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Toxicology of Chloral Hydrate in the Mouse

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Chloral hydrate has been found in our drinking water supplies at levels up to 5 μg/l. The purpose of this study was to evalute the acute and subchronic toxicology of chloral hydrate in the random-bred CD-1 mouse, to provide data for risk assessment. The acute oral LD_{50} of this compound was 1442 and 1265 mg/kg in male and female mice, respectively. Acute toxicity appeared to be related to depression of the central nervous system. Fourteen-day exposure by gavage in male mice at doses 1/10 and 1/100 the LD_{50} caused an increase in liver weight and a decrease in spleen weight at the highest dose level. Based on the data derived from 14 days of exposure, a 90-day study was performed. The compound was delivered via the drinking water; levels of the compound delivered per day were equivalent to those dosed in the 14-day study. The target organ in both sexes appeared to be the liver, with the males most affected. Male mice demonstrated a dose-related hepatomegaly accompanied by significant changes in serum chemistries and hepatic microsomal parameters. The females did not demonstrate the hepatomegaly observed in males, but did show alterations in hepatic microsomal parameters. No other significant toxicological changes were observed in either sex following 90 days of exposure.

Introduction

Chloral hydrate has been identified as a water contaminant in several of the water supplies surveyed by the EPA (*1*). Its presence in the drinking water stems from its industrial use in the preparation of polyurethanes. After its introduction as a therapeutic agent in 1869, it was commonly used as a hypnotic until well into the twentieth century, when it was largely supplanted by other drugs. However, it is still used as a sedative in doses usually ranging from 0.5 to 1.0 g, but sometimes as high as 2 g (*2*). Aside from this, exposure to chloral hydrate may be secondary to exposure to trichloroethylene, another water contaminant which is transformed into chloral hydrate during its metabolism (*3*).

While the toxic effects of chloral hydrate as a therapeutic agent have been well documented, the effects of long-term oral exposure at lower levels are not known. The purpose of this study was to describe the toxicity of chloral hydrate administered in the drinking water.

Methods

Animals and Housing

Random-bred CD-1 mice were obtained from Charles River Breeding Laboratories, Wilmington, MA. They were housed four per cage in plastic shoebox cages containing sawdust bedding (PWI

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Hardwood Sawdust, Lowville, N.Y.), and maintained on Agway Lab Chow *ad libitum*. The animal room was maintained at 21–24°C and 40–60% relative humidity. The light/dark cycle was maintained on 12-hr intervals. All mice were quarantined for one week prior to use and individually identified by earpunching.

For acute toxicity studies, 6-week-old male and female mice were used. For the 14-day study, 5-week-old mice were purchased, and exposure began at six weeks of age. For the 90-day study, 3-week-old mice were purchased, and exposure began at 4 weeks of age.

Chemical

Chloral (U.S.P. crystalline, J. T. Baker Co., Phillipsburg, N.J. 08865, lot #925086) was used in these investigations and the structure confirmed by infrared spectroscopy. The manufacturer stated purity was 99+%.

Chemical Administration

Solutions of chloral hydrate were prepared fresh daily in deionized water for acute and 14-day studies, and the appropriate concentrations were administered by gavage in a volume of 0.01 ml/g body weight to achieve the desired dose. The doses used in the 14-day study were 14.4 and 144 mg/kg. For the 90-day study, chloral hydrate was diluted in deionized water to concentrations of 0.07 and 0.7 mg/ml and administered as the drinking water. Drinking water solutions were maintained at room temperature in amber-colored bottles fitted with sipper spouts and were changed twice weekly. Less than 10% of the chloral hydrate was lost during the 3-4 days in water bottles. The analytical method employed GLC and head space analysis.

For the 90-day study, fluid consumption was calculated by weighing the water bottles at the time solutions were initially placed in them, then weighing the bottles once again before solutions were changed to determine the amount consumed over the 3- or 4-day period. Twelve cages of control mice and eight cages of each treatment group per sex were used to estimate fluid consumption. Chemical consumption was calculated from fluid consumption and is reported as grams of chemical/mouse/day and grams of chemical/kg body weight/day.

Acute Toxicity

Chloral hydrate was administered to male and female mice by an 18-gauge stainless steel stomach tube 18 hr after fasting from food. Seven doses were administered: 0, 300, 600, 900, 1200, 1500 and 1800 mg/kg. Eight mice of each sex were in each dose group. Following gavage, the mice were observed for behavioral and toxicological effects continuously for 4 hr and then twice daily for 14 days. Mice dying during the 14-day experimental period were necropsied, and gross pathological changes were described. Mice surviving the 14-day observation period were sacrificed and gross pathology was described. Log probit analysis (4) was used to determine the LD_{50}, 95% confidence limits and the LOG probit slope.

Necropsy

Animals were anesthetized with chloroform, and blood was collected immediately by cardiac puncture into 3.2% sodium citrate to be used for hematological evaluation. The organs (brain, liver, spleen, lungs, thymus, kidneys, and testes) were removed, trimmed, weighed, and gross pathological examinations were performed on all mice. Livers and spleens were used in further studies.

Urinalysis

Urine was collected at sacrifice and analyzed using Labstix reagent strips from Ames Co. (Elkhart, Ind.).

Hematology

Blood samples were taken as described above. Leukocyte, erythrocyte, and platelet counts were performed in a Coulter counter, Model ZBI. Hematocrits were performed with microhematocrit equipment, and hemoglobins were determined as cyanomethemoglobin. Leukocyte differentials were evaluated by the standard Wright's-Giemsa staining procedure. Bone marrow cells were flushed from the femur into α-Minimal Essential Medium (Gibco) with 5% fetal calf serum and enumerated on the Coulter counter.

Coagulation

The plasma from the blood samples was assayed for extrinsic activity by prothrombin time and for intrinsic activity by activated partial thromboplastin time (APTT). Reagents were obtained from General Diagnostics (Morris Plains, N.J.). Fibrinogen levels were determined by the kinetic method, using reagents from Dade Diagnostics, Inc. (Miami, Fla.)

Serum and Liver Chemistries

For serum chemistries, additional blood samples were drawn by cardiac puncture from animals involved in a study on humoral immunity (*5*) and allowed to clot. The sera from these samples were loaded onto the Abbott Bichromatic Analyzer, Model 100, and processed according to the procedures described in the Abbott Operator's Manual. Liver non-protein sulfhydryl levels were determined as described previously (*6*).

Preparation and Assay of Microsomal Parameters

Livers were removed, weighed, rinsed and homogenized at 4°C in 4 volumes of 0.15*M* KCl containing 0.01*M* potassium phosphate buffer, pH 7.4. Individual homogenates were prepared for each mouse. The homgenate was centrifuged at 9000 *g* for 20 min and the supernatant fluid recentrifuged at 100,000 *g* for 1 hr in a Beckman Model L5-50 ultracentrifuge. The microsomal pellet was resuspended in 0.05*M* potassium phosphate buffer, pH 7.4 (0.5 g of liver/ml), and samples were taken for immediate study.

Microsomal protein was assayed by the method of Lowry (*7*). The cytochrome P-450 content was determined from the CO difference spectrum, in dithionite reduced microsomes by using an extinction coefficient (*8*) of 91 cm^{-1} mM^{-1}. Aminopyrine *N*-demethylase activity was determined by measuring formaldehyde production by the Nash reaction as described by Cochin and Axelrod (*9*). Aniline hydroxylase activity was measured as *p*-aminophenol production (*10*).

Results

Acute Toxicity

The acute toxicity data for chloral hydrate based upon the 14-day observation period are shown in Table 1. The LD_{50} for the females was 1265 mg/kg and for males 1442 mg/kg. The slope of the dose-response curves was 32.6 for females and 30.6 for males. Within 10 min of gavage, the mice became sedated at the low doses, while at the intermediate and higher doses, the animals became lethargic and exhibited loss of righting reflex. Respiration was markedly inhibited with the higher dosages; this inhibition appeared to be the immediate cause of death. Animals necropsied after death showed gastric hypermia, but were otherwise unremarkable. Most deaths occurred within 4 hr at the highest dose. At lower dosages, some deaths occurred after 4 hr, with all deaths occurring within 24 hr.

Statistical Evaluation

If a one-way analysis of variance of the means showed treatment effects, a Dunnett's *T*-test was performed (*11*). Values which differ from vehicle control at $p < 0.05$ are noted in the tables. Each of the values in the tables is given as the mean ± standard error (SE) of the mean.

Fourteen-Day Range Finding Study

Chloral hydrate solutions were administered to male mice by daily gavage for 14 consecutive days at doses of 14.4 and 144 mg/kg, which were 1/100 and 1/10 the LD_{50}.

Table 1. Acute toxicity of chloral hydrate in CD-1 mice.

Exposure group, mg/kg[a]	Sex	Number dead	LD_{50}, mg/kg[b]	Log probit slope
0	M	0	1442	30.6
300	M	0	(1290–1605[b])	
600	M	0		
900	M	0		
1200	M	1		
1500	M	4		
1800	M	8		
0	F	0	1265	32.6
300	F	0	(1097–1405)	
600	F	0		
900	F	0		
1200	F	3		
1500	F	7		
1800	F	8		

[a]There were eight animals in each dose group, with a total of 56 mice per sex.
[b]The numbers in parentheses represent the 95% confidence limits.

Table 2. Body weights of CD-1 male mice over 14 days of gavaging with chloral hydrate.

Exposure group	Day 1		Day 8		Day 15[b]	
	Number of mice	BW, g[a]	Number of mice	BW, g[a]	Number of mice	BW, g[a]
Deionized water	68	25.1 ± 0.2	68	27.5 ± 0.3	66	30.3 ± 0.3
14.4 mg/kg	60	25.2 ± 0.2	60	27.9 ± 0.3	59	30.7 ± 0.3
144 mg/kg	60	25.5 ± 0.2	60	27.8 ± 0.3	59	30.5 ± 0.3

[a]Body weight expressed as mean ± SE.
[b]Day 15 was 24 hr after the last day of gavage.

No significant changes in body weight were seen between the control and chloral hydrate-exposed mice over the 14-day exposure period (Table 2). One mouse of 60 died in the second week of gavaging in each chloral hydrate exposure group. These deaths were probably not compound-related since two mice of 68 died in the control group.

Of the general toxicological parameters measured, it appears that the livers and spleens of mice gavaged at 144 mg/kg were primarily affected. When expressed as percentage of body weight, the liver size increased 18%, while the spleen size decreased 27% compared to the vehicle control (Table 3). These changes were also seen when the organ weights were expressed in milligrams or as organ to brain ratios. Mice that received the lower dose (14.4 mg/kg) also demonstrated the same findings but not at values significantly different from control at $p < 0.05$.

Hematological and coagulation values were within control values for both dose groups (Table 4).

Serum glutamic pyruvic transaminase activity and blood urea nitrogen levels were within control

Table 3. Body and organ weights of CD-1 male mice exposed for 14 days to chloral hydrate.[a]

Exposure group	Body weight, g	Organ	Weight, mg	% of body weight	Organ/brain
Deionized water	31.0 ± 0.6	Brain	456 ± 7	1.48 ± 0.03	—
		Liver	1923 ± 96	6.18 ± 0.22	4.23 ± 0.22
		Spleen	227 ± 18	0.73 ± 0.06	0.50 ± 0.04
		Lungs	230 ± 5	0.74 ± 0.02	0.51 ± 0.02
		Thymus	89 ± 5	0.29 ± 0.01	0.19 ± 0.01
		Kidneys	597 ± 15	1.93 ± 0.05	1.31 ± 0.02
		Testes	229 ± 8	0.74 ± 0.02	0.50 ± 0.01
14.4 mg/kg	31.6 ± 0.6	Brain	446 ± 9	1.41 ± 0.04	—
		Liver	2109 ± 53	6.66 ± 0.12	4.75 ± 0.15
		Spleen	197 ± 14	0.62 ± 0.12	0.44 ± 0.03
		Lungs	228 ± 5	0.72 ± 0.02	0.51 ± 0.01
		Thymus	98 ± 7	0.31 ± 0.02	0.22 ± 0.02
		Kidneys	549 ± 24	1.73 ± 0.06[b]	1.24 ± 0.06
		Testes	240 ± 7	0.76 ± 0.02	0.54 ± 0.02
144 mg/kg	31.0 ± 0.9	Brain	441 ± 9	1.43 ± 0.04	—
		Liver	2255 ± 74[b]	7.30 ± 0.17[b]	5.12 ± 0.14[b]
		Spleen	166 ± 9[b]	0.54 ± 0.02[b]	0.38 ± 0.02[b]
		Lungs	223 ± 6	0.72 ± 0.02	0.51 ± 0.01
		Thymus	82 ± 6	0.26 ± 0.02	0.18 ± 0.01
		Kidneys	563 ± 21	1.82 ± 0.06	1.28 ± 0.04
		Testes	229 ± 5	0.74 ± 0.02	0.52 ± 0.02

[a]Values represent the mean ± SE derived from 11-12 mice/group.
[b]Significantly different from control at $p < 0.05$ as determined by Dunnett's T-test.

Table 4. Hematological and coagulation values for CD-1 male mice exposed for 14 days to chloral hydrate.[a]

Exposure group	Hematocrit, %	Hemoglobin, g-%	Leukocytes × 10^{-3}/mm^3	Fibrinogen, mg-%	Prothombin time, sec
Deionized water	40 ± 1	12.9 ± 0.3	6.79 ± 0.50	284 ± 7	8.2 ± 0.1
14.4 mg/kg	39 ± 0.5	12.4 ± 0.2	5.84 ± 0.36	284 ± 15	8.1 ± 0.1
144 mg/kg	41 ± 1	12.9 ± 0.2	6.36 ± 0.48	272 ± 7	8.4 ± 0.1

[a]Values represent the mean ± SE derived from 11-12 mice/group.

Table 5. Serum enzyme and urea nitrogen levels in CD-1 male mice exposed for 14 days to chloral hydrate.[a]

Exposure group	LDH, IU/l.[b]	SGPT, IU/l.[c]	BUN, mg-%[d]
Deionized water	908 ± 72	53.5 ± 5.3	27.8 ± 0.9
14.4 mg/kg	923 ± 46	78.2 ± 10.9	29.2 ± 1.5
144 mg/kg	727 ± 44[e]	73.5 ± 10.7	27.6 ± 1.0

[a]Values represent the mean ± SE derived from 10-12 mice/group.
[b]LDH = lactic dehydrogenase.
[c]SGPT = serum glutamic pyruvic transaminase.
[d]BUN = blood urea nitrogen.
[e]Significantly different from control at $p < 0.05$ as determined by Dunnett's T-test.

ranges. However, lactic dehydrogenase activity was depressed 20% compared to control in mice gavaged at 144 mg/kg (Table 5). This is difficult to interpret, since most reported abnormalities exhibit elevations, and not depressions.

Ninety-Day Study

Since the toxicity observed in the range-finding study was minimal, the concentrations of the chemicals administered in the 90-day drinking water study were calculated to deliver the same doses as the 14-day study. These concentrations were 0.07 and 0.7 mg chloral hydrate/ml. A group receiving deionized water served as controls. There were 260 mice of each sex in the control group and 140 of each sex in each treatment group. Forty-eight mice of each sex in the control group and 32 mice of each sex in the treatment groups were randomly selected and their body weight and fluid consumption were recorded twice weekly. The time-weighted average for chloral hydrate intake was 18 and 173 mg/kg for the females and 16 and 160 mg/kg for the males at 0.07 and 0.7 mg/ml, respectively. Table 6 shows the 30-day breakdown of daily fluid and chemical consumption per mouse and per kilogram. For consistency, all the toxicological data are presented according to concentration of chloral hydrate in the drinking water.

The growth curves for mice exposed to chloral hydrate are shown in Figures 1 and 2. Male mice exposed to chloral hydrate showed a dose-dependent increase in body weight (Fig. 1) over the 90-day period. Weight gains over this period were 14.1 for controls, 15.4 for the 0.07 mg/ml group, and 17.1 for the 0.7 mg/ml group. These weight increases were confirmed in mice used for gross pathology, which had increases above control of 8% for the 0.07 mg/ml group and 12% for the 0.7 mg/ml group (Table 7). There was no apparent effect of chloral hydrate on the growth rate of females (Figure 2). The average changes in female body weight over the 90-day period was 10.5 g for controls, 9.7 g for the 0.07 mg/ml group, and 11.7 g for the 0.7 mg/ml group. However, in half of the female mice (those used for gross pathology), the ones exposed to the highest concentration of chloral hydrate were 11% heavier than the controls (Table 7).

There was no compound-related gross pathology seen at the time of necropsy in either male or female mice. However, liver weights, as percentage of body weight, were increased in male mice receiving 0.07 mg/ml (12%) and 0.7 mg/ml (20%) (Table 7). This increase in liver size was also seen when weights were expressed on a milligram basis. No significant hepatomegaly occurred in the females. Spleen, thymus, kidney, and testes weights were not altered by exposure to chloral hydrate. There was a slight decrease in the lung weights of male mice, but the effect was not dose dependent and was significant only when the weights were expressed as percentage of body weight. This trend was also

Table 6. Time-weighted averages of consumption of fluid and chloral hydrate by CD-1 mice in a subchronic 90-day study.[a]

Sex	Concentration, mg/ml	Day 0–30		Days 31–60		Days 61–90		Days 0–90		
		ml/kg/day	mg/kg/day	ml/kg/day	mg/kg/day	ml/kg/day	mg/kg/day	ml/kg/day	mg/kg/day	ml/mouse/day
M	0	351 ± 10	0	217 ± 6	0	256 ± 10	0	273 ± 10	0	8.5 ± 0.3
M	0.07	276 ± 7	19.3 ± 0.5	201 ± 5	14.1 ± 0.3	191 ± 4	13.4 ± 0.3	225 ± 9	15.7 ± 0.6	7.3 ± 0.3
M	0.7	347 ± 8	242.9 ± 5.5	178 ± 3	124.9 ± 3.5	183 ± 3	128.0 ± 2.2	228 ± 5	159.8 ± 3.8	7.7 ± 0.2
F	0	327 ± 6	0	234 ± 6	0	194 ± 6	0	256 ± 9	0	7.1 ± 0.3
F	0.07	319 ± 9	22.3 ± 7	253 ± 7	17.7 ± 0.5	232 ± 7	16.3 ± 0.5	261 ± 11	18.2 ± 0.8	7.1 ± 0.3
F	0.7	329 ± 10	230.3 ± 6.8	210 ± 5	147.0 ± 3.3	203 ± 5	142.2 ± 3.7	248 ± 7	173.4 ± 5.2	6.9 ± 0.2

[a]Values represent the mean ± SE derived from 48 mice in the deionized water group and 32 mice in the other groups.

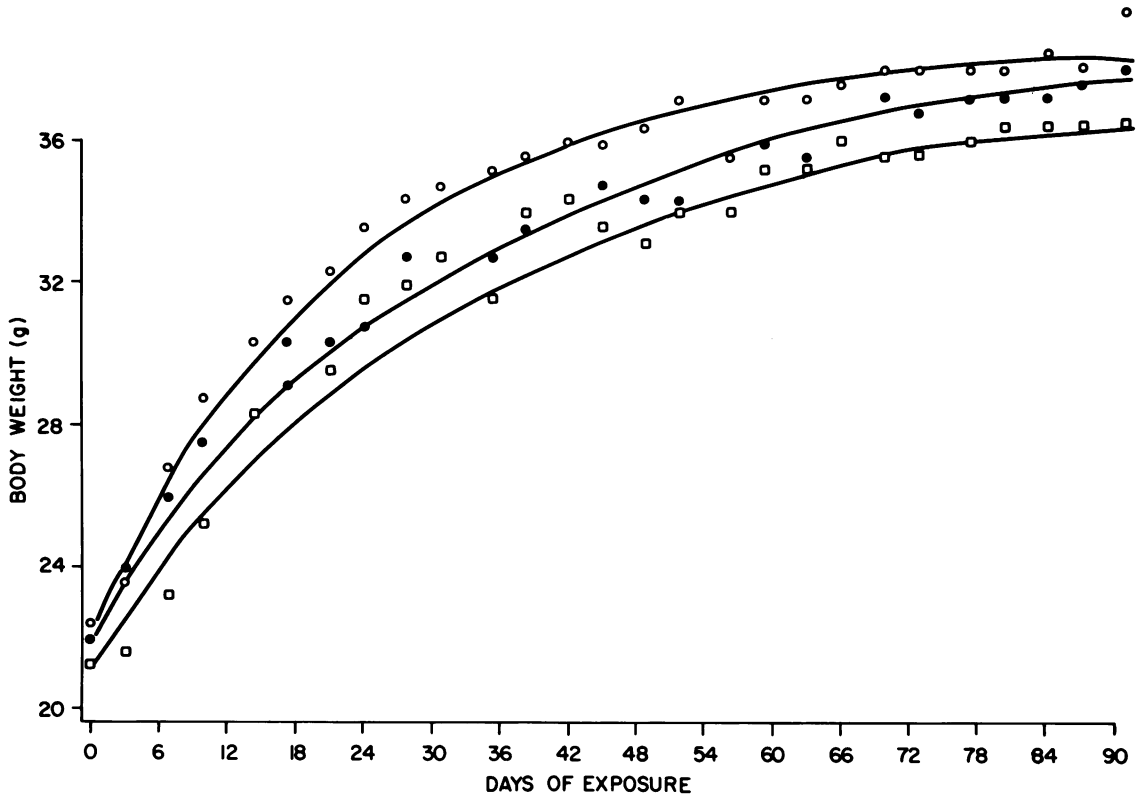

FIGURE 1. Growth chart of male CD-1 mice receiving chloral hydrate in the drinking water for 3 months. The mice received one of the following: (□) deionized water (48 mice) (0 chloral hydrate); (●) chloral hydrate (32 mice), 0.07 mg/ml (16 mg/kg/day); (○) chloral hydrate (32 mice), 0.7 mg/ml (160 mg/kg/day).

Table 7. Body and organ weights of CD-1 mice exposed to chloral hydrate in the drinking water for 3 months.[a]

	Males			Females		
Exposure Group	Deionized water (n = 21)	0.07 mg/ml (n = 15)	0.7 mg/ml (n = 16)	Deionized water (n = 22)	0.07 mg/ml (n = 13)	0.7 mg/ml (n = 16)
Body weight, g	33.9 ± 1.1	36.8 ± 1.0[b]	38.0 ± 0.8[b]	29.8 ± 1.0	29.9 ± 0.7	33.2 ± 0.6[b]
Brain, mg	453 ± 6	447 ± 22	448 ± 6	476 ± 8	473 ± 11	493 ± 5
(% body weight)	(1.36)	(1.23)	(1.18)[b]	(1.63)	(1.59)	(1.49)[b]
Liver, mg	1571 ± 81	1879 ± 83[b]	2107 ± 74[b]	1537 ± 47	1439 ± 90	1703 ± 57
(% body weight)	(4.59)	(5.12)[b]	(5.53)[b]	(5.22)	(4.77)	(5.13)
Spleen, mg	137 ± 12	147 ± 9	142 ± 6	159 ± 9	162 ± 10	162 ± 8
(% body weight)	(1.40)	(0.40)	(0.37)	(0.54)	(0.54)	(0.49)
Lungs, mg	204 ± 7	191 ± 8	204 ± 8	215 ± 11	190 ± 8	223 ± 11
(% body weight)	(0.61)	(0.52)[b]	(0.54)[b]	(0.73)	(0.64)	(0.68)
Thymus, mg	34 ± 2	45 ± 5	43 ± 4	54 ± 3	49 ± 3	61 ± 3
(% body weight)	(0.10)	(0.12)	(0.11)	(0.18)	(0.16)	(0.18)
Kidneys, mg	527 ± 18	557 ± 15	568 ± 15	410 ± 10	386 ± 16	420 ± 11
(% body weight)	(1.56)	(1.52)	(1.50)	(10.40)	(1.29)	(1.27)
Testes, mg	230 ± 9	241 ± 9	238 ± 12	—	—	—
(% body weight)	(0.68)	(0.66)	(0.63)			

[a]Values represent the mean ± SE.
[b]Significantly different from control at $p < 0.05$ as determined by Dunnett's T-test.

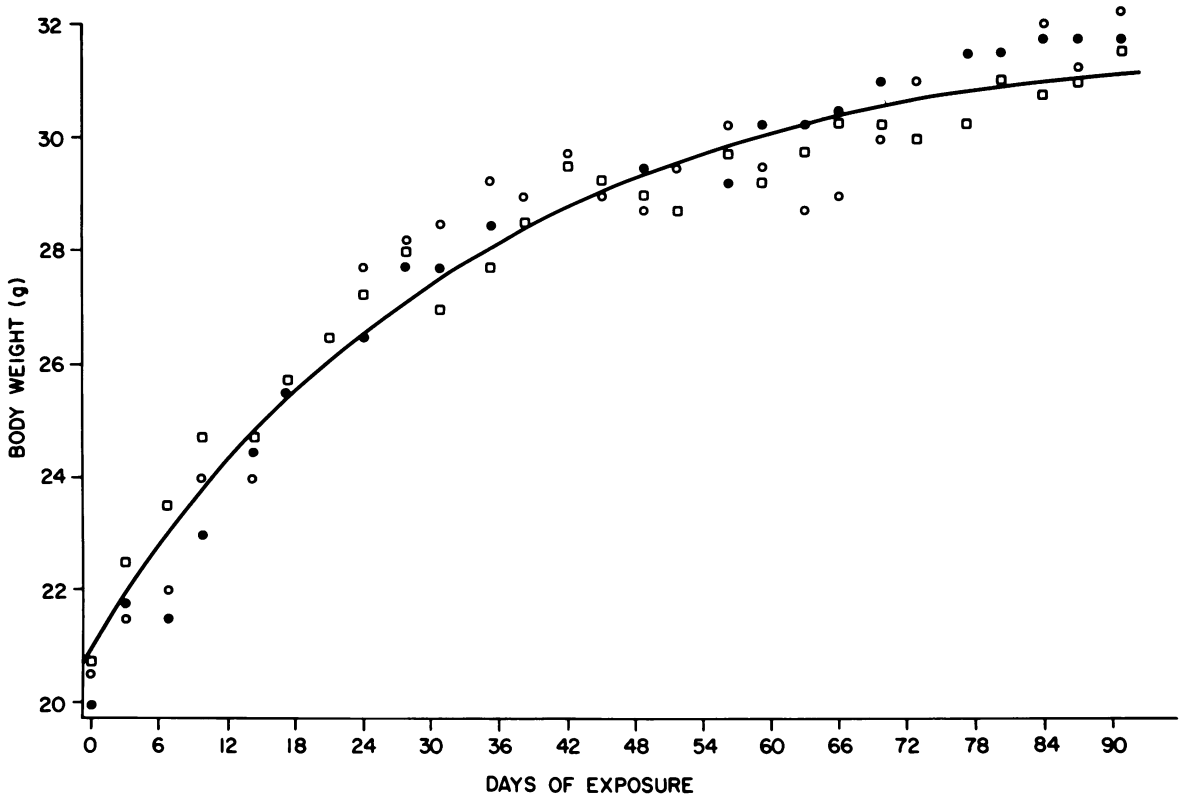

FIGURE 2. Growth chart of female CD-1 mice receiving chloral hydrate in the drinking water for 3 months. The mice received one of the following: (□) deionized water (48 mice) (0 chloral hydrate); (●) chloral hydrate (32 mice), 0.07 mg/ml (18 mg/kg/day); (○) chloral hydrate (32 mice), 0.7 mg/ml (173 mg/kg/day).

seen in the brain weights, which decreased proportionally with the increase in body weight.

Hematology

A summary of hematological findings is presented in Tables 8 and 9. Erythrocyte counts were 14% lower than control in female mice exposed to 0.7 mg/ml. This decrease was not confirmed with hemoglobin or hematocrit values, and was not evident in the males. Fibrinogen levels were elevated 9% above control in males exposed to 0.7 mg/ml, while a 14% elevation above control was seen in females exposed to 0.07 mg/ml. Slight increases were evi-

Table 8. Hematology of CD-1 mice exposed to chloral hydrate in the drinking water for 3 months.[a]

	Males			Females		
Exposure Group	Deionized water (n = 21)	0.07 mg/ml (n = 14)	0.7 mg/ml (n = 16)	Vehicle (n = 22)	0.07 mg/ml (n = 13)	0.7 mg/ml (n = 16)
Hematocrit, %	45.5 ± 0.9	43.4 ± 0.9	43.4 ± 0.8	42.4 ± 0.4	42.6 ± 0.4	42.3 ± 0.8
Hemoglobin, g-%	12.7 ± 0.3	12.4 ± 0.3	11.9 ± 0.2	11.4 ± 0.2	12.0 ± 0.2	11.9 ± 0.3
Erythrocytes, 10^6/mm^3	11.0 ± 0.3	11.4 ± 0.3	11.0 ± 0.3	12.4 ± 0.4	11.9 ± 0.6	10.7 ± 0.2[b]
Leukocytes, 10^3/mm^3	9.09 ± 0.90	9.38 ± 0.87	10.23 ± 0.60	11.43 ± 0.61	12.7 ± 0.90	11.02 ± 0.49
Platelets, 10^5/mm^3	3.98 ± 0.21	3.47 ± 0.22	4.20 ± 0.12	3.85 ± 0.19	3.94 ± 0.25	3.70 ± 0.18
Fibrinogen, mg-%	310 ± 10	321 ± 7	339 ± 9[b]	223 ± 5	265 ± 9[b]	244 ± 5
Prothrombin time, sec	9.7 ± 0.1	9.9 ± 0.4	9.7 ± 0.1	10.4 ± 0.2	10.4 ± 0.5	10.2 ± 0.3
APTT, sec	32.7 ± 1.8	34.2 ± 3.1	37.1 ± 1.8	33.7 ± 1.5	33.9 ± 2.4	38.1 ± 2.4

[a]Values represent the mean ± SE.
[b]Significantly different from control at $p < 0.05$ as determined by Dunnett's T-test.

Table 9. Differential cell counts of CD-1 mice exposed to chloral hydrate in the drinking water for 3 months.[a]

Sex	Exposure group	Number of mice n	Lymphocytes	Polymorpho-nuclears	Monocytes	Eosinophils
M	Deionized water	21	78.9 ± 2.6	18.8 ± 2.6	1.9 ± 0.5	0.6 ± 0.2
M	0.07 mg/ml	14	85.5 ± 1.7	10.6 ± 1.8[b]	2.4 ± 0.4	0.9 ± 0.3
M	0.7 mg/ml	16	83.0 ± 1.8	13.7 ± 1.7	1.5 ± 0.4	1.8 ± 0.3[b]
F	Deionized water	22	82.3 ± 1.9	14.0 ± 1.8	1.9 ± 0.3	1.9 ± 0.3
F	0.07 mg/ml	13	87.4 ± 1.2	9.5 ± 0.9	1.5 ± 0.3	1.5 ± 0.5
F	0.7 mg/ml	16	87.6 ± 1.6	9.4 ± 1.2	1.7 ± 0.4	1.4 ± 0.4

[a]Values represent the mean ± SE of the percent total of white cells.
[b]Significantly different from control at $p < 0.05$ as determined by Dunnett's T-test.

dent in the APTT times in both sexes; however, these increases were not significantly different from control. No consistent changes were observed in the differential counts.

Hepatic Microsomal Parameters

The status of the hepatic microsomal mixed functional oxidase parameters in mice exposed to chloral hydrate is summarized in Table 10. Microsomal protein was increased 10% above control in females exposed to 0.7 mg/ml, while no change was evident in males. Cytochrome b_5 levels increased 26% in males exposed to 0.07 mg/ml and 40% in males exposed to 0.7 mg/ml. In contrast, female mice exposed to 0.7 mg/ml had cytochrome b_5 levels 12% lower than vehicle control. Aminopyrine N-demethylase activity was increased in males exposed to 0.07 mg/ml (28%) and to 0.7 mg/ml (19%). No change in aminopyrine N-demethylase activity was evident in females. Aniline hydroxylase activity increased in both sexes. In males exposed to 0.07 and 0.7 mg/ml, the increase in aniline hydroxylase activity was 24 and 30%, respectively; while a 23% increase was observed in females exposed to 0.7 mg/ml.

Serum and Liver Chemistries

Serum and liver chemistry values are presented in Table 11. In the sera of male mice receiving 0.7 mg/ml, calcium decreased 8%, sodium decreased 5%, phosphorous decreased 19%, LDH activity increased 48%, and SGOT levels increased 45% above controls. Blood urea nitrogen levels decreased 19% in males exposed to 0.07 and 32% in males exposed to 0.7 mg/ml. Female mice demonstrated a different pattern of changes in chemistry. In the sera of female mice exposed to 0.7 mg/ml, potassium increased 9%, glucose increased 16%, cholesterol increased 21%, and phosphorus increased 11% above vehicle control. No changes in serum enzyme

Table 10. Hepatic microsomal activities in CD-1 mice exposed to chloral hydrate in the drinking water for 3 months.[a]

	Males			Females		
Exposure group	Deionized water (n = 4)	0.07 mg/ml (n = 8)	0.7 mg/ml (n = 8)	Deionized water (n = 8)	0.07 mg/ml (n = 4)	0.7 mg/ml (n = 8)
Microsomal protein						
mg/g liver	23.0 ± 0.7	21.8 ± 0.8	21.0 ± 0.4	19.7 ± 0.4	21.0 ± 0.5	21.6 ± 0.2[b]
mg/mouse	44.1 ± 2.6	41.1 ± 1.5	44.5 ± 2.7	31.9 ± 0.9	37.7 ± 3.8	39.7 ± 1.7
Cytochrome P-450						
nmole/mg/protein	1.21 ± 0.13	1.32 ± 0.05	1.37 ± 0.05	1.23 ± 0.05	1.26 ± 0.06	1.14 ± 0.03
nmole/mouse	60.0 ± 2.1	54.1 ± 2.5	61.1 ± 5.0	39.3 ± 2.1	47.8 ± 6.4	44.9 ± 2.2
Cytochrome b_5						
nmole/mg protein	0.402 ± 0.025	0.508 ± 0.022[b]	0.564 ± 0.017[b]	0.640 ± 0.021	0.662 ± 0.032	0.564 ± 0.024[b]
nmole/mouse	20.1 ± 6.9	20.2 ± 0.9	25.2 ± 1.9	20.4 ± 1.0	24.8 ± 2.0	22.0 ± 0.6
Aminopyrine N-demethylase						
nmole/mg/min	9.47 ± 0.50	12.09 ± 0.27[b]	11.29 ± 0.32[b]	13.06 ± 0.46	13.09 ± 0.52	12.66 ± 0.31
nmole/mouse/min	418 ± 33	499 ± 34	500 ± 28	405 ± 19	490 ± 33	502 ± 30
Aniline hydroxylase						
nmole/mg/min	1.35 ± 0.05	1.68 ± 0.05[b]	1.75 ± 0.04[b]	1.70 ± 0.04	1.83 ± 0.17	2.09 ± 0.06[b]
nmole/mouse/min	59.6 ± 5.2	67.9 ± 3.1	77.1 ± 3.4	54.2 ± 1.3	68.3 ± 6.9	82.1 ± 1.6[b]

[a]Values represent the mean ± SE.
[b]Significantly different from control at $p < 0.05$ as determined by Dunnett's T-test.

Table 11. Clinal chemistry values of in CD-1 mice exposed to chloral hydrate in the drinking water for 3 months.[a]

	Males			Females		
Exposure group	Deionized water (n = 22)	0.07 mg/ml (n = 14)	0.7 mg/ml (n = 16)	Deionized water (n = 23)	0.07 mg/ml (n = 14)	0.7 mg/ml (n = 16)
Calcium, mg-%	11.74 ± 0.27	11.50 ± 0.39	10.76 ± 0.29[b]	11.19 ± 0.20	11.08 ± 0.24	11.62 ± 0.19
Sodium, mq/l.	151 ± 1	148 ± 1	143 ± 2[b]	144 ± 2	145 ± 2	151 ± 2
Chloride, mq/l.	107 ± 2	109 ± 1	111 ± 1	108 ± 1	114 ± 2[b]	108 ± 2
Potassium, mq/l.	6.24 ± 0.17	5.83 ± 0.22	5.97 ± 0.27	5.90 ± 0.10	6.07 ± 0.20	6.45 ± 0.24[b]
Protein, g-%	7.93 ± 0.19	9.08 ± 0.39	9.04 ± 0.71	8.14 ± 0.30	8.16 ± 0.40	8.26 ± 0.38
Glucose, mg-%	173 ± 5	166 ± 6	156 ± 10	120 ± 4	122 ± 6	139 ± 5[b]
Cholesterol, mg-%	87.8 ± 2.9	90.3 ± 3.4	88.2 ± 5.3	55.4 ± 1.8	61.0 ± 2.0	66.8 ± 2.3[b]
Phosphorus, mg-%	7.27 ± 0.14	6.99 ± 0.20	5.92 ± 0.25[b]	6.14 ± 0.11	6.10 ± 0.21	6.79 ± 0.18[b]
BUN, mg-%[c]	30.9 ± 1.0	24.9 ± 1.0[b]	21.0 ± 1.1[b]	19.9 ± 0.5	19.4 ± 0.6	21.2 ± 1.1
LDH, IU/l.[d]	741 ± 38	716 ± 40	1096 ± 171[b]	685 ± 33	704 ± 52	724 ± 36
SGPT, IU/l.[e]	38.0 ± 1.6	36.0 ± 2.6	40.6 ± 2.7	28.9 ± 1.4	28.1 ± 1.5	32.6 ± 3.6
SGOT, IU/l.[f]	94.1 ± 5.3	89.2 ± 6.6	136 ± 14[b]	89.4 ± 4.7	81.6 ± 6.2	93.8 ± 9.4
ALP, IU/l.[g]	48.2 ± 4.3	45.6 ± 2.0	42.6 ± 4.3	57.4 ± 2.4	53.3 ± 2.4	50.7 ± 3.4
GLU, μmole/g liver[h]	6.84 ± 0.52	7.21 ± 0.23	6.83 ± 0.27	6.01 ± 0.19	6.13 ± 0.21	5.34 ± 0.15[b]

[a]Values represent the mean ± SE.
[b]Significantly different from control at $p < 0.05$ as determined by Dunnett's T-test.
[c]BUN = blood urea nitrogen.
[d]LDH = lactic dehydrogenase.
[e]SGPT = Serum glutamic pyruvic transaminase.
[f]SGOT = Serum glutamic oxaloacetic transaminase.
[g]ALP = Alkaline phosphatase.
[h]Glutathione.

activities were evident in female mice exposed to chloral hydrate. Nonprotein sulfhydryl levels decreased 11% in females receiving 0.7 mg/ml, while no change was evident in male mice.

Discussion

The toxicity studies on chloral hydrate administered via the drinking water were undertaken because of its presence in human drinking water supplies (*1*). Acute, 14- and 90-day studies were performed on CD-1 random-bred mice. The acute lethal effects of chloral hydrate appear related to depression of the central nervous system. After oral administration of chloral hydrate, mice died within 24 hr without gaining consciousness; if they returned to consciousness, they survived. There were no sex differences in the LD_{50}'s of chloral hydrate. Boitsov (*12*) reported the LD_{50} in rats to be 285 mg/kg and the LD_{50} in mice to be 1100 mg/kg, which is very similar to the LD_{50} for mice obtained in our laboratories (see Table 1).

The 14-day study was performed in male mice with the chemical administered by stomach tube at doses 1/100 and 1/10 the LD_{50}. The exposure-related effects were an 18% increase in liver weight and a 27% decrease in spleen weight at the highest dose (144 mg/kg). These effects were not accompanied by any gross pathological changes.

In the 90-day drinking water studies, the highest time-weighted average dose the mice received per day was 173 mg/kg, based on fluid consumption and body weight. This is equivalent to a dose of about 14 mg/kg in humans when extrapolation is based on body surface area. For the average 60 kg person, this is less than 1 g/day. Thus, the doses administered in this study were high from an environmental standpoint, but were within the range of human therapeutic exposure. The currently recommended human therapeutic dose of chloral hydrate is 500-1000 mg, although doses of 2 g are not uncommon (*2*).

Chloral hydrate caused no deaths in the 90-day drinking water study, nor did it cause a decrease in weight gain. In the males a slight dose-dependent increase in body weight was observed.

In the male, the target organ appeared to be the liver. This is suggested by dose-related hepatomegaly, elevation in SGOT and LDH (but not in SGPT) activity, increased hepatic microsomal aminopyrine *N*-demthylase and aniline hydroxylase activity and increased ctyochrome b_5 content. However, there was no increase in cytochrome P-450 content.

Female mice did not exhibit the hepatomegaly observed in the males. However, at the highest dose, aniline hydroxylase activity was increased, while liver nonprotein sulfhydryl and cytochrome b_5 levels were decreased.

Neither sex showed exposure-related changes in hematological, coagulation, or urinalysis parame-

ters. From the data presented the target organ for chloral hydrate appears to be the liver, particularly in the male mouse. The lowest adverse effect level was 0.07 mg chloral hydrate/ml of drinking water. The next report (*5*) will present evidence that the immune system may be more sensitive than the liver to the effects of chloral hydrate.

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REFERENCES

1. U.S. EPA. Preliminary assessment of suspected carcinogens in drinking water. Report to Congress, Washington, D.C., 1975.
2. Gilman, A. G., Goodman, L. S., and Gilman, A. The Pharmacological Basis of Therapeutics. Macmillan, New York, 1980.
3. Waters, E. M., Gerstner, H. B., and Huff, J. E. Trichloroethylene. I. An Overview. J. Toxicol. Environ. Health, 2: 671-707 (1977).
4. Goodnight, J. H. Probit procedure. In: SAS User's Guide, 1979 ed., J. T. Helwig and K. A. Council, eds., SAS Institute Inc., Raleigh, N.C.
5. Kauffmann, B. M., White, K. L., Jr., Sanders, V. M., Douglas, K. A., Sain, L. E., Borzelleca, J. F., and Munson, A. E. Humoral and cell-mediated immune status in mice exposed to chloral hydrate. Environ. Health Perspect. 44: 147-151 (1982).
6. Barnes, D. W., Morahan, P. S., and Munson, A. E. The effects of maleic anyhydride vinyl ether polymers on hepatic microsomal mixed function oxidase parameters and other biological activities. J. Pharmacol. Exptl. Therap. 208: 392-398 (1979).
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randell, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275 (1951).
8. Omura, T., and Sato, R. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J. Biol. Chem. 239: 2370-2378 (1964).
9. Cochin, J., and Axelrod, J. Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalophene, and normorphine. J. Pharmacol. Exptl. Therap. 125: 105-110 (1959).
10. Imai, Y., Ito, A., and Sato, R. Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. J. Biochem. 60: 417-428 (1966).
11. Dunnett, C. W. New tables for multiple comparisons with a control. Biometrics. 20: 482-491 (1964).
12. Boitsov, A. N., Rotenberg, Y. S., and Mulenbova, V. G. Toxicological evaluation of chloral in the process of its liberation during spraying and pouring of polyurethane foams. Gig. Trud. Prof. Zabol. 14: 26-29 (1970).