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Species and Strain Sensitivity to the Induction of Peroxisome Proliferation by Chloroacetic Acids[1]

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Trichloroacetic acid (TCA) and dichloroacetic acid (DCA) are present in finished drinking water as by-products of the chlorine disinfection process at concentrations ranging from 34 to 160 μg/liter (Uden and Miller, 1983). They result from the reaction between chlorine and humic substances in the surface waters (Miller and Uden, 1983; Coleman *et al.*, 1984). TCA and DCA were also found to be the major chlorinated organic compounds formed in the stomach of experimental animals following the ingestion of water containing sodium hypochlorite (Mink *et al.*, 1983). The widespread exposure of large segments of the human population to low levels of TCA and DCA prompted an examination of the toxicological effects of these chemicals.

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Species and Strain Sensitivity to the Induction of Peroxisome Proliferation by Chloroacetic Acids. DEANGELO, A. B., DANIEL, F. B., MCMILLAN, L., WERNSING, P., AND SAVAGE, R. E., JR. (1989). *Toxicol. Appl. Pharmacol.* **101**, 285–298. B6C3F$_1$ mice and Sprague–Dawley rats were provided drinking water containing 6–31 mM (1–5 g/liter) trichloroacetic acid (TCA), 8–39 mM (1–5 g/liter) dichloroacetic acid (DCA), or 11–32 mM (1–3 g/liter) monochloroacetic acid (MCA) for 14 days. TCA and DCA, but not MCA, increased the mouse relative liver weight in a dose-dependent manner. Rat liver weights were not altered by TCA or DCA treatment, but were depressed by MCA. Hepatic peroxisome proliferation was demonstrated by (1) increased palmitoyl-CoA oxidase and carnitine acetyl transferase activities, (2) appearance of a peroxisome proliferation-associated protein, and (3) morphometric analysis of electron micrographs. Mouse peroxisome proliferation was enhanced in a dose-dependent manner by both TCA and DCA, but only the high DCA concentration (39 mM) increased rat liver peroxisome proliferation. MCA was ineffective in both species. Three other mouse strains (Swiss–Webster, C3H, and C57BL/6) and two strains of rat (F344 and Osborne–Mendel) were examined for sensitivity to TCA. TCA (12 and 31 mM) effectively enhanced peroxisome proliferation in all mouse strains, especially the C57BL/6. A more modest enhancement in the Osborne–Mendel (288%) and F344 rat (167%) was seen. Dosing F344 rats with 200 mg/kg TCA in water or corn oil for 10 days increased peroxisome proliferation 179 and 278%, respectively, above the vehicle controls. These studies demonstrate that the mouse is more sensitive than the rat with respect to the enhancement of liver peroxisome proliferation by TCA and DCA and suggest that if peroxisome proliferation is critical for the induction of hepatic cancer by TCA and DCA, then the rat should be less sensitive or refractory to tumor induction.

Trichloroacetic acid (TCA) and dichloroacetic acid (DCA) are present in finished drinking water as by-products of the chlorine disinfection process at concentrations ranging from 34 to 160 μg/liter (Uden and Miller, 1983). They result from the reaction between chlorine and humic substances in the surface waters (Miller and Uden, 1983; Coleman *et al.*, 1984). TCA and DCA were also found to be the major chlorinated organic compounds formed in the stomach of experimental animals following the ingestion of water containing sodium hypochlorite (Mink *et al.*, 1983). The widespread exposure of large segments of the human population to low levels of TCA and DCA prompted an examination of the toxicological effects of these chemicals.

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Recent studies from our laboratory (Herren-Freund *et al.*, 1987) have demonstrated that the administration of both TCA and DCA in the drinking water increased the incidence of liver cancer in male B6C3F$_1$ mice. Prior initiation with a genotoxic carcinogen was not required for the TCA and DCA to produce tumors. On the other hand, the chloroacetic acids do not appear to act as tumor initiators by directly damaging DNA since neither TCA nor DCA possessed mutagenic activity when tested with various *Salmonella* strains (Rapson *et al.*, 1980; Herbert *et al.*, 1980). Likewise the two compounds only weakly increased sister chromatid exchanges in cultured Chinese hamster ovary cells at high concentrations and in the absence of metabolic activation (Meier and Blazak, 1985). Neither TCA nor DCA produced mouse bone marrow sister chromatid exchanges or micronucleus formation *in vivo* (J. Meier, personal communication), and TCA failed to initiate enzyme-altered foci in rat liver (Parnell *et al.*, 1986). One notable exception is the study of Nelson and Bull (1988) who, using an alkaline unwinding assay, found that TCA and DCA increased the number of DNA single-strand breaks in the liver of B6C3F$_1$ mouse and the Sprague–Dawley rat.

Our interest in the mechanism by which TCA and DCA enhance liver cancer has focused on several parameters including the ability of the chloroacetic acids to stimulate hepatic peroxisome proliferation in rats and mice. Since many peroxisome proliferators have been found to increase the incidence of liver cancer in rodents, these agents have been proposed to constitute a unique class of chemical carcinogens (Reddy *et al.*, 1980a). The mechanism(s) by which peroxisome proliferator chemicals induce liver neoplasia is not well defined. One theory is that the heightened production and subsequent metabolism of hydrogen peroxide and other oxygen metabolites resulting from the activities of peroxisomal enzymes initiate DNA strand breakage, crosslinkage, and amelorative repair processes which may yield mutations and chromosomal aberrations (Cadet and Teoule, 1978).

The objective of this study was to measure the ability of TCA and DCA to stimulate hepatic peroxisome proliferation in several strains of rats and mice. In addition, the effect of the vehicle (corn oil vs water) on the TCA induction of hepatic peroxisome proliferation was measured in order to compare the results from these studies with those already in the literature.

MATERIALS AND METHODS

Chemicals

Trichloroacetic acid (CAS No. 76-03-9), dichloroacetic acid (CAS No. 79-43-6), and monochloroacetic acid (CAS No. 79-11-8) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). The chloroacetic acids (purity ≥ 99%) were dissolved in distilled water at concentrations between 1 and 5 g/liter (6–39 mM), and the pH was adjusted to 6.8–7.2 by the addition of an appropriate volume of 10 N sodium hydroxide. All solutions were prepared at the beginning of each study and stored at 5°C. They were administered to the animals in glass water bottles fitted with teflon stoppers and double-balled sipper tubes. Drinking waters were changed weekly. All reagents used in the preparation of liver extracts, analysis of peroxisomal enzymes, and gel electrophoresis, unless otherwise specified, were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals

The various strains of young adult male rats (Sprague–Dawley, Osborne–Mendel, and Fisher 344) weighing 225–275 g and male mice (B6C3F$_1$, C57BL/6, C3H, and Swiss–Webster) weighing 20–25 g were purchased from Charles River Laboratories (Portage, MI). The animals were maintained on Purina Laboratory Chow and water *ad libitum* at 22 ± 2°C and 40–60% humidity under a 12-hr light–dark cycle. Following 2 weeks in quarantine, the animals were divided into treatment groups and the exposure to the various concentrations of chloroacetic acids (6–39 mM) in the drinking water was started. Control animals were given drinking water containing 34 mM sodium chloride. After 14 days, the animals were killed by CO_2 asphyxiation and the body and liver weights were recorded. The livers were quickly removed and minced and pieces were preserved in 2.5% glutaraldehyde for ex-

amination by electron microscopy. The remainder of each liver was quick-frozen in liquid nitrogen and stored at −70°C until it could be processed for analysis of peroxisomal enzymes. In order to evaluate the effect of the vehicle upon the induction of peroxisome proliferation by TCA, a group of F344 rats was given by gavage 200 mg/kg TCA dissolved in either corn oil or distilled water on each of 10 days. The animals were killed 24 hr following the last dose.

Peroxisomal Enzyme Analysis

Portions of the frozen livers were homogenized (1:10 w/v) in a buffer containing 0.25 M sucrose, 0.05 M sodium EDTA, and 0.02 M Tris–HCl, pH 7.4. The homogenates were centrifuged at 800*g* for 5 min, the fatty layers were removed by aspiration, and the extracts were stored at −70°C until assay. Previous work had shown that the enzyme activities in the frozen extracts did not differ significantly from the activities in liver extracts not frozen prior to assay. Cyanide-insensitive palmitoyl-Coenzyme A (PCO) activity was measured according to the method of Osumi and Hashimoto (1978). Carnitine acetyl-CoA transferase (CAT) activity was assayed according to the method of Gray *et al.* (1982).

Gel Electrophoresis

The induction of the 80,000 kDa peroxisome proliferation-associated (PP-A) protein (Reddy and Kumar, 1977) in the liver extracts from rats and mice exposed to the highest drinking water concentrations of the chloroacetic acids (31 mM TCA, 39 mM DCA, and 32 mM MCA) was demonstrated by polyacrylamide gel electrophoresis (PAGE) using the procedure of Laemmli (1970) as described by Irwin and Dauphinais (1979). A 10% acrylamide separating gel was overlayed with a 3% stacking gel. The liver extracts (30 μg protein) were made up to 100 μl with sample buffer (final concentrations: 0.01 M Tris–HCl, pH 6.8, 1% 2-mercaptoethanol, 2% sodium dodecylsulfate, 6% sucrose, 0.001% bromphenyl blue) and heated in a boiling water bath for 5 min. The samples were loaded into the wells, and electrophoresis was carried out at constant amperage and temperature (30 mA/gel; 20°C) in a vertical slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA) until the bromphenol blue dye front had moved approximately 11 cm into the separating gel. The gels were removed and stained overnight in a solution containing 0.05% Coomassie brilliant blue R (Serva Fine Biochemicals, Inc., Heidelberg, Federal Republic of Germany), 25% 2-propanol, and 10% acetic acid. The gels were thoroughly destained with a solution containing 25% 2-propanol and 10% acetic acid.

Electron Microscopy

For the morphometric measurement of peroxisome proliferation, one liver section from each of two B6C3F$_1$ mice or Sprague–Dawley rats from the high-dose treatment groups was randomly selected. The number of peroxisomes, peroxisomal areas, and the cytoplasmic area in six electron micrograph fields from each section (12 fields/treatment group) were measured using a Bioquant System IV image analysis system. The number of peroxisomes per 100 μm^3 of liver and volume percentage of cytoplasm occupied by the peroxisomes were calculated according to the methods described by Loud (1968).

Statistical Analysis

Statistical analysis of the data was carried out using the Tukey means multiple comparison or the one-way analysis of variance.

RESULTS

Although water consumption measurements were not made during this study, earlier analysis in our laboratory had shown that rats and mice will limit their water consumption with increasing concentrations of the chloroacids. The calculated average daily intakes in mg/kg body wt/day for the B6C3F$_1$ mouse over a 14-day period were: 265, 386, and 482 for a drinking water containing 11, 21, and 32 mM MCA; 90, 166, and 346 for 8, 16, and 39 mM DCA; and 131, 261, and 442 for 6, 12, and 31 g/liter TCA. For the Sprague–Dawley rat the 14-day average daily intakes were: 170, 321, and 501 for 11, 21, and 32 mM MCA; 166, 294, and 666 for 8, 16, and 39 mM DCA; and 212, 327, and 719 for 6, 12, and 31 mM TCA.

Body and Liver Weight Changes

Table 1 shows the effect of a 14-day drinking water administration to the three chloroacetic acids on the body weights and liver weights (expressed as percentage of the body weight) of male B6C3F$_1$ mice and Sprague–Dawley rats. Rats drinking water containing the three acids experienced a dose-dependent

TABLE 1

BODY WEIGHT AND LIVER WEIGHT CHANGES FOR SD RATS AND B6C3F1 MICE GIVEN TCA, DCA, OR MCA IN THEIR DRINKING WATER FOR 14 DAYS

Chloroacetic acid		Drinking water concentration	Body weight[a] (g)	Liver weight (g/100 g body wt)
		Sprague–Dawley rat		
TCA	(6)[b]	31 mM	285 ± 14*,[c]	4.0 ± 0.4
	(6)	21 mM	319 ± 15	4.4 ± 0.4
	(6)	6 mM	304 ± 8*	3.8 ± 0.4
DCA	(3)	39 mM	262 ± 16**	4.7 ± 0.9
	(5)	16 mM	270 ± 22**	3.8 ± 0.2
	(5)	8 mM	282 ± 20*	4.7 ± 0.4
MCA	(5)	32 mM	194 ± 10**	3.1 ± 0.1*
	(6)	21 mM	220 ± 10**	3.3 ± 0.1*
	(6)	11 mM	283 ± 7**	3.5 ± 0.2*
NaCl	(6)	34 mM	341 ± 6	4.4 ± 0.4
		B6C3F1 mouse		
TCA	(6)	31 mM	21 ± 1	7.1 ± 0.4**
	(6)	12 mM	21 ± 0	5.9 ± 0.3
	(6)	6 mM	22 ± 0	5.5 ± 0.2
DCA	(6)	39 mM	21 ± 1	7.9 ± 0.3**
	(6)	16 mM	21 ± 1	6.5 ± 0.2**
	(6)	8 mM	20 ± 1	6.2 ± 0.1**
MCA	(6)	32 mM	20 ± 1	4.9 ± 0.1
	(6)	21 mM	20 ± 1	5.2 ± 0.1
	(5)	11 mM	21 ± 1	5.2 ± 0.2
NaCl	(6)	34 mM	21 ± 3	5.1 ± 0.2

[a] Average ± SEM.

[b] (*N*) = the number of animals surviving and used for analysis.

[c] $p < 0.05$ (*) or $p < 0.01$ (**) as determined by the Tukey means multiple comparison test when compared to the NaCl control.

depression in body weight gain. The severity of the weight gain depression was greatest for MCA (57% of the control value at 32 mM) and least for TCA (84% of control at 31 mM). The depression of the growth rate by the high concentration DCA (39 mM) was intermediate (75% of control). TCA and DCA did not affect the relative liver weights. However, MCA treatment depressed liver to body weight ratios and these declines were dose dependent. In marked contrast, the body weight gains of the B6C3F$_1$ mice were not altered by drinking water containing any of the three chloroacetic acids. However, DCA and to a lesser extent TCA treatment did increase relative liver weights in the mice. MCA in the drinking water had no effect on the relative liver weights of the mice.

Alteration of Peroxisomal Enzyme Activities

The effects of the chloroacetic acids on CAT activities in the Sprague–Dawley rat and B6C3F$_1$ mouse are shown in Figs. 1A (rat) and 1B (mouse). MCA did not alter CAT activity in either rats or mice at any of its drinking water concentrations (11–32 mM).

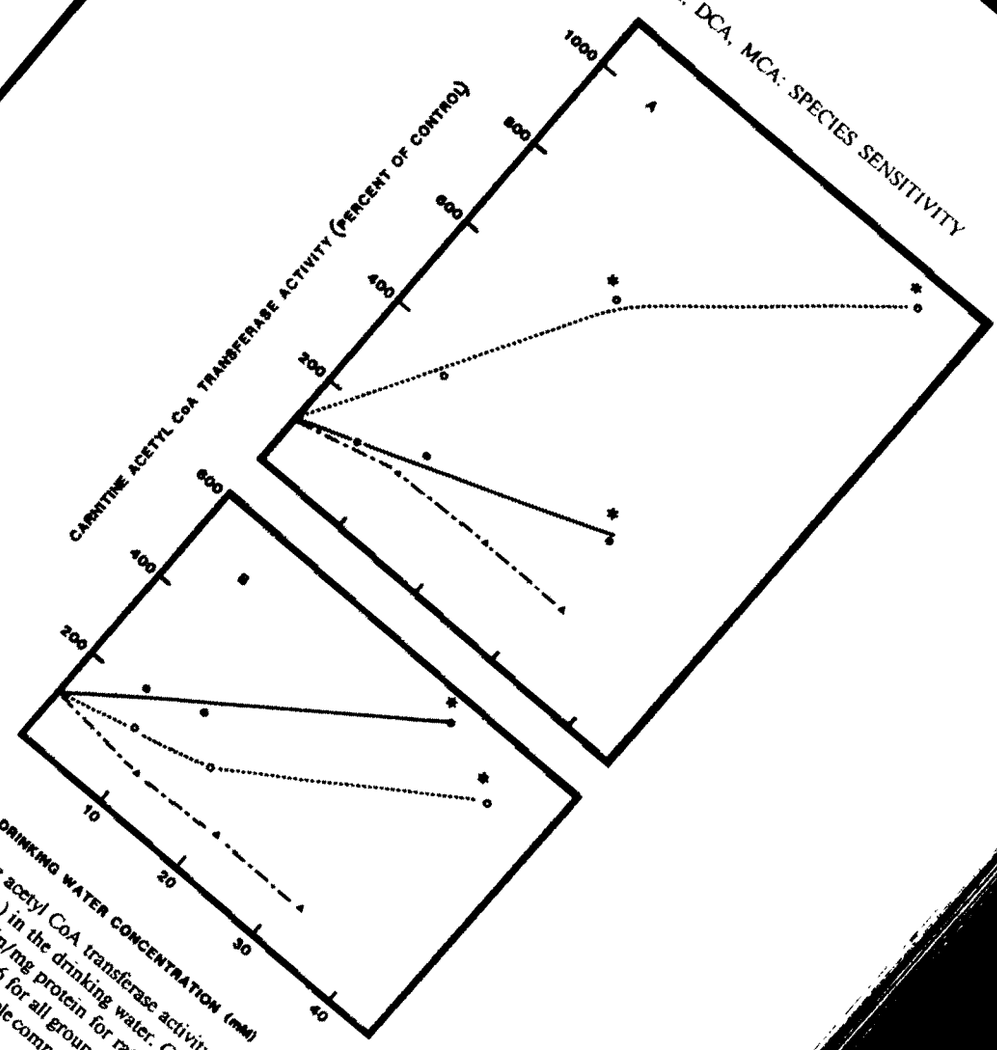

FIG. 1. The enhancement of carnitine acetyl CoA transferase activity in the livers of (A) rats and (B) mice by TCA (●), DCA (○), and MCA (▲) in the drinking water. Control enzyme activities ± SE were 1.81 ± 0.23 nmoles carnitine transferred/min/mg protein for rats and 3.58 ± 0.39 nmoles of carnitine transferred/min/mg protein for mice. $N = 5$ or 6 for all groups except rats on 39 mM DCA where $N = 3$. *$p < 0.05$ as determined by the Tukey means multiple comparison test when compared to the NaCl control.

DCA at 39 mM increased the liver CAT activity in both rats (1033% of control) and mice (450% of control). The results obtained for TCA were the reverse of those obtained for DCA. TCA increased CAT activity in the mice (561% of control) at 31 mM to a greater extent than in the rats (321% of control).

The results obtained for the alteration of hepatic cyanide-insensitive PCO, a specific peroxisomal enzyme marker, by the chloroacetic acids differed markedly from those seen for CAT. The Sprague–Dawley rat appeared relatively insensitive to the induction of peroxisome PCO activity by the chloroace-

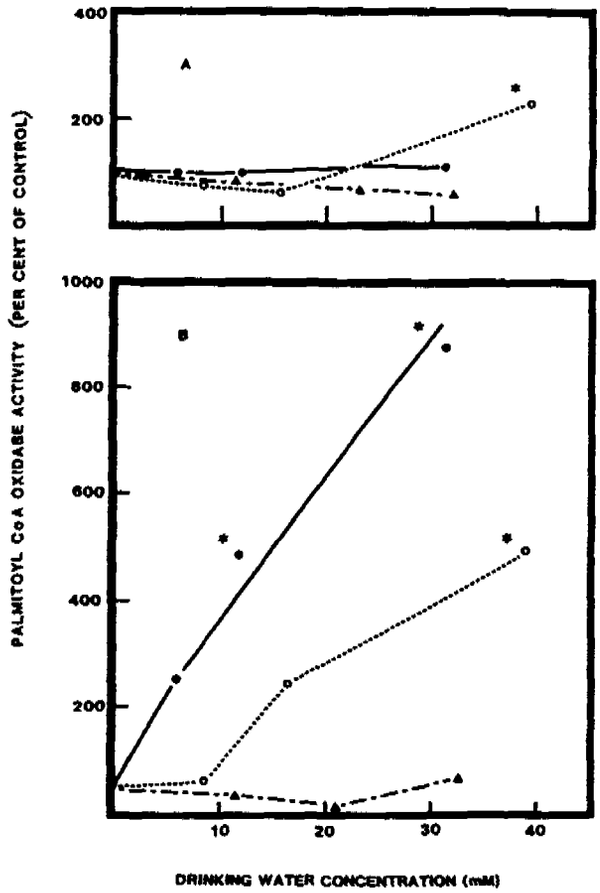

FIG. 2. The alteration of cyanide-insensitive palmitoyl-CoA oxidase activity in the livers of (A) rats and (B) mice by TCA (●), DCA (○), and MCA (▲), in the drinking water. The enzyme activity for control groups ± SE were 3.91 ± 0.23 nmoles NAD reduced/min/mg protein for rats and 1.40 ± 0.34 nmoles of NAD reduced/min/mg protein for mice. $N = 5$ or 6 for all groups except rat on 39 mM DCA where $N = 3$. *$p < 0.05$ as determined by the Tukey means multiple comparison test when compared to the NaCl control.

tic acids. Only the highest concentration of DCA (39 mM) in the drinking water increased the activity of this marker enzyme (234% of the control value, Fig. 2A). The mouse, on the other hand, responded with an increased PCO activity to both TCA and DCA (Fig. 2B). TCA was the more potent of the two acids. At 31 mM it increased the PCO activity to 959% of the control value in a dose-dependent manner; this compared to a 430% increase seen for the highest concentration of DCA (39 mM). No statistically significant increases in PCO activity were measured at the lower DCA concentrations. MCA did not alter PCO specific activity in either species.

Gel Electrophoresis

SDS–polyacrylamide gel electrophoresis was also used to evaluate liver peroxisome proliferation following chloroacetic acid treatment by demonstrating the presence of a

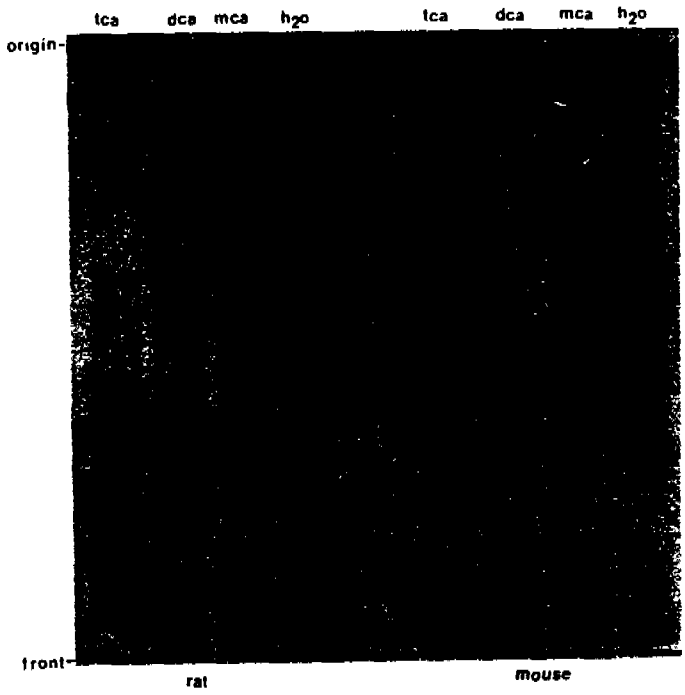

FIG. 3. Polyacrylamide gel electrophoretic separation of proteins in liver extracts derived from rats and mice treated for 14 days with 31 mM TCA, 39 mM DCA, 32 mM MCA, or 34 mM NaCl (H_2O). Trypsinogen (24 kDa), ovalbumin (45 kDa), and bovine albumin (66 kDa), were corun as molecular weight markers.

PP-A protein (Fig. 3). No PP-A protein band was seen for control or MCA (32 mM)-treated mice and rats, or for TCA (31 mM)-treated rats. TCA induced the PP-A protein only in the mouse, while DCA (39 mM) was active in both species. The molecular weight of the PP-A protein was determined by comparing its migration to those of marker proteins. A molecular weight of 74,000 kDa was obtained compared to the value of 80,000 kDa reported in the literature (Reddy *et al.*, 1980b).

Quantitative Morphometry

The enhancement of peroxisome proliferation by the highest concentration of the chloroacetic acids was scored by determining the number of peroxisomes per unit volume of cytoplasm and calculating the volume percentage of the cytoplasm they occupied. A cellular structure was identified as a peroxisome if it met three of the four following criteria: (1) it was round to oval in shape; (2) had a single limiting membrane; (3) contained granular material; and (4) had an electron dense nucleoid(s). If any structure possessed a double limiting membrane it was excluded, even if the other three criteria were met. The variable size of the peroxisomes could be related to sectioning or to the stage of development of the peroxisome. Good agreement was obtained between the morphometric measurements (Table 2) and the two other indicators of peroxisome proliferation. In the rat, only DCA increased the peroxisome number per 100 μm^3 and the volume percentage of cytoplasm occupied. In the mouse, both TCA and DCA increased the peroxisome number per μm^3 (30.75 ± 2.80 and 30.77 ± 2.85, respectively, vs 6.89 ± 1.23 for the control) and the volume percentage of the

TABLE 2

MORPHOMETRIC ANALYSIS OF HEPATIC PEROXISOME PROLIFERATION INDUCED BY CHLOROACETIC ACIDS

Treatment	Number of peroxisomes per 100 μm^3 cytoplasm	Volume fraction of cytoplasm occupied by peroxisomes
	Sprague–Dawley rat	
31 mM TCA	7.13 ± 1.13[a]	0.89 ± 0.17*
39 mM DCA	16.75 ± 2.05*	2.80 ± 0.31*
32 mM MCA	8.32 ± 0.69	2.27 ± 0.26
34 mM NaCl	6.60 ± 0.75	1.94 ± 0.72
	B6C3F1 mouse	
31 mM TCA	30.75 ± 2.80*	4.92 ± 0.54*
39 mM DCA	30.77 ± 2.85*	3.75 ± 0.46*
32 mM MCA	16.67 ± 3.33	1.02 ± 0.17
34 mM NaCl	6.89 ± 1.23	0.61 ± 0.12

[a] Average ± SEM for 12 fields examined (6 fields/liver section).

* $p < 0.05$ as determined by the analysis of variance when compared to the NaCl control.

cytoplasm occupied by peroxisomes (4.92 ± 0.54 and 3.75 ± 0.46, respectively, vs 0.61 ± 0.12 for the control). MCA slightly increased the peroxisome number, but not to a level of significance ($p < 0.05$).

Taking all the data together, the conclusion may be drawn that the Sprague–Dawley rat is relatively insensitive to the induction of peroxisome proliferation by the chloroacetic acids since only the highest concentration of DCA (39 mM) for 14 days increased the various markers of peroxisomal proliferation. In contrast, both DCA and TCA were active in the B6C3F$_1$ mouse with TCA appearing to be more potent than DCA. MCA was ineffective in increasing liver peroxisome proliferation in both rodent species.

TCA Induction of Peroxisome Proliferation in Other Rat and Mouse Strains

The ability of TCA in the drinking water to alter peroxisome proliferation was tested in two other strains of rat, Osborne–Mendel and F344, and four mouse strains, Swiss–Webster, B6C3F$_1$, C57BL/6, and C3H. The latter two are the parental strains of the B6C3F$_1$ hybrid strain. Groups of six animals were given 34 mM NaCl, 12 mM TCA, or 31 mM TCA in the drinking water for 14 days. TCA treatment depressed the body weight of the F344 rat but the effect was not significant (94% of the control value). Only a slight increase (112%) in the relative liver weight for the F344 rat was recorded (Table 3). Dose-dependent increases in the relative liver weights were obtained for all mouse strains. The magnitude of the hepatotrophic response was the same for the Swiss–Webster, B6C3F$_1$, and C3H strains (126–138% of control). A greater hepatotrophic response was observed in the C57BL/6 where the relative liver weight increased to 164% of the control value at both the low and high TCA concentrations. Hepatic peroxisome proliferation was monitored by measuring the PCO activity (Table 4). Unlike the results obtained for the Sprague–Dawley rat, a modest increase was measured for the F344 rat (163% of the control value) at the high TCA concentration. A greater response of 238% of the control value for exposure to 31 mM TCA was seen in the Osborne–Mendel rat. Relative to the rat, TCA was a good inducer of PCO activity associated with peroxisome proliferation in all of the mouse strains examined. TCA increased PCO activity in a dose-dependent manner and was equally effective in the C3H, B6C3F$_1$, and Swiss–Webster strains showing increases of 746, 778, and 749%, respectively, at the 31 mM concentration. The C57BL/6 strain was particularly sensitive to the enhancing effects of TCA with a more than 2000% increase over the control value at both TCA concentrations.

Corn Oil Effect on Peroxisome Proliferation

The vehicle used for TCA induction of peroxisome proliferation in the liver of the F344

TABLE 3

BODY WEIGHT AND LIVER WEIGHT CHANGES IN RATS AND MICE GIVEN TCA IN THE DRINKING WATER FOR 14 DAYS

Species/strain	Drinking water concentration	Body weight[a] (g)	Liver weight (g/100 g body wt)
Rat			
Osborne–Mendel	31 mM	323 ± 7	5.5 ± 0.2
	12 mM	324 ± 8	5.6 ± 0.2
	NaCl	333 ± 8	5.6 ± 0.1
F344	31 mM	252 ± 3	5.6 ± 0.1*
	12 mM	264 ± 4	5.3 ± 0.2
	NaCl	269 ± 3	5.0 ± 0.2
Mouse			
Swiss–Webster	31 mM	28 ± 1	8.6 ± 0.2*
	12 mM	27 ± 2	8.0 ± 0.3*
	NaCl	28 ± 1	6.8 ± 0.3
B6C3F1	31 mM	25 ± 1	8.8 ± 0.2*
	12 mM	25 ± 1	8.2 ± 0.2*
	NaCl	24 ± 1	6.5 ± 0.2
C57BL/6	31 mM	24 ± 1	11.1 ± 0.3*
	12 mM	25 ± 1	10.9 ± 0.3*
	NaCl	26 ± 0	6.7 ± 0.3
C3H	31 mM	24 ± 0	9.1 ± 0.2*
	12 mM	23 ± 1	8.3 ± 0.2*
	NaCl	23 ± 0	6.6 ± 0.1

[a] Average ± SEM; $N = 6$.

* $p < 0.05$ as determined by the Tukey means multiple comparison test when compared to the NaCl control.

rat can have an effect (Table 5). The animals were dosed with 200 mg/kg TCA in either 1 ml distilled water or corn oil for 10 days. Corn oil and TCA independently increased the PCO activity to 140% and 179%, respectively, of the water control. The TCA dissolved in the corn oil increased hepatic PCO activity to 228% of the corn oil alone and 314% of the value obtained for the water control.

DISCUSSION

TCA and DCA were more effective enhancers of hepatic peroxisome proliferation in the mouse than in the rat. MCA was inactive in both species. Following a 14-day exposure to the chloroacetic acids in the drinking water, three indices of the peroxisome proliferation, PCO activity, the 80,000 kDa PP-A protein, and peroxisome number and volume, were increased in the $B6C3F_1$ mouse. Only the highest concentration of DCA (39 mM) increased these markers in the Sprague–Dawley rat. TCA was stronger than DCA as an inducer of peroxisomes in the mouse.

The patterns of DCA and TCA enhancement of hepatic CAT activity were similar to that seen for PCO except that the response in rats to DCA was stronger. CAT is found primarily in hepatic mitochondrial fractions where it constitutes approximately 60% of the total CAT activity (Markwell *et al.*, 1973). Peroxisomal CAT activity is about 3-fold lower. In view of the study by Leighton *et al.* (1982) who reported a 21-fold increase in mitochondrial C_4-fatty acid specific CAT activity compared to a 4- to 5-fold enhancement

TABLE 4

TCA INDUCTION OF PEROXISOME PROLIFERATION

Species/strain	Drinking water concentration	PCO activity[a,b]	% Control
Rat			
Osborne–Mendel	31 mM	9.65 ± 0.76*	238
	12 mM	6.26 ± 0.50	154
	NaCl	4.05 ± 0.32	100
F344	31 mM	15.40 ± 1.08*	163
	12 mM	9.21 ± 0.77	97
	NaCl	9.46 ± 1.22	100
Mouse			
Swiss–Webster	31 mM	47.98 ± 4.44*	748
	12 mM	24.10 ± 3.19*	376
	NaCl	6.41 ± 0.36	100
B6C3F1	31 mM	34.21 ± 2.87*	778
	12 mM	24.44 ± 1.02*	556
	NaCl	4.40 ± 0.37	100
C57BL/6	31 mM	69.55 ± 2.65*	2213
	12 mM	81.45 ± 2.35*	2592
	NaCl	3.14 ± 0.73	100
C3H	31 mM	19.31 ± 1.64*	744
	12 mM	11.02 ± 1.34*	425
	NaCl	2.59 ± 0.59	100

[a] Activity expressed as nanomoles NAD reduced/min/mg protein.
[b] Average ± SEM; $N = 6$.
* $p < 0.05$ as determined by the Tukey means multiple comparison test when compared to the NaCl control.

of the peroxisomal enzyme in response to ciprofibrate, further investigation into the subcellular distribution of DCA-induced CAT activity appears warranted.

TABLE 5

CORN OIL EFFECT ON HEPATIC PEROXISOME INDUCTION IN F344 RATS

Treatment	PCO activity[a]	Percent control
Corn oil	4.68 ± 0.21[b]	100
TCA in corn oil[c]	10.50 ± 0.86*	228
Water	3.34 ± 0.22	100
TCA in water[c]	5.97 ± 0.59*	179

[a] Nanomoles NAD reduced/min/mg protein.
[b] Average ± SEM; $N = 4$.
[c] Two hundred milligrams per kilogram po for 10 days.
* $p < 0.05$ as determined by the Tukey means multiple comparison test when compared to the vehicle control.

In three other mouse strains, Swiss–Webster, C3H, and C57BL/6 examined TCA was a good enhancer of PCO activity. It was particularly effective in the C57BL/6 strain. TCA was a relatively weak inducer of peroxisomes in the two other rat strains, the Osborne–Mendel and F344 rats. Only the highest concentration increased the PCO activity to 238% and 163% of the control, respectively. These results are in contrast to those reported by Elcombe (1985) who found that 200 mg/kg TCA administered in corn oil by gavage for 10 days increased hepatic PCO activity to 650% of the control value in male Alderley Park (Wistar-derived) rats. The estimated average daily intake for the rats in our study was 712 mg/kg/day. Goldsworthy and Popp (1987) found that male F344 rats given 500 mg/kg TCA in corn oil for 10 consecutive days had a liver PCO activity of 284%

above the control value. Parnell and coworkers (1986) reported that male Sprague–Dawley rats given 31 mM TCA in the water for up to 6 months showed only a slight increase in the PCO activity (113–119% above the control value). Finally, Mather *et al.* (unpublished observations) in a 90-day study of the effects of TCA and DCA in the drinking water on the male Sprague–Dawley rat found that 31 mM TCA stimulated hepatic PCO 115% above the control value while DCA increased it nearly fivefold.

The differences observed in these studies relative to the extent of rat hepatic PCO activity by TCA may be explained in part by a consideration of the vehicle used to deliver the compound. In this study, the administration of 200 mg/kg TCA via a corn oil gavage for 10 days stimulated the PCO activity to 224% of corn oil alone. By comparison we observed a 179% stimulation of PCO activity when the same dose of TCA was given in water. Corn oil alone raised PCO activity to 140% of the value of water alone ($p < 0.05$). Thus corn oil appeared to potentiate the TCA enhancement of hepatic PCO activity (175% of the water control, $p < 0.05$).

Both TCA and DCA increased the relative liver weights in the mouse. DCA was a particularly effective hepatotrophic agent. With one exception (high dose TCA, F344 strain) no increases in the relative liver weights of rats by the chloroacetic acids were measured. The extent to which the liver enlargement in DCA- and TCA-treated mice is due to hyperplasia or hypertrophy is not known. We recently found that mouse hepatocyte turnover, as assessed by autoradiography of [^{3}H]-thymidine incorporation, was not increased after exposure to DCA concentrations up to 39 mM (5 g/liter) for 4 weeks while hepatocyomegaly and increased liver weights were obtained (data not shown). This would suggest that hypertrophy is the main mechanism for the DCA-induced increase in relative liver weight in the mouse.

The role played by peroxisome proliferation in the carcinogenicity of DCA and TCA, if any, in the mouse liver is not known. It has been proposed that chemicals which enhance peroxisome proliferation increase the level of hydrogen peroxide generation by inducing the activity of several oxidases including the multienzyme system for the β-oxidation of long-chain fatty acids (Osumi and Hashimoto, 1978; Reddy and Lalwani, 1984). While peroxisome catalase activity is also increased during peroxisome proliferation, its induction is disproportionately smaller than the rise in hydrogen peroxide production (Reddy *et al.*, 1969). The higher cellular levels of hydrogen peroxide, a relatively mild and stable oxidant, could in turn give rise to the production of more reactive oxygen species (e.g., the hydroxyl radical, OH·, Chance *et al.*, 1979). These active oxygen species (AOS) are potent oxidants of many essential cellular components such as lipids, proteins, and nucleic acids (Tappel, 1975; Fridovich, 1978).

It seems clear that AOS produce DNA damage. AOS such as OH· and O_2^- have produced DNA strand breaks in bacterial (Hagensee and Moses, 1986; Imlay *et al.*, 1988) and mammalian cells (Mello-Filho *et al.*, 1984; Birnboim and Kanabus-Kaminska, 1985), mutations (Moody and Hassan, 1982; Levin *et al.*, 1982), chromosomal aberrations (Oya *et al.*, 1986), and sister chromatid exchanges (Larramendy *et al.*, 1987). Thus there is ample reason to speculate that peroxisome proliferation could lead, via the production of AOS, to the formation of cellular DNA damage and hence the initiation of the neoplastic process (Rao and Reddy, 1987).

However, attempts to relate peroxisome proliferation to hepatic DNA damage have not been convincing. For example, Kornbrust *et al.* (1984) reported that a sustained feeding of di(2-ethylhexyl) phthalate did not induce any detectable DNA repair activity in hepatocytes. Similarly, treatment with clofibrate, methyl clofenopate, or di(2-ethylhexyl) phthalate, all effective peroxisome prolifera-

tors, did not lead to increased DNA strand breaks in liver DNA when evaluated by an alkaline elution assay (Elliot and Elcombe, 1987). Using a structure activity comparison, the observation that DCA is the more potent carcinogen and TCA the more effective inducer of peroxisomes does not directly support peroxisome proliferation as the dominant mechanism for the hepatocarcinogenicity of the chloroacetic acids.

Chemicals which have peroxisome proliferator properties also possess mitogenic and hepatomegalic properties which are also characteristic of many tumor promoters (Beckett *et al.*, 1972; Moody *et al.*, 1977). Several peroxisome proliferators (nafenopen, di(2-ethylhexyl) phthalate) have been shown to increase the incidence of liver cancer in rats which were previously initiated with diethylnitrosamine (Reddy and Rao, 1978). However, the fact that some peroxisome proliferators increase the incidence of liver neoplasia in rodents previously initiated with a carcinogen does not prove that their role in carcinogenesis is confined to that mechanism, i.e., enhancement of the growth rate of cells initiated by ambient environmental carcinogens. For example, they may act as complete carcinogens in that they possess both initiating and promoting activities. It will be interesting to determine the carcinogenic potential of TCA or DCA in the rat since they do not induce high levels of hepatic peroxisome proliferation nor do they stimulate liver growth. These data encourage the prediction that this species might be refractive to the carcinogenicity of the chloroacetic acids. The same argument can be put forward for MCA in both rodent species.

The role, if any, of peroxisome proliferative processes in the induction of cancer by the chlorinated acetic acids remains unknown. Likewise, the mechanistic basis for chloroacetic acid-induced hepatocarcinogenesis in the mouse (the results of Nelson and Bull, 1988 notwithstanding) are still uncertain. The possibility that peroxisome proliferation might play a necessary, albeit insufficient, role in the etiology of this disease is still a viable hypothesis. The determination of the carcinogenic potential of these compounds in the rat may provide additional, valuable insight into this enigma.

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