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Toxicology of Organic Drinking Water Contaminants: Trichloromethane, Bromodichloromethane, Dibromochloromethane and Tribromomethane

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This study evaluated the subchronic toxicity of selected halomethanes which are drinking water contaminants. The compounds studied were trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane. Subchronic 14-day gavage studies were performed with the use of doses encompassing one-tenth the LD_{50} for the compounds. A 90-day gavage study of one of the compounds, trichloromethane, was also done. Parameters observed included body and organ weights, histopathology, hematology, clinical chemistries, and hepatic microsomal enzyme activities. Toxicity to the humoral immune system was assessed by measuring the number of splenic IgM antibody-forming cells and the serum antibody level to sheep erythrocytes. Cell-mediated immunity was evaluated by measuring the delayed type hypersensitivity response and popliteal lymph node proliferation response to sheep red blood cells. The functional activity of the reticuloendothelial system, as measured by the vascular clearance rate and tissue uptake of ^{51}Cr sheep red blood cells was also determined.

The major effects of the halomethanes were increased liver weights, elevations of SGPT and SGOT, decreased spleen weights and a decrease in the number of splenic IgM antibody-forming cells. The humoral immune system appeared to be an indicator of halomethane toxicity. There is evidence that subchronic 14-day exposure may be of greater value than long-term studies in determining the toxicity of these compounds.

Introduction

In a study involving 80 U.S. cities, trichloromethane, bromodichloromethane, dibromochloromethane, or tribromomethane were found in all finished drinking water supplies at levels up to 311 μg/l. (*1*). Finished water supplies for which surface water was the original source and in which disinfection was accomplished by chlorination contained the highest levels of halomethanes. Total trihalomethane concentrations were, for the most part, related to the chlorination of raw water containing organic material.

At present the effects of chronic low level ingestion of trihalomethanes in drinking water are not known, although it has been suggested by Cantor (*2*) that an association between bladder cancer mortality rates and trihalomethane levels in drinking water exists. To understand the toxicology of the trihalomethanes better, this study investigated their effects upon the immune system in addition to the usual toxicological parameters. We performed

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subchronic 14-day studies in male and female mice with the selected trihalomethanes, as well as a 90-day study with trichloromethane, to determine if effects seen with short-term exposure would increase or diminish with long-term administration. It was found that the trihalomethanes affected primarily the liver and the spleen, with resulting implications for the immune system, and that certain effects diminished with long-term exposure.

Materials and Methods

CD-1 male and female mice were purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts, and housed four per plastic shoebox cage with sawdust bedding and free access to Agway Lab Chow and deionized drinking water. The mice were individually tagged by earpunching and quarantined for one week prior to experimentation. The room was maintained at 21–24°C and a relative humidity of 40–60%. The light-dark cycle was set on 12 hr intervals.

Trichloromethane (Fisher Scientific Co., Richmond, Va. 23230; lot #790372), tribromomethane (Aldrich Chemical Co., Milwaukee, Wisc. 53233; lot #13294–2), bromodichloromethane (Pflatz and Bauer Inc., Stamford, Conn. 06902; lot #320355), and dibromochloromethane (Columbia Organic Chemical Co., Columbia, S.C.; lot #020580) were obtained from commercial sources. Solutions for gavage were prepared fresh daily in 10% Emulphor in deionized water, and appropriate concentrations were administered in a volume of 0.01 ml/g body weight to deliver the desired dose. The dose levels chosen for these studies were 50, 125 and 250 mg/kg/day for 14 days. A 90-day subchronic study was also done for one of the chemicals, trichloromethane, using the same dosages.

Animals were anesthetized with chloroform and blood was collected immediately by cardiac puncture. Gross pathological examinations were performed on all mice. The organs (brain, liver, lungs, spleen, thymus, kidneys, and testes) were then removed, trimmed, and weighed. Material for histologic analysis (kidney, liver, spleen) was fixed in 10% buffered formalin, infiltrated and embedded in paraffin, sectioned at 6 microns, and stained with hematoxylin and eosin. Livers and spleens not destined for histologic study were used in additional procedures.

Blood samples for hematological study were taken in 3.2% sodium citrate. Leukocyte, erythrocyte and platelet counts were performed on a Coulter Counter, Model ZBI. Hematocrits were performed with microhematocrit equipment and hemoglobins determined as cyanomethemoglobin. Leukocyte differentials were evaluated using the standard Wright's-Giemsa staining procedure. Bone marrow cells were flushed from the femur in α-MEM with 5% fetal calf serum and enumerated on the Coulter Counter.

The plasma from the blood samples was assayed for extrinsic activity by prothrombin time. Reagents for this assay were obtained from General Diagnostics. Fibrinogen levels were determined by the kinetic method, using reagents from Dade Diagnostics Inc., Miami, Florida.

For clinical chemistry studies, additional blood samples were drawn by cardiac puncture from animals used for studies on humoral immunity, then allowed to clot. The sera from these samples were loaded onto an Abbott Bichromatic Analyzer, Model 100, and processed according to procedures described in the Operator's Manual.

Livers for microsomal assay were removed, weighed, rinsed, and homogenized at 4°C in four volumes of 0.15*M* potassium chloride containing 0.1*M* potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 9000*g* for 20 min and the supernatant recentrifuged at 100,000*g* for 1 hr in a Beckman Model L5-50 ultracentrifuge. The microsomes in the pellet were resuspended in 0.05*M* potassium phosphate buffer, pH 7.4 (0.5 g of liver/ml), and aliquots were taken for immediate study.

Microsomal protein was assayed by the method of Lowry et al. (*3*). The cytochrome P-450 content was determined from the reduced CO versus reduced difference spectrum, using an extinction coefficient of 91 $cm^{-1}mm^{-1}$ (*4*). Aminopyrine *N*-demethylase activity was determined by measuring formaldehyde production by the Nash reaction as described by Cochin and Axelrod (*5*). Aniline hydroxylase activity was measured as *p*-aminophenol production (*6*).

The primary IgM response to sheep erythrocytes (sRBC), a measure of humoral immunity, was estimated by the hemolytic plaque assay of Jerne and Nordin (*7*) as modified by Cunningham and Szenberg (*8*). Mice were immunized with 4×10^8 sRBC by IP injection 4 days prior to sacrifice. Spleen cell suspensions were prepared in RPMI 1640 culture medium using stainless steel mesh screens and adjusted to a cell concentration of 10^6/ml for assay of antibody-forming cells (AFC).

As a complement to the plaque assay, the plasma antibody titer was measured by the hemagglutination technique. Seven days after IP injection of 10^9 sRBC, blood was collected by cardiac puncture from chloroform-anesthetized animals into 3.2% sodium citrate. After centrifugation, the plasma was heat inactivated, and serial (1:1) dilutions were made in phosphate-buffered saline. To each of the

dilutions in a microtiter well, an equal volume of a 0.5% suspension of sRBC was added. After incubation for 2 hr at 37°C, the plates were observed on a magnifying mirror for agglutination of the sRBC. The antibody titers were expressed as $\log_2$ of the reciprocal of the first dilution with no visible agglutination.

Cell-mediated immunity was evaluated by measuring the delayed type hypersensitivity (DTH) response to sRBC. This is a modification of the methods of Lagrange et al. (*9*) and Paranjpe and Boone (*10*). Sensitization was accomplished by injecting 10^8 sRBC in a volume of 0.02 ml into the left footpad (LFP). Four days following sensitization, the mice were challenged in the LFP with 4×10^8 sRBC in a volume of 0.04 ml. At 17 hr following challenge, the mice were injected intravenously with 0.3 ml of ^{125}I-human serum albumin (HSA) (80,000 cpm/0.1 ml). Two hours later, the mice were sacrificed by cervical dislocation, and both hind feet were removed at the ankle joint and radioassayed in a gamma counter. The right footpad (RFP) served as an unchallenged control for background ^{125}I-HSA. A group of mice which was not sensitized, but was challenged as above, acted as unsensitized controls to determine nonspecific swelling. Results are expressed as a stimulation index (SI), which is calculated as follows:

$$SI = \left(\frac{\text{cpm LFP sensitized}}{\text{cpm RFP sensitized}}\right) - \left[\bar{x}\left(\frac{\text{cpm LFP unsensitized}}{\text{cpm RFP unsensitized}}\right)\right]$$

Popliteal lymph node proliferation in response to sRBC was determined to further evaluate cellular immunity. Mice were sensitized 4 days prior to the last day of gavage by injection of 1×10^8 sRBC in 0.02 ml of Alsever's solution into the left hind footpad (LFP). One group of control mice was injected with saline to measure nonspecific stimulation. Four days later, the animals were challenged by injecting 4×10^8 sRBC in 0.04 ml Alsever's solution into the LFP. At 1.5 hr after challenge, the mice were given an IP injection of 0.2 ml FUdR ($8 \times 10^{-6} M$). An IV injection of 2.0 μCi ^{125}I-labeled iododeoxyuridine in 0.2 ml saline was administered 30 min later. The mice were sacrificed 20 hr after labeling, and the popliteal lymph nodes were removed and counted in a gamma counter. The stimulation index was calculated as shown below:

$$SI = \left(\frac{\text{SENSITIZED}\ \text{cpm left popliteal node}}{\text{cpm right popliteal node}}\right) - \left[\bar{x}\left(\frac{\text{UNSENSITIZED}\ \text{cpm left popliteal node}}{\text{cpm right popliteal node}}\right)\right]$$

Several particles for measuring the phagocytic activity of the fixed macrophage system had been compared previously (*11*). For these studies we used sRBC. Freshly-drawn sRBC (5×10^9 cells/ml) were radiolabeled with ^{51}sodium chromate in a 37°C shaker bath with 1 mCi/5 ml cells for 30 min. After chromation, the sRBC were washed with Alsever's solution until the supernatant was virtually radioactivity free. Unlabeled sRBC (5×10^9 ml) were added to the labeled cells until the hematocrit was 10%. The resulting cpm's were approximately 200,000/0.1 ml. The sRBC were refrigerated and used the following day. Before use, the cells were washed to remove any free chromium released overnight.

Mice used for evaluation of RES activity were weighed and placed in shoebox cages maintained at 21°C. At zero time, 0.1 ml of labeled particle/10 g body weight was injected intravenously. Blood samples (10 μl) were taken from the tip of the tail at 2, 4, 6, 8, 10, and 15 min. The blood samples were put into 1 ml of distilled water and radioassayed. At the end of 60 min, the mice were sacrificed by decapitation and drained of blood. The liver, spleen, lungs, thymus, and kidneys were removed, weighed, and placed in tubes for counting in a gamma counter. Blood clearance is expressed as the phagocytic index, which is determined by the slope of the clearance curve. Organ distribution is expressed as percent organ uptake and cpm/mg tissue (specific activity).

In order to determine whether or not tolerance to certain halomethanes could be induced, the following experiment was conducted. Groups of male and female mice were gavaged with trichloromethane at the doses previously stated for 90 days. They were then given a single gavaged dose of 1000 mg/kg, an amount approximating the LD_{50} dose, and observed for lethality.

If a one-way analysis of variance of the means showed treatment effects, a Dunnett's *T*-test was performed (*12*). Values which differ from vehicle control at $p < 0.05$ are noted in the tables. Each of the values is given as the mean ± SE of the mean (SEM).

Results

Subchronic Fourteen-Day Studies

CD-1 male and female mice were given trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane by gavage at 50, 125 or 250 mg/kg/day for 14 days. No chemically induced deaths were noted with any of the compounds. At the end of this period the animals were necropsied. Table 1 lists all the parameters observed in the subchronic 14- and 90-day studies. The data pre-

Table 1. General toxicological and immunological parameters observed following halomethane exposure.

Body weights	Clinical chemistry
	Calcium
Organ weights	Sodium
Brain	Chloride
Liver	Potassium
Spleen	Protein
Lungs	Glucose
Thymus	Cholesterol
Kidneys	Bilirubin
Testes	Creatinine
	BUN
Hematology	LDH
WBC	SGPT
RBC	SGOT
HCT	ALP
HGB	
Platelets	Hepatic microsomal activities
	Protein content
Hexobarbital sleeping time	Glutathione
Bone marrow	Cytochrome P-450
DNA synthesis	Cytochrome b_5
Stem cell culture	Aminopyrine N-demethylase
Cell-mediated immunity	Aniline hydroxylase
Delayed type hypersensitivity	Humoral immunity
Popliteal lymph node proliferation	Enumeration of antibody-forming cells
Functional status of RES	Hemagglutination

Table 2. Effects of trichloromethane upon CD-1 male mice following 14 days of exposure by gavage.

Parameter	Vehicle (12)[a]	50 mg/kg (8)[a]	125 mg/kg (8)[a]	250 mg/kg (7)[a]
Body weights, g	31.3 ± 0.5	29.6 ± 0.5	31.2 ± 0.5	26.3 ± 1.2[b]
Organ weights				
Liver, mg	1632 ± 48	1734 ± 34	2031 ± 63[b]	2111 ± 126[b]
(% body weight)	(5.22)	(5.87)	(6.50)[b]	(8.01)[b]
Spleen, mg	141 ± 8	178 ± 7[b]	149 ± 13	110 ± 6
(% body weight)	(0.45)	(0.60)[b]	(0.40)	(0.42)
Clinical chemistry				
SGPT, IU/l.	60 ± 9	81 ± 12	95 ± 22	2163 ± 27[b]
Humoral immunity				
AFC/spleen × 10^5	2.89 ± 0.28	1.44 ± 0.18[b]	1.35 ± 0.24[b]	1.02 ± 0.12[b]
AFC/10^6 cells	3253 ± 171	2057 ± 152[b]	1700 ± 190[b]	1405 ± 101[b]
Hemagglutination ($\log_2$ titer)	7.74 ± 0.23	7.82 ± 0.27	7.32 ± 0.19	7.32 ± 0.22

[a]Number of mice per group given in parentheses following each dose level.
[b]Significant at $p < 0.05$ level, Dunnett's T-test.

sented in this manuscript represents alteration which occurred in a dose-dependent fashion or at the total AFC and at the intermediate and high doses when expressed as AFC/10^6 spleen cells; however, trends are omitted in an effort to minimize presentation of data.

Trichloromethane. Males exposed to the high dose of trichloromethane showed a decrease in body weight (Table 2). Liver weights were increased at 125 and 250 mg/kg regardless of the method of data expression. Spleen weight was significantly elevated at the low dose alone. SGPT was increased in the high dose group. The most striking change occurring in the males was a decrease in AFC expressed as total number and per 10^6 spleen cells at all levels of treatment. Hemagglutination titer was not significantly affected, nor were any alterations in cell-mediated immunity observed.

No significant change in body weight was seen in the female mice (Table 3). Liver weights were increased at all dose levels when the results were expressed as percent of body weight and in the high dose regardless of the method of expression. Spleen weights showed a decreasing trend with increasing dose weight levels but did not attain significance at the $p < 0.05$ level. Changes in clinical chemistry parameters included increased SGOT and SGPT values at the high dose and a decrease in serum glucose at the intermediate and high doses. Humoral immunity was depressed at all doses in terms of highest dose level only. Parameters which were essentially unchanged or did not reveal relevant

Table 3. Effects of trichloromethane upon CD-1 female mice following 14 days of exposure by gavage.

Parameter	Vehicle (12)[a]	50 mg/kg (12)[a]	125 mg/kg (8)[a]	250 mg/kg (12)[a]
Body weights, g	26.3 ± 0.6	25.0 ± 0.6	25.2 ± 0.6	25.6 ± 0.8
Organ weights				
Liver, mg	1442 ± 44	1518 ± 27	1517 ± 57	1786 ± 52[b]
(% body weight)	(5.49)	(6.08)[b]	(6.03)[b]	(6.99)[b]
Spleen, mg	151 ± 9	138 ± 8	140 ± 14	134 ± 6
(% body weight)	(0.57)	(0.56)	(0.55)	(0.52)
Clinical chemistry				
SGPT, IU/l.	53 ± 5	47 ± 4	58 ± 4	154 ± 30[b]
SGOT, IU/l.	97 ± 7	78 ± 5	76 ± 4	143 ± 15[b]
Glucose, mg-%	133 ± 4	136 ± 3	114 ± 4[b]	83 ± 6[b]
Humoral immunity				
AFC/spleen × 10^5	1.35 ± 0.3	0.89 ± 0.05[b]	0.58 ± 0.6[b]	0.41 ± 0.05[b]
AFC/10^6 cells	1692 ± 67	1661 ± 84	1157 ± 87[b]	1253 ± 76[b]
Hemagglutination ($\log_2$ titer)	8.07 ± 0.22	7.70 ± 0.18	7.82 ± 0.19	7.40 ± 0.26

[a]Number of mice per group given in parentheses following each dose level.
[b]Significant at $p < 0.05$ level, Dunnett's T-test.

Table 4. Effects of bromodichloromethane upon CD-1 male mice following 14 days of exposure by gavage.

Parameter	Vehicle (12)[a]	50 mg/kg (8)[a]	125 mg/kg (8)[a]	250 mg/kg (9)[a]
Body weights, g	32.9 ± 0.6	33.2 ± 0.6	31.1 ± 1.0	25.8 ± 1.2[b]
Organ weights				
Liver, mg	1904 ± 65	2050 ± 72	2083 ± 115	2851 ± 79
(% body weight)	(5.78)	(6.17)	(6.70)[b]	(7.21)[b]
Spleen, mg	160 ± 12	143 ± 6	136 ± 10	99 ± 11[b]
(% body weight)	(0.49)	(0.43)	(0.44)	(0.38)
Hematology				
Fibrinogen (mg %)	320 ± 14	345 ± 15	283 ± 10	276 ± 17[b]
Clinical chemistry				
Glucose, mg-%	179 ± 6	197 ± 9	186 ± 16	124 ± 11[b]
SGOT, IU/l.	70 ± 10	159 ± 28	172 ± 19	643 ± 125[b]
SGPT, IU/l.	35 ± 2	91 ± 17	118 ± 29	703 ± 12[b]
BUN, mg-%	23 ± 1	24 ± 2	28 ± 4	47 ± 4[b]
Humoral immunity				
AFC/spleen × 10^5	1.36 ± 0.12	0.96 ± 0.14	0.98 ± 0.13	0.66 ± 0.11[b]
AFC/10^6 cells	1196 ± 85	961 ± 60	1000 ± 78	1024 ± 72
Hemagglutination ($\log_2$ titer)	9.50 ± 0.18	9.20 ± 0.13	8.95 ± 0.18[b]	8.77 ± 0.24[b]

[a]Number of mice per group given in parentheses following each dose level.
[b]Significant at $p < 0.05$ level, Dunnett's T-test.

hemagglutination titer was not significantly affected.

Bromodichloromethane. A decrease in body weight was seen in the high dose group of male mice after 14 days of bromodichloromethane administration (Table 4). Liver weights were increased at the intermediate and high dose levels when the data were expressed as percent of body weight. The decrease in spleen weight was significant only at the high dose, but a nonsignificant decrease could be observed at all dose levels. A decrease in the fibrinogen level at 250 mg/kg was seen. Clinical chemistry changes occurred only in the high dose group and included a decrease in glucose and increases in SGOT, SGPT and BUN. The immune system was affected as evidenced by decreases in AFC/spleen and hemagglutination titer, both in the high dose group animals.

Female mice in the high dose group showed a significant reduction in body weight (Table 5). An increase in liver size was manifest in the intermediate and high dose groups only when the data were expressed as percent of body weight. Spleen weight was decreased at the 125 and 250 mg/kg dosages, both as total milligrams and as percent of body weight. Hematological studies revealed a decrease in fibrinogen levels at the intermediate and high dose regimens. Changes in clinical chemistry parameters were observed in the high dose group and included elevations in SGOT, SGPT, and BUN serum levels. The humoral immunity alterations seen as a result of bromodichloromethane administration were decreases in AFC/spleen and hemagglutination titer, observed in the intermediate and high dose groups.

Dibromochloromethane. Fourteen-day administration of dibromochloromethane caused a reduc-

Table 5. Effects of bromodichloromethane upon CD-1 female mice following 14 days of exposure by gavage.

Parameter	Vehicle (12)[a]	50 mg/kg (8)[a]	125 mg/kg (8)[a]	250 mg/kg (11)[a]
Body weights, g	26.6 ± 0.4	26.0 ± 0.6	24.9 ± 0.6	21.5 ± 1.0[b]
Organ weights				
Liver, mg	1432 ± 46	1584 ± 73	1608 ± 59	1553 ± 58
(% body weight)	(5.36)	(6.08)	(6.46)[b]	(7.31)[b]
Spleen, mg	162 ± 7	136 ± 11	112 ± 7[b]	82 ± 10[b]
(% body weight)	(0.61)	(0.52)	(0.45)[b]	(0.37)[b]
Hematology				
Fibrinogen (mg %)	226 ± 8	209 ± 7	191 ± 12[b]	119 ± 11[b]
Clinical chemistry				
SGPT, IU/l.	50 ± 7	49 ± 3	184 ± 40	648 ± 73[b]
SGOT, IU/l.	94 ± 10	85 ± 7	215 ± 38	774 ± 51[b]
BUN, mg-%	29 ± 1	28 ± 2	31 ± 2	41 ± 2[b]
Humoral immunity				
AFC/spleen × 10^5	1.11 ± 0.10	1.19 ± 0.15	0.55 ± 0.08[b]	0.35 ± 0.05[b]
AFC/10^6 cells	1018 ± 73	1133 ± 61	791 ± 61	801 ± 69
Hemagglutination ($\log_2$ titer)	8.91 ± 0.15	9.18 ± 0.14	8.32 ± 0.27	7.91 ± 0.15[b]

[a]Number of mice per group given in parentheses following each dose level.
[b]Significant at $p < 0.05$ level, Dunnett's T-test.

Table 6. Effects of dibromochloromethane upon CD-1 male mice following 14 days of exposure by gavage.

Parameter	Vehicle (11)[a]	50 mg/kg (8)[a]	125 mg/kg (8)[a]	250 mg/kg (12)[a]
Body weights, g	32.3 ± 0.7	33.5 ± 1.0	31.7 ± 0.07	25.8 ± 0.8[b]
Organ weights				
Liver, mg	1851 ± 72	2148 ± 101	2376 ± 182	2007 ± 105
(% body weight)	(5.72)	(6.40)	(7.49)[b]	(7.74)[b]
Spleen, mg	162 ± 7	154 ± 10	145 ± 8	96 ± 8[b]
(% body weight)	(0.50)	(0.46)	(0.46)	(0.37)[b]
Hematology				
Fibrinogen (mg %)	309 ± 10	310 ± 5	296 ± 8	251 ± 15[b]
Clinical chemistry				
Glucose, mg-%	274 ± 14	289 ± 14	252 ± 12	151 ± 8[b]
SGPT, IU/l.	48.6 ± 5.8	68.4 ± 7.9	83.5 ± 11.1	486.1 ± 61.9[b]
SGOT, IU/l.	97.9 ± 10.8	101.9 ± 10.3	102.8 ± 11.1	474.6 ± 67.5[b]
Humoral immunity				
AFC/spleen × 10^5	1.50 ± 0.16	1.39 ± 0.13	1.01 ± 0.28	0.74 ± 0.16[b]
AFC/10^6 cells	1611 ± 115	1484 ± 165	1114 ± 191[b]	1088 ± 105[b]
Hemagglutination ($\log_2$ titer)	9.41 ± 0.08	9.57 ± 0.16	9.32 ± 0.00	8.82 ± 0.26[b]
Cell-mediated immunity				
Popliteal lymph node (Stimulation Index)	11.06 ± 0.95	8.33 ± 1.85	8.27 ± 1.12	4.30 ± 0.93[b]

[a]Number of mice per group given in parentheses following each dose level.
[b]Significant at $p < 0.05$ level, Dunnett's T-test.

tion in body weight in the males at the high dose (Table 6). Liver weights were increased at 125 and 250 mg/kg/day when expressed as percent of body weight. Spleen and thymus values decreased significantly at the high dose whether the data was expressed as total milligrams or as percent of body weight. Of the hematological parameters, only fibrinogen was affected, with a decrease occurring at the high dose. Clinical chemistry alterations were increases in SGPT and SGOT and a decrease in serum glucose, all in the high dose group. Both humoral and cell-mediated immunity were affected by dibromochloromethane. The number of AFC was significantly reduced when expressed as total cells or as AFC/10^6 spleen cells. This was noted at the high dose, as was a reduction in hemagglutination titer. Cell-mediated immunity, as measured by the popliteal lymph node stimulation index, was depressed at the high dose. It should be noted that even though the changes were significant only at the 250 mg/kg/day level, the decreasing trend can be observed with the lower doses.

No significant body weight change was observed in females receiving dibromochloromethane. The major organ weight change in the females was an increase in liver size in the intermediate and high dose groups (Table 7). The ability of the liver to metabolize hexobarbital was impaired at the inter-

Table 7. Effects of dibromochloromethane upon CD-1 female mice following 14 days of exposure by gavage.

Parameter	Vehicle (12)[a]	50 mg/kg (8)[a]	125 mg/kg (8)[a]	250 mg/kg (12)[a]
Body weights, g	29.9 ± 0.5	27.1 ± 0.7	26.1 ± 0.9	26.3 ± 0.7
Organ weights				
Liver, mg	1528 ± 56	1622 ± 61	1735 ± 93	1923 ± 62[b]
(% body weight)	(5.67)	(5.98)	(6.63)[b]	(7.33)[b]
Hematology				
Fibrinogen (mg %)	260 ± 6	259 ± 6	248 ± 10	233 ± 7[b]
Clinical chemistry				
Glucose, mg-%	157 ± 4	148 ± 10	160 ± 8	105 ± 17[b]
SGPT, IU/l.	54 ± 6	73 ± 14	107 ± 16	449 ± 44[b]
SGOT, IU/l.	112 ± 8	124 ± 17	156 ± 17	526 ± 53[b]
Hexobarbital sleeping time, min[c]	15.1 ± 0.9	17.3 ± 1.4	27.7 ± 1.9[b]	37.4 ± 2.5[b]
Humoral immunity				
AFC/spleen × 10^5	1.34 ± 0.11	1.00 ± 0.12	0.79 ± 0.19[b]	0.45 ± 0.07[b]
AFC/10^6 cells	1641 ± 85	1418 ± 80	1071 ± 195[b]	1013 ± 100[b]
Hemagglutination (log_2 titer)	9.49 ± 0.17	9.70 ± 0.18	9.20 ± 0.30	8.82 ± 0.15[b]

[a]Number of mice per group given in parentheses following each dose level.
[b]Significant at $p < 0.05$ level, Dunnett's T-test.
[c]Induced by 80 mg/kg hexobarbital.

Table 8. Effects of tribromomethane upon CD-1 male mice following 14 days of exposure by gavage.

Parameter	Vehicle (10)[a]	50 mg/kg (7)[a]	125 mg/kg (7)[a]	250 mg/kg (10)[a]
Body weights, g	23.0 ± 0.9	24.5 ± 0.8	26.1 ± 0.3[b]	25.8 ± 0.52[b]
Organ weights				
Liver, mg	1337 ± 68	1485 ± 65	1682 ± 28[b]	1779 ± 63[b]
(% body weight)	(5.79)	(6.06)	(6.44)[b]	(6.90)[b]
Hematology				
Fibrinogen (mg %)	302 ± 24	283 ± 34	274 ± 16	218 ± 17[b]
Prothrombin time, sec	7.4 ± 0.1	6.8 ± 0.2[b]	7.0 ± 0.1[b]	6.8 ± 0.2[b]
Clinical chemistry				
Glucose, mg-%	233 ± 9	223 ± 11	204 ± 12	195 ± 8[b]
SGOT, IU/l.	92 ± 6	110 ± 19	80 ± 8	159 ± 33[b]
BUN, mg-%	36 ± 1	36 ± 1	34 ± 1	31 ± 1[b]
Humoral immunity				
AFC/spleen × 10^5	6.07 ± 0.6	5.95 ± 0.6	6.69 ± 1.1	3.44 ± 0.5[b]
AFC/10^6 cells	1310 ± 75	1279 ± 99	1368 ± 52	936 ± 79[b]
Cell-mediated immunity				
Delayed type hypersensitivity (Stimulation Index)	3.97 ± 0.53	2.22 ± 0.26	4.11 ± 1.21	1.96 ± 0.48[b]

[a]Number of mice per group given in parentheses following each dose level.
[b]Significant at $p < 0.05$ level, Dunnett's T-test.

mediate and high dose as evidenced by increased hexobarbital sleeping times. The only hematological change was a slight decrease in fibrinogen at the high dose. Altered clinical chemistry parameters were increases in SGPT and SGOT and a decrease in serum glucose, all occurring at 250 mg/kg/day. A decrease in AFC/spleen and AFC/10^6 spleen cells was noted in the intermediate and high dose groups, whereas a reduction of hemagglutination titer occurred only at the high dose.

Tribromomethane. Tribromomethane caused an increase in body weight in the intermediate and high dose groups of the males (Table 8). Liver weight, expressed as total milligrams or percent of body weight, increased at the 125 and 250 mg/kg dosages. Spleen weight was unchanged. Hematological changes included a decrease in fibrinogen at the highest dose and a reduction in prothrombin time occurring at all dosages. Clinical chemistry changes all appeared at the 250 mg/kg level and included a decrease in serum glucose and BUN and an increase in SGOT. Both humoral and cellular immunity were depressed in the high dose group. The change in humoral immunity was evident whether expressed as AFC/spleen or AFC/10^6 cells.

In contrast to the males' change in body weight, females showed a significant decrease at the high dose (Table 9). An increase in liver weight was evident at the high dose regardless of the method of expression, whereas the decrease in spleen weight was significant only when expressed as total milligrams and occurred at the intermediate and high

Table 9. Effects of tribromomethane upon CD-1 female mice following 14 days of exposure by gavage.

Parameter	Vehicle (12)[a]	50 mg/kg (7)[a]	125 mg/kg (6)[a]	250 mg/kg (7)[a]
Body weights, g	22.3 ± 0.6	20.4 ± 0.5	20.6 ± 0.5	19.5 ± 0.9[b]
Organ weights				
Liver, mg	1253 ± 48	1298 ± 47	1248 ± 46	1524 ± 69[b]
(% body weight)	(5.61)	(6.37)	(6.07)	(7.88)[b]
Spleen, mg	119 ± 9	99 ± 11	89 ± 6[b]	53 ± 5[b]
(% body weight)	(0.53)	(0.48)	(0.46)	(0.27)
Clinical chemistry				
SGOT, IU/l.	80.4 ± 6.2	75.5 ± 8.6	65.8 ± 13.5	129.7 ± 17.2[b]

[a]Number of mice per group given in parentheses following each dose level.
[b]Significant at $p < 0.05$ level, Dunnett's T-test.

Table 10. Effects of trichloromethane upon CD-1 male mice following 90 days of exposure by gavage.

Parameter	Vehicle (11)[a]	50 mg/kg (8)[a]	125 mg/kg (7)[a]	250 mg/kg (12)[a]
Body weights, g	36.1 ± 0.9	35.5 ± 0.8	34.1 ± 1.3	36.3 ± 0.8
Organ weights				
Liver, mg	1921 ± 69	2020 ± 70	2025 ± 130	2428 ± 115[b]
(% body weight)	(5.31)	(5.69)	(5.91)	(6.65)[b]
Spleen, mg	156 ± 13	139 ± 12	141 ± 12	180 ± 9
(% body weight)	(0.43)	(0.39)	(0.41)	(0.44)
Clinical chemistry				
Glucose, mg-%	225 ± 11	265 ± 9	286 ± 13	318 ± 15[b]
SGPT, IU/l.	63 ± 4	58 ± 6	76 ± 6	81 ± 6
SGOT, IU/l.	90 ± 8	105 ± 12	106 ± 14	131 ± 21
Humoral immunity				
AFC/spleen × 10^5	2.27 ± 0.20	1.50 ± 0.21[b]	2.41 ± 0.27	1.34 ± 0.18[b]
AFC/10^6 cells	1723 ± 91	1371 ± 151	1620 ± 115	1081 ± 105[b]
Hemagglutination ($\log_2$ titer)	9.50 ± 0.18	9.20 ± 0.13	9.32 ± 0.22	9.16 ± 0.17
Hepatic microsomal activities				
Microsomal protein, mg/g liver	26.9 ± 0.64	24.4 ± 0.60	26.4 ± 0.69	23.5 ± 1.09[b]
Glutathione, mmole/g liver	10.0 ± 0.39	10.6 ± 0.36	10.6 ± 0.37	10.1 ± 0.48
Aniline hydroxylase, nmole/mg/min	1.86 ± 0.06	2.22 ± 0.11[b]	1.81 ± 0.02	1.52 ± 0.06[b]
Hexobarbital sleeping time (min)[c]	36.2 ± 4.0	41.8 ± 4.1	46.8 ± 4.6	47.4 ± 4.9

[a]Number of mice per group given in parentheses following each dose level.
[b]Significant at $p < 0.05$ level, Dunnett's T-test.
[c]Induced by 80 mg/kg hexobarbital.

doses. The only alteration in clinical chemistry values was an increase in SGOT, which occurred in the 250 mg/kg group. The immune system was not affected by tribromomethane as measured by the humoral and cell mediated responses to sRBC.

Subchronic Ninety-Day Study

CD-1 male and female mice were gavaged with trichloromethane at 50, 125 or 250 mg/kg/day for 90 days. No chemically induced deaths were attributed to the compound during treatment.

Male mice showed no significant changes in body weight due to treatment (Table 10). Liver weight was increased both as total milligrams and as percent of body weight at the high dose. Spleen weight was not affected. Clinical chemistry values were essentially normal except for serum glucose, which was elevated at the intermediate and high doses. Humoral immunity was affected at the 250 mg/kg dose in that AFC/spleen and AFC/10^6 cells were decreased. Total AFC/spleen also decreased at the low dose. Hemagglutination titer was not affected.

Hepatic microsomal activities were evaluated in the 90-day exposure study. Table 10 shows a suppression of microsomal protein at the high dose. There were also changes in the microsomal enzyme aniline hydroxylase, but they were not dose-related, increasing at the low dose and decreasing at the high dose.

Ninety-day trichloromethane administration did not alter body weights at any level of treatment in the female mice (Table 11). Liver weights were increased at all levels of treatment when expressed either as total milligrams or as percent of body weight. Serum glucose was increased in the females at the high dose only. The only change occurring in humoral immunity was a decrease in AFC/10^6 spleen cells at the low dose; not a dose-related effect. A

Table 11. Effects of trichloromethane upon CD-1 female mice following 90 days of exposure by gavage.

Parameter	Vehicle (12)[a]	50 mg/kg (7)[a]	125 mg/kg (7)[a]	250 mg/kg (12)[a]
Body weights, g	28.6 ± 0.5	29.2 ± 1.1	30.6 ± 0.5	30.8 ± 0.9
Organ weights				
Liver, mg	1293 ± 47	1606 ± 60[b]	1835 ± 42[b]	1991 ± 66[b]
(% body weight)	(4.51)	(5.54)[b]	(5.99)[b]	(6.48)
Spleen, mg	168 ± 12	158 ± 14	187 ± 25	161 ± 8
(% body weight)	(0.59)	(0.55)	(0.62)	(0.52)
Clinical chemistry				
Glucose, mg-%	232 ± 6	248 ± 15	261 ± 16	295 ± 9[b]
Humoral immunity				
AFC/spleen × 10^5	2.01 ± 0.24	1.56 ± 0.27	1.58 ± 0.14	1.76 ± 0.23
AFC/10^6 cells	1324 ± 63	984 ± 59[b]	1191 ± 79	1086 ± 79
Hemagglutination ($\log_2$ titer)	9.74 ± 0.19	9.66 ± 0.21	9.32 ± 0.22	9.66 ± 0.19
Cell-mediated immunity				
Delayed type hypersensitivity (Stimulation Index)	4.12 ± 0.56	3.14 ± 0.41	3.39 ± 0.57	2.48 ± 0.30[b]
Hepatic microsomal activities				
Microsomal protein, mg/g liver	28.0 ± 0.79	24.9 ± 0.84	22.9 ± 1.11[b]	22.3 ± 1.03[b]
Glutathione, mmole/g liver	6.91 ± 0.24	9.99 ± 0.47[b]	10.79 ± 0.50[b]	11.20 ± 0.51[b]
Aniline hydroxylase, nmole/mg/min	1.48 ± 0.04	1.41 ± 0.08	1.11 ± 0.06[b]	1.09 ± 0.06[b]
Hexobarbital sleeping time (min)[c]	19.2 ± 2.3	34.0 ± 2.9	35.0 ± 1.8[b]	46.5 ± 4.4[b]

[a]Number of mice per group given in parentheses following each dose level.
[b]Significant at $p < 0.05$ level, Dunnett's T-test.
[c]Induced by 80 mg/kg hexobarbital.

Table 12. Hyporesponsiveness to lethal effects of trichloromethane.[a]

	Females		Males	
Group	Survivors/N	% Mortality	Survivors/N	% Mortality
Vehicle	7/11	36	1/9	89
50 mg/kg	8/8	0	5/9	44
125 mg/kg	8/8	0	5/6	17
250 mg/kg	7/7	0	7/8	13

[a]Animals were given a single gavage of 1000 mg/kg after 90 days of exposure to the doses listed.

decrease was noted in the cell-mediated delayed type hypersensitivity assay, occurring in the high dose group.

The kidneys, livers and spleens of male and female mice gavaged with vehicle did not exhibit histopathological changes. Histologic examination of the selected organs of mice gavaged with chloroform revealed normal spleens; however, slight histopathologic changes were seen in the kidneys and livers of both males and females. The kidneys exhibited small intertubular collections of chronic inflammatory cells, mainly lymphocytes. The liver exhibited generalized hydropic degeneration of hepatocytes and occasional small focal collections of lymphocytes. Small amounts of extravasated bile were occasionally noticed in the females. Most of this extravasated material was present within sinusoidal Kupffer cells.

The females displayed a slight decrease in microsomal protein at the intermediate and high doses (Table 11). Aniline hydroxylase activity also decreased at these doses. Glutathione increased at all three levels of treatment, a change not observed in the males.

Tolerance Study

As shown in Table 12, a degree of protection to the lethal effects of trichloromethane was induced by subchronic 90-day exposure. A single 1000 mg/kg dose was lethal to 36% of the females who had received vehicle for 90 days but none of the treated mice died. Mortality of vehicle-treated males was 89%, while in the trichloromethane-treated groups, 13–44% died.

Discussion

A result of industrialization and urban development has been an increased need for a monitoring system to assess environmental contamination pertaining to air, food, and water supplies. Since it is impractical, if not impossible, to completely eliminate many low-level contaminants, it is imperative that we understand the effects of exposure to these contaminants. Our efforts to insure potable water supplies through chemical treatment have contributed to the ever-increasing number of compounds

to which we are exposed. The purpose of this study was to examine the effects of low-level exposure to selected halomethanes which are found in finished water supplies as a result of chlorination or industrial wastes. In addition to standard toxicological evaluation, the main intent was to investigate the effects of these chemicals upon the immune system. The use of the random-bred CD-1 mouse was a compromise between the random-bred rat, which is used for conventional toxicity testing, and the inbred mouse, which is most suitable for studies involving immune system function. Gavage was selected as the route of administration to control the amount of chemical to which the animals were exposed.

The major effect seen in subchronic 14-day administration of the compounds in regard to organ weights was an increase in liver weight. This was evident for all compounds and usually occurred in the intermediate and high dose groups of both sexes. This effect seemed to carry over into the 90-day study as was more evident in the female groups, in which liver weight was increased at all doses, whereas a significant change was only seen at the high dose in the males. It should be noted that in a number of instances the spleen weight decreased. This is of importance since the spleen is intimately involved with immune system function.

Hematological studies did not reveal any major changes in the cellular components of this system. However, a decrease in fibrinogen levels was noted with dibromochloromethane, tribromomethane and bromodichloromethane administration. This may reflect decreased capacity of the liver to synthesize the protein.

Alterations which occurred in the clinical chemistry parameters were indicative of hepatic disturbances. SGPT or SGOT were increased in both sexes with all compounds administered in the 14-day studies. In the 90-day study, this effect was not seen, perhaps indicative of recovery or tolerance to the compound used in the long-term experiment. The elevation in serum glucose level in the subchronic 90-day exposure may also reflect alteration in the ability of the liver to control glucose levels.

Although cell-mediated immunity was depressed in a few instances, these changes occurred only with the highest dose. Suppression of the humoral immune system was observed with all the chemicals under investigation in both sexes, the only exception being the females receiving tribromomethane for 14 days. Furthermore, a dose response was evident in almost all instances and, even when the decreases were not statistically significant, a definite trend toward reduction could be noticed. Thus it appears that the humoral immune system may be an indicator of halomethane toxicity. Further studies involving functional aspects of immunity are necessary to strengthen this observation.

The observation that subchronic 90-day exposure to trichloromethane did not exacerbate the changes seen to occur as a result of 14-day administration of the compound and, in fact, were less severe, points out the need for short-term studies to reveal effects which although transient, may have serious consequences under given circumstances, e.g., exposure to pathogens while the immune system is depressed. This is supported by the tolerance experiment, which revealed compensatory mechanisms being activated during subchronic 90-day exposure.

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