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Carcinogenicity of Chlorinated Methane and Ethane Compounds Administered in Drinking Water to Mice

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The chlorinated hydrocarbons chloroform ($CHCl_3$), 1,1-dichlorethane (1,1-DCE) and 1,2-dichloroethane (1,2-DCE) have been detected in finished drinking water. When administered to B6C3F1 mice by gavage in corn oil, these compounds have been shown to induce hepatic tumors. The present study examines the effect on liver tumor incidence of continuous treatment of $CHCl_3$ (600 mg/L and 1800 mg/L), 1,1-DCE (835 mg/L and 2500 mg/L), and 1,2-DCE (835 mg/L and 2500 mg/L) administered in drinking water to male B6C3F1 mice using a two-stage (initiation/promotion) treatment protocol. Seventy 4-week-old male B6C3F1 mice constituted each treatment group. Of these mice, 35 were initiated by treatment with diethylnitrosamine (DENA) (10 mg/L) in the drinking water for 4 weeks. The remaining 35 received deionized drinking water. Each group was subsequently treated with one of two concentrations of $CHCl_3$, 1,1-DCE, or 1,2-DCE in drinking water for 52 weeks. An additional group received phenobarbital (PB) (500 mg/L) and served as the positive control for liver tumor promotion. Mice were sampled after 24 weeks (10 mice) and 52 weeks (25 mice). At sampling, liver and lung tumors were detected. None of the compounds increased the number or incidence of lung or liver tumors by themselves. PB promoted liver tumor formation (but not lung tumors) in the DENA- initiated mice. 1,1-DCE and 1,2-DCE did not affect the incidence or number of liver or lung tumors in the DENA-initiated animals. $CHCl_3$, however, inhibited liver and lung tumorigenesis in the DENA-initiated mice.

Introduction

Chloroform ($CHCl_3$), 1,1-dichloroethane (1,1-DCE), and 1,2-dichloroethane (1,2-DCE) have been detected in finished drinking water (*1*). These compounds induced neoplasms in Osborne-Mendel rats and B6C3F1 hybrid mice when administered chronically by daily gavage. Chloroform produced kidney-epithelial tumors in rats and a high incidence of hepatocellular carcinomas in mice (*2*). 1,2-DCE treatment caused stomach-squamous cell carcinomas in rats and hepatocellular carcinomas in mice (*2*). 1,1-DCE exposure induced hepatocellular carcinomas in mice and a slight increase in thyroid tumors in rats (*2*). Therefore, these three compounds, when administered chronically to B6C3F1 mice by daily gavage (in a corn oil vehicle), have been shown to induce hepatocellular neoplasms (*2*). The hepatocarcinogenicity of these compounds administered in drinking water to mice has, however, not been established. $CHCl_3$ and 1,1-DCE appear to be nongenotoxic, producing their carcinogenic effects through epigenetic mechanisms (*3*). 1,2-DCE has been shown to be mutagenic (*4*) and capable of binding to cellular macromolecules (*5*) and thus probably functions through genotoxic mechanisms.

The present study was undertaken to examine the carcinogenicity of $CHCl_3$, 1,1-DCE, and 1,2-DCE when chronically administered to B6C3F1 male mice in drinking water, using a two-stage carcinogenesis protocol to determine if $CHCl_3$, 1,1-DCE, and 1,2-DCE may be expressing their hepatocarcinogenicity through tumor promotion mechanisms.

Materials and Methods

Chemicals

Diethylnitrosamine (DENA) and sodium phenobarbital (PB) were purchased from Sigma Chemical Co. (St. Louis, MO). $CHCl_3$, 1,1-DCE, and 1,2-DCE (glass-distilled, no preservatives), were purchased from Burdick and Jackson Laboratories, Inc. (Muskegon, MI).

Animals

Male C3H mice (8 weeks old) and female C57BL mice (8 weeks old) were purchased from Charles River Breeding Labs (Wilmington, MA). These mice were

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Table 1. Experimental design.

Group	Treatment	
	Initiation[a]	*Promotion*[b]
1	–	—
2	–	Phenobarbital
3	+	—
4	+	Phenobarbital
5	–	$CHCl_3$ (low dose)
6	+	$CHCl_3$ (low dose)
7	–	$CHCl_3$ (high dose)
8	+	$CHCl_3$ (high dose)
9	–	1,1-DCE (low dose)
10	+	1,1-DCE (low dose)
11	–	1,1-DCE (high dose)
12	+	1,1-DCE (high dose)
13	–	1,2-DCE (low dose)
14	+	1,2-DCE (low dose)
15	–	1,2-DCE (high dose)
16	+	1,2-DCE (high dose)

[a] For tumor initation, DENA was placed in drinking water (10 mg/L) for 4 weeks (+ indicates DENA was present in drinking water; – indicates untreated deionized drinking water was present).

[b] For tumor promotion, compounds were placed in the drinking water until sacrifice (at 24 or 52 weeks' exposure). Concentrations used included PB, 500 mg/L; $CHCl_3$, low dose, 600 mg/L; $CHCl_3$ high dose, 1800 mg/L; 1,1-DCE, low dose, 835 mg/L; 1,1-DCE, high dose, 2500 mg/L; 1,2-DCE, low dose, 835 mg/L; and 1,2-DCE, high dose, 2500 mg/L (— indicates that untreated, deionized drinking water was present).

bred at the Medical College of Ohio animal facility to produce B6C3F1 hybrid mice, which were used in subsequent experiments. All mice were maintained in plastic cages with corncob bedding and given certified Purina Laboratory Chow and water *ad libitum*. Male B6C3F1 hybrid mice were weaned at 30 days of age and treated as noted below.

Experimental Design

Thirty-day-old male hybrid mice were randomly placed into 16 groups of 35 mice each. The design of the study is shown in Table 1. Eight of the groups received drinking water containing DENA (10 mg/L) for 4 weeks. The remaining eight groups received deionized drinking water. The DENA-treated animals constituted the initiated groups, and the deionized-drinking-water-treated animals constituted the noninitiated groups. After 4 weeks, the eight initiated groups received drinking water containing either deionized water alone or PB (500 mg/L), $CHCl_3$ (600 mg/L; low dose), $CHCl_3$ (1800 mg/L; high dose), 1,1-DCE (835 mg/L; low dose), 1,1-DCE (2500 mg/L; high dose), 1,2-DCE (835 mg/L; low dose), or 1,2-DCE (2500 mg/L; high dose). Similarly, the eight noninitiated groups received drinking water containing either deionized water or PB, high-dose $CHCl_3$, low-dose $CHCl_3$, high-dose 1,1-DCE, low-dose 1,1-DCE, high-dose 1,2-DCE, or low-dose 1,2-DCE.

All compounds were added to deionized/distilled water and placed in amber glass drinking water bottles containing Teflon stoppers and double sipper tubes. Mice were sacrificed after 24 weeks or 52 weeks of promotion. Ten mice were sacrificed at the 24-week sampling time, and the remaining 25 were sacrificed at the 52-week sampling time. At sacrifice, complete necropsies were conducted on each mouse, and all tissues were examined for the presence of gross pathologic lesions. Liver, lungs, and kidneys were weighed, fixed in 10% phosphate-buffered formalin, and processed for routine hematoxylin and eosin (H&E) slide preparation. Histologic sections of liver, kidneys, and lungs from each mouse were examined, and the number of tumors were quantitated.

Drinking water was freshly made and changed weekly, and its consumption was monitored weekly for each cage. Intake was calculated by weighing water bottles before and after each weekly water change. The mean water intake (in grams) of each mouse per day was calculated by subtracting the weight of water after 1 week from the weight of the water at time of filling and dividing this number by the number of days and the number of mice per cage. Body weights of mice during the treatment process were determined on a monthly basis for each cage.

Dose levels of $CHCl_3$, 1,1-DCE, and 1,2-DCE used in the present study were chosen through a preliminary toxicity study. Maximum tolerated dose (MTD) drinking water levels of $CHCl_3$, 1,1-DCE, and 1,2-DCE were 1800 mg/L, 2500 mg/L, and 2500 mg/L, respectively. The MTD was the highest concentration of compound that failed to cause mortality in 8-week-old male B6C3F1 mice after a 4-week exposure period. For the subsequent long-term treatment studies, MTD and ⅓ MTD were used as the dose levels. Dose levels for DENA of 10 mg/L and PB (500 mg/L) were determined through previous studies in our laboratory.

Results

Body Weight and Drinking Water Intake

Body weight determinations made during the treatment period are shown in Figure 1. Untreated mice displayed a steady increase in body weight over time. All treated mice displayed mean body weight gains that paralleled those of the control mice but were lower. Only mice receiving DENA and $CHCl_3$ (low dose), DENA and $CHCl_3$ (high dose), and $CHCl_3$ (high dose) by itself exhibited a statistically significant ($p < 0.05$) decrease in mean body weight from that of the untreated mice from 8 weeks to 56 weeks of treatment.

The pattern of drinking water intake in the mice during the treatment times paralleled that of the body weight determinations (Fig. 2). Mice receiving $CHCl_3$ (both high and low doses; with and without DENA initiation) and 1,2-DCE (high doses; with and without DENA initiation) displayed a statistically significant ($p < 0.05$) decrease in drinking water intake at all times sampled from 8 weeks until 48 weeks of treatment over that of the other treatment groups.

Survival

All untreated, PB-treated, DENA-untreated, and DENA-PB-treated mice survived until the 52-week

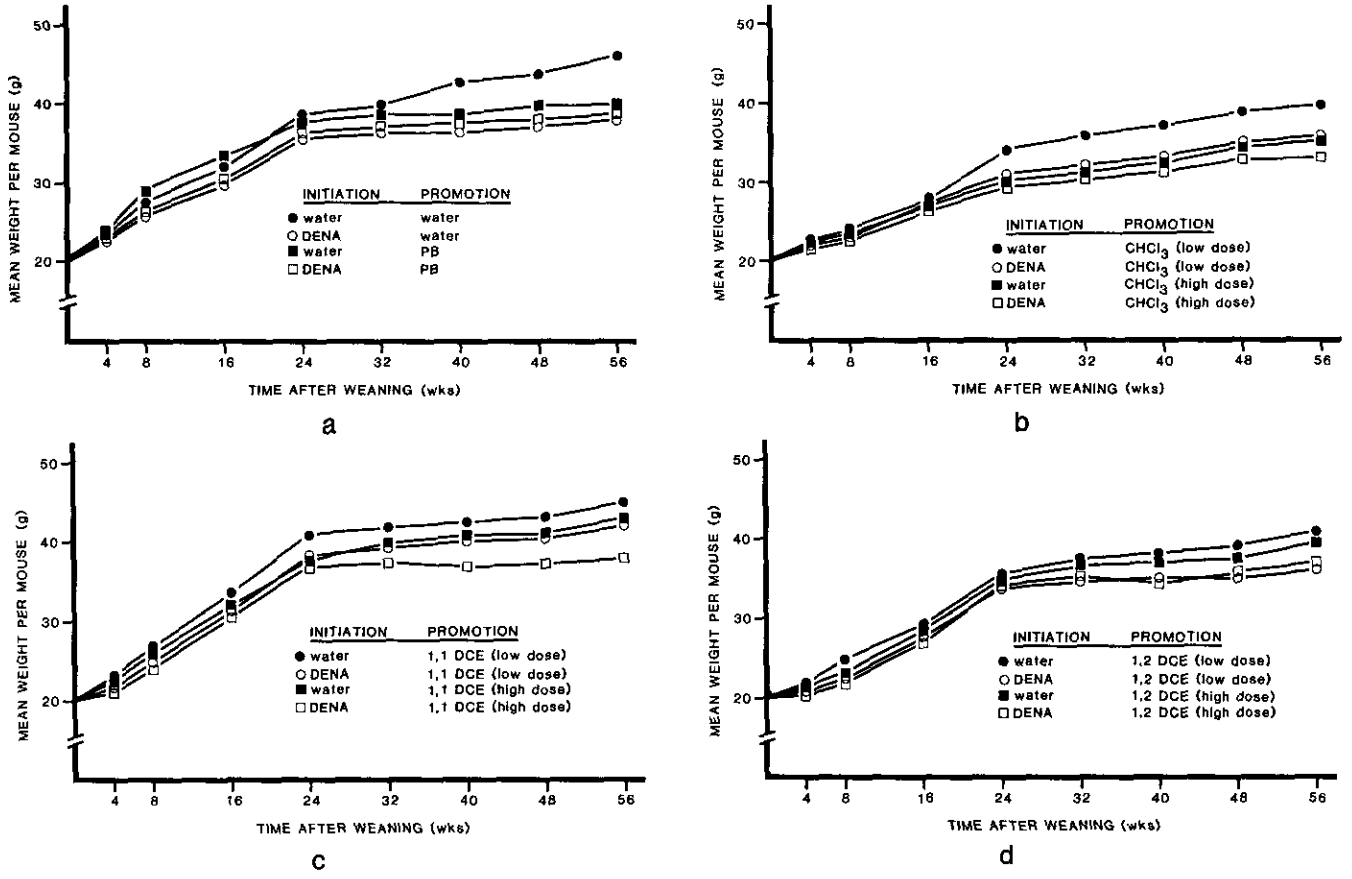

FIGURE 1. Mean body weights of mice (in grams) sampled after weaning until sacrifice at week 56: (*a*) mean body weight per mouse of groups receiving deionized water only, DENA only, PB only, and DENA + PB; (*b*) body weights of mice that received high- and low-dose $CHCl_3$ with and without prior DENA initiation; (*c*) body weights of mice that received 1,1-DCE (high and low dose) with and without DENA treatment; (*d*) mean body weights of mice that received 1,2-DCE (high and low dose) with and without DENA treatment.

sampling time. Death occurred in week 40 to 52 of treatment in mice treated with DENA-$CHCl_3$ (high dose) (4 mice), DENA-$CHCl_3$ (low dose) (3 mice), $CHCl_3$ (high dose) (3 mice), $CHCl_3$ (low dose) (2 mice) and DENA-1,2-DCE (high dose) (3 mice). These mice were necropsied, and the findings were included with the liver and lung tumor data at the 52-week sampling time.

Liver Tumors

Liver tumors detected in the sampled mice included adenomas and carcinomas. The liver tumor incidence in treated and control mice reflects the total number of tumors (adenomas and carcinomas) found by histologic examination (Table 2). Untreated male B6C3F1 mice exhibited no liver tumors at the 24-week sampling time. However, at 52 weeks, 5 of 25 mice were found to have liver tumors, with an average of 0.08 tumors per mouse. Treatment with $CHCl_3$, 1,2-DCE, or 1,1-DCE (both high and low doses) without initiation resulted in no significant increase or decrease either in liver tumor incidence or in the number of tumors per liver from that of the untreated control mice at either sampling time. Phenobarbital exposure without initiation, however, produced a significant increase in both liver tumor incidence and the mean number of liver tumors per mouse over that of the untreated controls at both the 24-week and the 52-week sampling times.

Mice initiated with DENA for 4 weeks followed by treatment with deionized drinking water exhibited a 70% liver tumor incidence with a mean number of 3.0 tumors per liver at the 24-week sampling time and a 100% liver tumor incidence with a mean of 29.3 tumors per liver at the 52-week sampling period. Mice initiated with DENA followed by exposure to PB in drinking water displayed a significant increase over that of the DENA-initiated mice in both the incidence of liver tumors at the 24-week sampling time and in the mean number of liver tumors at both the 24-week and 52-week sampling times. Mice initiated with DENA followed by promotion with either 1,1-DCE or 1,2-DCE at both high and low dose levels exhibited no significant difference in liver tumor incidence or mean liver tumor number at either of the two sampling times. $CHCl_3$ administered at both low and high dose levels in the drinking water following DENA initiation produced a significant decrease in the mean number of liver tumors per mouse from that of the group initiated with DENA

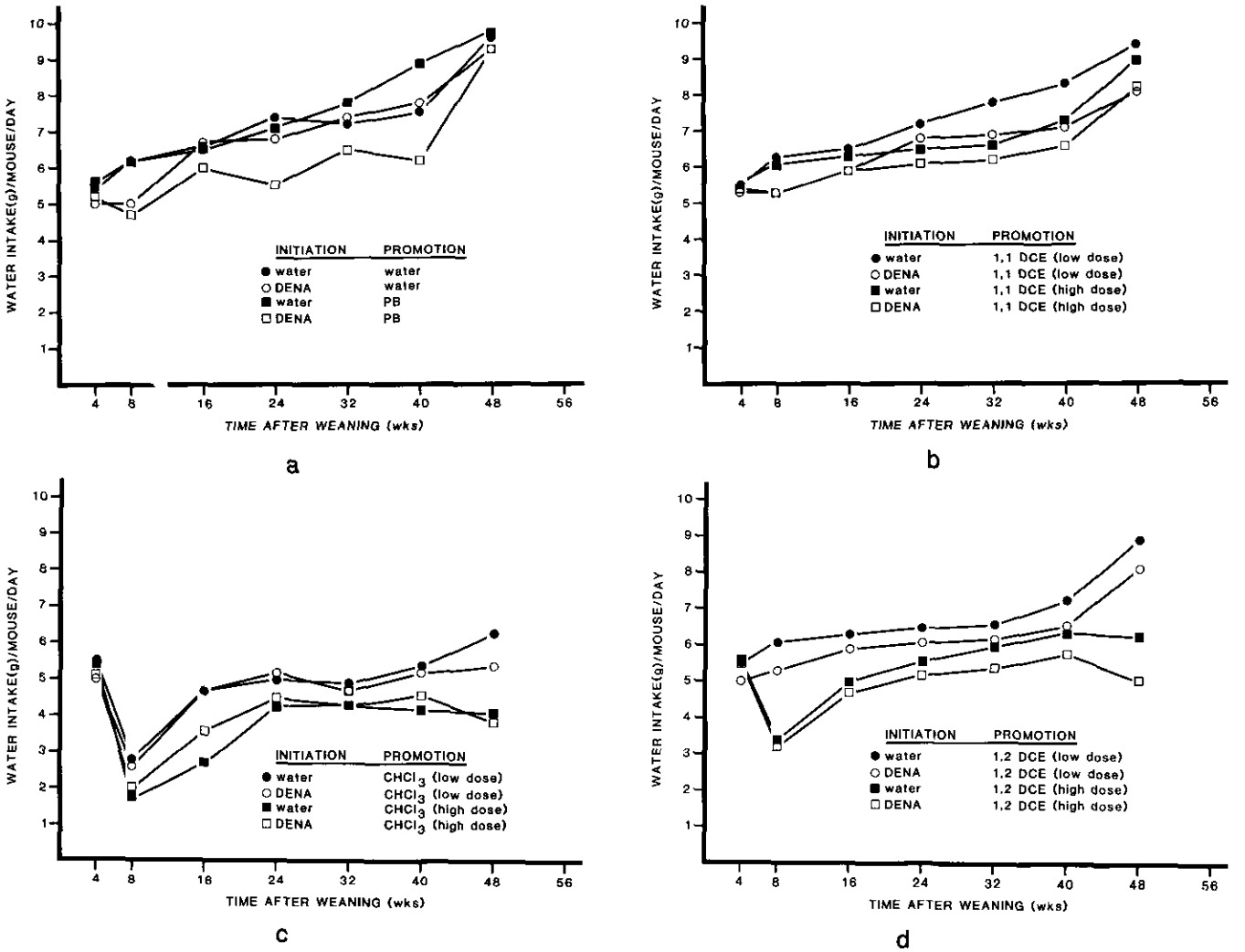

FIGURE 2. Average drinking water intake (in grams), for each mouse per day sampled from 4 weeks post-weaning until 4 weeks before sacrifice: (*a*) *B6C3F1 male mice treated with water only, PB only, DENA initiation, and DENA + PB;* (*b*) *DENA-initiated and -noninitiated* mice subsequently treated with $CHCl_3$ (high and low dose); (*c*) mice that received 1,1-DCE (high or low dose) with or without DENA initiation for 4 weeks; (*d*) mice that received 1,2-DCE (high or low dose) subsequent to DENA or no treatment.

and administered deionized drinking water at both the 24-week and 52-week sampling period.

Lung Tumors

Lung tumors were also detected in treated mice. When examined histologically, all lung tumors proved to be lung adenomas. No significant differences in lung tumor incidence or in the mean number of lung tumors per mouse was found in noninitiated mice receiving either chloroform, 1,1-DCE, 1,2-DCE, or PB (Table 3). Mice initiated with DENA displayed a significant increase in lung tumor incidence and in the mean number of lung tumors per mouse over that of the noninitiated mice. However, mice treated with 1,1-DCE, 1,2-DCE, $CHCl_3$, PB, or deionized water exhibited no significant difference in either tumor incidence or the number of tumors per mouse when compared to mice initiated with DENA and administered deionized drinking water.

Morphometric Determination of Liver Tumor Area

Histologic examination of the $CHCl_3$-treated mice (both DENA-initiated and -noninitiated) suggested that the liver tumors in the $CHCl_3$-treated mice were smaller in size when compared to those of the other treated mice. To quantitate this observation, tumors in $CHCl_3$-treated mice (both DENA-initiated and -noninitiated at both high and low dose) were measured using a computerized planimetry system (*6*). The liver tumor area and total liver area was quantitated in H&E-stained, randomly selected liver sections from the 52-week mouse sample. Similar measurements were made on

Table 2. Liver tumor incidence.

Treatment		24 Weeks sampling time		52 Weeks sampling time	
Initiation	Promotion	Incidence (%)[a]	No. of tumors/ mouse (range)	Incidence (%)[a]	No. of tumors/ mouse (range)
−	—	0/10 (0)	0	5/25 (20)	0.08 ± 0.28 (0–2)
+	—	7/10 (70)	3.00 ± 2.50 (0–8)	25/25 (100)	29.30 ± 15.40 (6–61)
−	$CHCl_3$ (600 mg/L)	0/10 (0)	0	3/25 (12)	0.10 ± 0.30 (0–1)
+	$CHCl_3$ (600 mg/L)	4/10 (40)	0.60 ± 0.84 (0–2)	25/25 (100)	19.40 ± 14.10 (1–44)
−	$CHCl_3$ (1800 mg/L)	0/10 (0)	0	4/25 (16)	0.30 ± 0.90 (0–4)
+	$CHCl_3$ (1800 mg/L)	3/10 (30)	0.78 ± 1.20 (0–2)	20/25 (80)	18.40 ± 14.70 (0–49)
−	1,1-DCE (835 mg/L)	0/10 (0)	0	7/25 (28)	0.20 ± 0.40 (0–1)
+	1,1-DCE (835 mg/L)	7/10 (70)	6.60 ± 8.70 (1–27)	25/25 (100)	38.33 ± 20.40 (6–67)
−	1,1-DCE (2500 mg/L)	0/10 (0)	0	2/25 (8)	0.10 ± 0.40 (0–2)
+	1,1-DCE (2500 mg/L)	6/10 (60)	2.80 ± 3.10 (2–8)	23/25 (92)	31.60 ± 34.70 (0–48)
−	1,2-DCE (835 mg/L)	0/10 (0)	0	4/25 (16)	0.20 ± 0.50 (0–2)
+	1,2-DCE (835 mg/L)	8/10 (80)	5.60 ± 5.90 (1–17)	25/25 (100)	34.50 ± 17.40 (7–59)
−	1,2-DCE (2500 mg/L)	0/10 (0)	0	3/25 (12)	0.10 ± 0.30 (0–1)
+	1,2-DCE (2500 mg/L)	3/10 (30)	0.90 ± 1.90 (1–6)	23/25 (92)	25.20 ± 16.70 (0–53)
−	Phenobarbital (500 mg/L)	2/10 (20)	0.30 ± 0.67 (1–2)	9/25 (36)	0.70 ± 1.10 (0–3)
+	Phenobarbital (500 mg/L)	9/10 (90)	16.00 ± 17.10 (7–43)	25/25 (100)	42.80 ± 1.30 (9–60)

[a] Incidence values represent the number of mice with liver tumors per total number of mice sampled. The number of tumors per mouse represents the mean ± SD of the number of liver tumors per mouse for all mice in that group at that sampling time.

histologic sections from mice initiated with DENA and administered deionized water, mice initiated with DENA and promoted with PB, and untreated mice sampled at 52 weeks. Quantitation of the relative tumor area and the mean area of each tumor is shown in Table 4.

Other Findings

In addition to lung and liver tumors, focal areas of cellular necrosis were found in the kidneys and liver of $CHCl_3$-treated mice at the 24-week and 52-week sampling times. No extratumorous necrosis was observed in untreated, PB-treated, DENA-initiated, or DENA-PB-treated mice. Occasional focal areas of hepatic necrosis were seen in the high-dose, 1,2-DCE-treated mice (both initiated and noninitiated). Focal areas of hepatic lipid accumulation were seen in the $CHCl_3$-treated mice (high dose). Focal areas of necrosis (one to two areas per section) were observed in the kidney tubules of $CHCl_3$-treated mice.

Discussion

In the present study, $CHCl_3$, 1,1-DCE, and 1,2-DCE failed to induce hepatic tumors in male B6C3F1 mice when administered for 52 weeks in drinking water. Similarly, when these three chlorinated compounds were evaluated for liver tumor promotion activity, they failed to increase either the tumor incidence or the mean tu-

Table 3. Lung tumor incidence.

Treatment		24 Weeks sampling time		52 Weeks sampling time	
Initiation	Promotion	Incidence (%)[a]	No. of tumors/mouse (range)	Incidence (%)[a]	No. of tumors/mouse (range)
−	−	0/10 (0)	0	2/25 (8)	0.10 ± 0.30 (0–1)
+	−	1/10 (10)	0.10 ± 0.30 (0–1)	18/25 (72)	1.40 ± 1.40 (0–5)
−	$CHCl_3$ (600 mg/L)	0/10 (0)	0	0/25 (0)	0
+	$CHCl_3$ (600 mg/L)	0/10 (0)	0	13/25 (52)	0.80 ± 0.90 (0–3)
−	$CHCl_3$ (1800 mg/L)	0/10 (0)	0	0/25 (4)	0.04 ± 0.20 (0–1)
+	$CHCl_3$ (1800 mg/L)	1/10 (10)	0.10 ± 0.30 (0–1)	6/25 (24)	0.30 ± 0.60 (0–2)
−	1,1-DCE (835 mg/L)	0/10 (0)	0	0/25 (0)	0
+	1,1-DCE (835 mg/L)	0/10 (0)	0	20/25 (80)	2.50 ± 2.60 (0–1)
−	1,1-DCE (2500 mg/L)	0/10 (0)	0	1/25 (4)	0.04 ± 0.20 (0–1)
+	1,1-DCE (2500 mg/L)	2/10 (20)	0.20 ± 0.40 (0–1)	20/25 (80)	1.80 ± 1.60 (0–6)
−	1,2-DCE (835 mg/L)	1/10 (10)	0.10 ± 0.30 (0–1)	1/25 (4)	0.04 ± 0.20 (0–1)
+	1,2-DCE (835 mg/L)	2/10 (20)	0.20 ± 0.40 (0–1)	12/25 (48)	1.00 ± 1.10 (0–3)
−	1,2-DCE (2500 mg/L)	0/10 (0)	0	3/25 (12)	0.10 ± 0.30 (0–1)
+	1,2-DCE (2500 mg/L)	0/10 (0)	0	23/25 (88)	2.60 ± 2.00 (0–9)
−	Phenobarbital (500 mg/L)	0/10 (0)	0	0/25 (0)	0
+	Phenobarbital (500 mg/L)	1/10 (10)	0.10 ± 0.30 (0–1)	11/25 (44)	0.80 ± 1.00 (0–4)

[a] Incidence values represent the number of mice with lung tumors per total number of mice sampled. The number of tumors per mouse represents the mean ± SD of the number of tumors per mouse for all mice sampled at the sampling time.

Table 4. Morphometric measurement of liver tumor area.[a]

Initiation	Promotion	Relative liver tumor area, %	Mean tumor area, cm^2
−	—	0.50	8.65
+	—	12.10	12.82
+	Phenobarbital (500 mg/L)	22.31	16.37
−	$CHCl_3$ (high dose)	6.21	9.78
+	$CHCl_3$ (high dose)	9.27	9.54
−	$CHCl_3$ (low dose)	1.12	7.47
+	$CHCl_3$ (low dose)	6.02	11.97

[a] Values were determined by quantitating the liver tumor area and total liver area on randomly selected H&E-stained histologic sections from mice sampled at 52 weeks treatment. Measurements were made using a computer-assisted planimetry (Altos computer with bitpad and stereology software). The relative tumor area was determined by using the equation: (liver tumor area/total liver area) × 100. The mean tumor area was determined by using the equation: liver tumor area/number of liver tumors.

Table 5. Comparison of dose levels of $CHCl_3$, 1,1-DCE, and 1,2-DCE in B6C3F1 mice.

Compound	Mode of treatment	Dose[a]	Approximate weekly dose, mg/g body weight mouse[b]
$CHCl_3$	Drinking water	1800 mg/L	1.8
	Corn oil gavage	300 mg/kg	1.5
1,1-DCE	Drinking water	2500 mg/L	3.8
	Corn oil gavage	3000 mg/kg	15.0
1,2-DCE	Drinking water	2500 mg/L	3.3
	Corn oil gavage	200 mg/kg	1.0

[a] Corn oil gavage dose from previous study (2) represents the maximum tolerated dose administered daily five times per week. Drinking water dose taken from the present study.

[b] Approximate weekly dose calculated for the drinking water mode of treatment as follows: mean daily water uptake (mL) for 30 g mouse × concentration in drinking water (mg/mL) × 7 days/30 g (mouse body weight). Approximate weekly dose calculated for the corn oil gavage mode of treatment as follows: mean daily dose (mg/g) for 30 g mouse × 5 treatments.

mor number per mouse over that seen with DENA initiation alone. In fact, $CHCl_3$, when administered in the tumor promotion protocol, significantly inhibited the average number and average size of liver tumors compared to those of the DENA-only-treated mice. Phenobarbital, a liver tumor promoter in mice and rats, increased the mean number and mean size of the DENA-initiated liver tumors.

The observed liver tumor findings with $CHCl_3$, 1,1-DCE, and 1,2-DCE are in opposition to those previously reported (2) for these three compounds when administered to male B6C3F1 mice by gavage. In this previous study, $CHCl_3$ (300 mg/kg), 1,1-DCE (3000 mg/kg), and 1,2-DCE (200 mg/kg), administered in corn oil by daily gavage 5 times a week for 78 weeks, produced a significant increase in the incidence of liver tumors (2). Chloroform induced liver tumors in 44 out of 45 mice, 1,1-DCE induced liver tumors in 8 out of 47 mice, and 1,2-DCE induced liver tumors in 12 out of 47 mice (2). The difference in the liver tumor response between the corn oil gavage study and the present drinking water study may be related to the method of treatment (gavage vs. drinking water) and the vehicle (corn oil vs. water) used for administration. Three potential reasons for these differences include: the dose in drinking water may be lower than that given by the gavage protocol; the uptake, distribution, and elimination of the compounds associated with the two modes of treatment may vary; and the corn oil vehicle may be modifying the liver tumor response. With regard to dose in the mouse, in the present study, the average water intake for a 24-week-old mouse [mean body weight 30 g] given 1800 mg/L $CHCl_3$ in drinking water was 4.3 g of water per day. This amount is calculated as a weekly intake of 1.8 mg of $CHCl_3$/g body weight of mouse, which compares favorably with a weekly intake of 1.5 mg/g of chloroform body weight when given by gavage (Table 5). When an approximately weekly dose is calculated using this method, it appears that for the high-dose $CHCl_3$, the doses are similar (1.8 mg/g body weight for drinking water vs. 1.5 mg/g body weight for gavage); for the high dose of 1,1-DCE, the corn oil gavage resulted in a 5-fold higher dose than that of the drinking water; and for the 1,2-DCE, the high dose in drinking water resulted in a dose 3.3-fold higher than the gavage dose. Therefore, with the exception of the 1,1-DCE, the weekly drinking water doses are higher than the weekly gavage doses.

Whether the pharmacokinetics of the two treatments are similar remains to be examined. In the present study, focal kidney and liver necrosis was observed in the chloroform-treated mice, suggesting that the chloroform was getting to the liver and kidneys. However, the relative amount of compound taken up by the liver and kidney using the two modes of administration needs further study. The possibility of a tumor-promoting role for the corn oil vehicle used in the gavage administration of carcinogenic/toxic compounds has recently been raised (7). Whether corn oil may modify the response of the hepatocytes to chlorinated hydrocarbons also needs to be examined.

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