, 166. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SCHWETZ, B. A., LEONG, B. K. J., AND GEHRING, P. J. (1975). The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats. *Toxicol. Appl. Pharmacol.* 32, 84–96.

SIMMON, V. F., KAUHANEN, K., AND TARDIFF, R. G. (1977). Mutagenic activity of chemicals identified in drinking water. In *Progress in Genetic Toxicology* (D. Scott, B. A. Bridges, and F. H. Sobels, eds.), pp. 249, 258. Elsevier/North-Holland, New York.

U.S. EPA (1975). Preliminary assessment of suspected carcinogens in drinking water. Report to Congress, Washington, D.C. EPA-56014-75-005 PB. 260961.

WILSON, J. F. (1965). Methods of examining experimental animal fetuses for malformations. In *Teratology: Principles and Practices* (J. G. Wilson and J. Warkany, eds.), pp. 262, 277. Univ. Chicago Press, Chicago.