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Trichloroacetic Acid Effects on Rat Liver Peroxisomes and Enzyme-Altered Foci

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The initiating and promoting effects of trichloroacetic acid (TCA) were investigated using a rat hepatic enzyme-altered foci bioassay. The experimental protocol used has been shown to induce γ-glutamyltranspeptidase (GGT)-positive foci in hepatic tissue following an initiating dose with a genotoxic carcinogen. Twenty-four hours following ⅔ partial hepatectomy, rats received either a single oral dose (1500 mg/kg) or 5000 ppm TCA in drinking water for 10, 20, or 30 days. Two weeks after the end of TCA exposure, the rats were promoted for 3 or 6 months with 500 ppm phenobarbital in drinking water. TCA failed to induce GGT-positive foci using this initiation protocol.

In addition, groups of ⅔ partially hepatectomized rats were initiated with a single oral dose of diethylnitrosamine (10 mg/kg) and then administered 50, 500, or 5000 ppm TCA drinking water. In this promotion protocol, TCA exposure resulted in a significant increase in the number of GGT-positive foci.

The ability of TCA to stimulate peroxisomal-dependent palmitoyl-coenzyme A oxidation was also investigated. Only the 5000 ppm TCA treatment within the promotion protocol resulted in a significant, although minor, stimulation of peroxisomal enzyme activity.

The findings support the hypothesis that TCA may possess weak promoting activity in the rat liver.

Introduction

The presence of trichloroacetic acid and other nonvolatile halogenated organic products of water chlorination in drinking water has only been recently recognized (*1–6*). Consequently, very few data are available concerning expected environmental levels or what, if any, adverse effects these chemical products may have on biological systems.

Trichloroacetic acid (TCA), dichloroacetic acid (DCA), and chloral hydrate are major nonvolatile chlorinated products formed during chlorination of water containing organic material (*1–5*). What few data are available concerning levels of these compounds in finished drinking water indicate that their consistent presence ranges from ten to several hundred parts per billion (*2,3*). The environmental levels of these nonvolatile chlorination products will certainly vary with local conditions and are directly related to the concentration of humic materials present in the water (*3,5*). Enteric production of TCA and DCA following oral administration of sodium hypochlorite has also been demonstrated (*7*). Although TCA and DCA are structurally similar, chlorination studies of fulvic and humic acids indicate that TCA formation does not proceed through a DCA intermediate, but that both form independently (*3*). The relative concentration of each depends on the reaction conditions (*3,6*).

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Purified TCA and DCA are nonmutagenic in the Ames assay (*8–10*), although some of the nonvolatile by-products formed during water chlorination do exhibit mutagenic activity in the Ames assay (*2*).

TCA is also used as a pre-emergence herbicide, medically as a caustic agent for chemical cautery, and as a common laboratory reagent. These direct uses of TCA are not, however, considered major sources of environmental contamination and exposure.

DCA has direct uses in agriculture as a fungicide and is similarly classified toxicologically as a corrosive. However, during the last decade, DCA has been extensively investigated for potential therapeutic use as a hypoglycemic, hypolactatemic, and hypolipodemic agent (*10–14*). It has been used to treat diabetes mellitus, lactic acidosis, and hypercholestrolemia in man, but because of its toxicity at therapeutic doses, clinical trials have been halted (*10,14*). Although DCA exerts various metabolic effects on many tissues, its hepatic effects are the most prominent (*11,12*). DCA has also been shown to be a metabolite of various hepatotoxic organochlorines such as dichloroethylene, dichloroethane (a hepatic carcinogen in rats), and tetrachloroethane (a hepatic carcinogen in mice) (*15–18*). These halogenated organics are commonly found as pollutants in surface water and groundwater supplies (*19–22*).

TCA is metabolically related to trichloroethylene (TCE), an organic solvent with wide industrial application and a contaminant of surface water and ground-

Table 3. TCA promotion C^{14}-palmitoyl-CoA oxidation.

Group	Treatment	N	Palmitoyl CoA enzyme activity, μm/min/g liver[a]			
			2 weeks	1 month	3 months	6 months
M	PH/DEN/PB	6	0.37 ± 0.01*	0.36 ± 0.02*	0.45 ± 0.04*	0.54 ± 0.02*
N	Ph/DEN/50 ppm TCA	6	0.49 ± 0.02	0.53 ± 0.02	0.59 ± 0.03	0.67 ± 0.02
O	PH/DEN/500 ppm TCA	6	0.55 ± 0.01	0.49 ± 0.01	0.58 ± 0.01	0.64 ± 0.02
P	PH/DEN/5000 ppm TCA	6	0.61 ± 0.01†	0.64 ± 0.01†	0.70 ± 0.02†	0.76 ± 0.02†
Q	5000 ppm TCA	6	0.59 ± 0.02†	0.63 ± 0.01†	0.66 ± 0.02†	0.77 ± 0.02†
R	PH	4	0.52 ± 0.01	0.53 ± 0.01	0.58 ± 0.02	0.66 ± 0.02
S	PH/DEN	4	0.56 ± 0.02	0.51 ± 0.02	0.62 ± 0.01	0.68 ± 0.02

[a] Values are expresed as means ± standard error of the least-squares mean.
*Significantly lower than groups N, O, P, Q, R, and S by least-squares means comparisons ($p \leq 0.05$).
†*Significantly greater than groups M, N, O, R, and S by least-squares means comparisons ($p \leq 0.05$). All other comparisons were not* significant.

Table 4. TCA initiation C^{14}-palmitoyl-CoA oxidation.

Group	Treatment	N	Palmitoyl CoA enzyme activity, μm/min/g liver[a]			
			First	Second	3 months	6 months
A	PH/DEN/PB	6	0.49 ± 0.03	0.50 ± 0.02	0.40 ± 0.01	0.57 ± 0.02
B	PH/1 dose/PB	6	0.59 ± 0.04	0.44 ± 0.03	0.42 ± 0.02	0.55 ± 0.02
C	PH/10 days/PB	6	0.54 ± 0.02	0.57 ± 0.08	0.42 ± 0.02	0.58 ± 0.02
D	PH/20 days/PB	6	0.55 ± 0.03	0.48 ± 0.01	0.43 ± 0.03	0.55 ± 0.02
E	PH/30 days/PB	6	0.59 ± 0.03	0.48 ± 0.01	0.37 ± 0.03	0.55 ± 0.02
F	30 days/PB	6	0.57 ± 0.02	0.48 ± 0.01	0.37 ± 0.03	0.59 ± 0.02
G	PH/PB	4	0.55 ± 0.01	0.58 ± 0.05	0.45 ± 0.01	0.57 ± 0.02

[a] Values are expressed as means ± standard error of the least-squares mean. Significant differences are not present between groups by least-squares means comparisons ($p \leq 0.05$).

Table 5. TCA initiation/body weights.

Group	Treatment	N	Organ weight as % body weight[a]					
			3 months			6 months		
			Spleen	Liver	Kidney	Spleen	Liver	Kidney
A	PH/DEN/PB	6	0.22 ± 0.15	5.20 ± 0.22	0.43 ± 0.02	0.19 ± 0.01	5.20 ± 0.16	0.43 ± 0.02
B	PH/1 dose/PB	6	0.22 ± 0.15	5.70 ± 0.22	0.44 ± 0.02	0.19 ± 0.01	5.17 ± 0.16	0.40 ± 0.02
C	PH/10 days/PB	6	0.22 ± 0.15	5.96 ± 0.22	0.52 ± 0.02	0.20 ± 0.01	5.04 ± 0.16	0.41 ± 0.02
D	PH/20 days/PB	6	0.26 ± 0.15	5.51 ± 0.22	0.50 ± 0.02	0.20 ± 0.01	4.91 ± 0.16	0.40 ± 0.02
E	PH/30 days/PB	6	0.21 ± 0.15	5.44 ± 0.22	0.49 ± 0.02	0.21 ± 0.01	5.04 ± 0.16	0.43 ± 0.02
F	30 days/PB	6	0.20 ± 0.15	5.44 ± 0.22	0.49 ± 0.02	0.19 ± 0.01	4.97 ± 0.16	0.43 ± 0.02
G	PH/PB	4	0.22 ± 0.18	5.83 ± 0.27	0.44 ± 0.03	0.22 ± 0.01	4.76 ± 0.20	0.45 ± 0.02

[a] Values are expressed as means ± standard error of the least-squares mean. Significant differences are not present between groups by least-squares means comparisons ($p \leq 0.05$).

Table 6. TCA promotion organ/body weights.

Group	Treatment	N	Organ weight as % body weight[a]					
			3 months			6 months		
			Spleen	Liver	Kidney	Spleen	Liver	Kidney
M	PH/DEN/PB	6	0.23 ± 0.01	5.47 ± 0.14*	0.53 ± 0.02	0.19 ± 0.13	5.06 ± 0.15*	0.42 ± 0.02
N	PH/DEN/50 ppm TCA	6	0.23 ± 0.01	3.92 ± 0.14	0.52 ± 0.02	0.18 ± 0.13	3.76 ± 0.15	0.40 ± 0.02
O	PH/DEN/500 ppm TCA	6	0.20 ± 0.01	4.12 ± 0.14	0.55 ± 0.02	0.17 ± 0.13	4.41 ± 0.15	0.46 ± 0.02
P	PH/DEN/5000 ppm TCA	6	0.22 ± 0.01	4.25 ± 0.14	0.54 ± 0.02	0.19 ± 0.13	4.41 ± 0.15	0.48 ± 0.02
Q	5000 ppm	6	0.20 ± 0.01	4.19 ± 0.14	0.58 ± 0.02	0.18 ± 0.13	4.44 ± 0.15	0.53 ± 0.02
R	PH	4	0.21 ± 0.02	3.73 ± 0.17	0.53 ± 0.02	0.18 ± 0.15	3.83 ± 0.19	0.46 ± 0.02
S	PH/DEN	4	0.20 ± 0.02	3.90 ± 0.17	0.55 ± 0.02	0.15 ± 0.15	3.96 ± 0.19	0.49 ± 0.02

[a] Values are expressed as means ± standard error of the least-squares mean.
*Significantly greater than groups N, O, P, Q, R, and S by least-squares means comparisons ($p \leq 0.05$). All other comparisons were not significant.

Table 7. TCA initiation GGT-positive foci.[a]

Group	Treatment	N	No. of foci/cm² 3 months	No. of foci/cm² 6 months
A	PH/DEN/PB	6	2.05 ± 0.18*	9.93 ± 0.71*
B	PH/1 dose/PB	6	0.05 ± 0.18	0.32 ± 0.71
C	PH/10 days/PB	6	0.08 ± 0.18	0.28 ± 0.71
D	PH/20 days/PB	6	0.07 ± 0.18	0.30 ± 0.71
E	PH/30 days/PB	6	0.06 ± 0.18	0.33 ± 0.71
F	/30 days/PB	6	0.10 ± 0.18	0.49 ± 0.71
G	PH/PB	4	0.07 ± 0.22	0.14 ± 0.86

[a] Values are expressed as means ± standard error of the least-squares mean.

*Significantly greater than groups B, C, D, E, F, and G by least-squares means comparisons ($p \leq 0.05$). All other comparisons were not significant.

Table 8. TCA promotion GGT-positive foci.[a]

Group	Treatment	N	No. of foci/cm² 3 months	No. of foci/cm² 6 months
M	PH/DEN/PB	6	1.65 ± 0.23*	7.61 ± 0.72*
N	PH/DEN/50 ppm TCA	6	0.71 ± 1.16†	1.83 ± 0.32‡
O	PH/DEN/500 ppm TCA	6	0.39 ± 0.16	1.63 ± 0.32‡
P	PH/DEN/5000 ppm TCA	6	0.70 ± 0.16†	2.45 ± 0.32‡
Q	5000 ppm TCA	6	0.23 ± 0.16	0.03 ± 0.32
R	PH	4	0.23 ± 0.20	0.41 ± 0.39
S	PH/DEN	4	0.05 ± 0.20	0.30 ± 0.39

[a] Values are expressed as means ± standard error of the least-squares mean.

*Significantly greater than groups N, O, P, Q, R, and S by least-squares means comparisons ($p \leq 0.05$).

†Significantly greater than groups Q and S by least-squares means comparisons ($p \leq 0.05$). Group M excluded from comparisons.

‡Significantly greater than groups Q, R, and S by least-squares means comparison ($p \leq 0.05$). Group M excluded from comparisons.

ducers (*31*). No differences in body or organ weights could be attributed to TCA administration. Additionally, no necrosis was observed in the liver in any groups treated with TCA.

A significant ($p < 0.05$) increase in liver weight was detected in the positive control (group M) of the promotion groups (Table 6). This increase is consistent with hepatomegaly because of microsomal induction commonly seen with PB treatment.

TCA Initiation

The results of the GGT-positive foci initiation bioassay are summarized in Table 7. Only the positive control (group A), which had approximately 2 and 10 foci/cm² at 3 and 6 months, respectively, showed a statistically significant effect. The initiation control (group G) had almost no induction of GGT-positive foci. These results are consistent with those of other investigators (*41–43*), who have shown that both PH and PB promotion are necessary to optimize the induction of DEN-initiated enzyme-altered foci. The four TCA treatment groups (B, C, D, and E) failed to demonstrate significant induction of GGT-positive foci. The differences in size of foci among the groups have not yet been statistically evaluated.

TCA Promotion

The results of the promotion experiment are summarized in Table 8. As with the initiation protocol results, the positive control (group M) had induced GGT-positive foci at a level significantly ($p < 0.05$) higher than that seen in the other groups at both the 3- and 6-month intervals. The lack of significant foci induction within the promotion controls (group S) or initiation/promotion controls (group R) again supports the need for both PH and PB promotion to optimize induction of DEN-initiated foci. The low-dose (50 ppm) TCA-promotion group (N) had significantly ($p < 0.05$) greater foci induction at 3 months than any of the negative controls (groups Q, R, and S), except for group R. This same level of foci induction is seen with high-dose (5000 ppm) TCA promotion (group P). The statistical differences between the low and high TCA dose groups (N and P) and control group R were $p < 0.06$ and $p < 0.07$, respectively. The level of GGT-positive foci induction seen at 3 months with 500 ppm TCA promotion (group O) was greater than all the negative controls but was not statistically significant. However, at the 6-month interval, all three dose levels of TCA promotion (groups N, O, and P) resulted in statistically significant ($p < 0.05$) greater levels of foci induction compared to any of the negative controls (groups Q, R, and S).

Discussion

It has been recently reported that TCA induces hepatic peroxisomal enzyme activities (*29,30*). This peroxisomal stimulating activity, along with increased metabolic TCA formation in the mouse compared to the rat following TCE administration has led several researchers to speculate that TCA levels may be important for explaining why TCE is carcinogenic in the mouse but not in the rat (*25,26*).

In this study, when TCA was investigated for its initiating potential in the rat hepatic foci bioassay, no evidence of significant genotoxicity was found. Short-term *in vitro* mutagenicity testing of TCA has also been negative (*8,9*). Although there appears to be little to support the notion of significant genotoxic TCA activity, the paucity of data does not allow a definitive determination at this time.

The promoting activity of TCA was also investigated using the rat hepatic system. After 3 months of TCA administration in drinking water, significant, although somewhat equivocal, promotion activity was observed for both the low dose (50 ppm) and high dose (5000 ppm) of TCA. The promoting activity associated with the medium dose of TCA (500 ppm), although resulting in more GGT-positive foci than those of the negative controls, was not significant. However, by 6 months, all three dose levels of TCA produced significant increases in the

number of GGT-positive foci. This promoting activity was not, however, of the magnitude seen with phenobarbital, a known potent hepatic tumor promotor. No dose-response relationship between the weak promoting activity and the concentrations of TCA used in this study was evident.

Although TCA is reported to cause hepatic peroxisomal stimulation in rats and mice, the results of this study indicate that it is unlikely that TCA's effects are related to the promoting ability seen here. The minimal stimulation, 10 to 20% over controls, of peroxisomal-associated, cyanide-insensitive, palmitoyl-CoA oxidation in TCA-exposed rats was seen only at the 5000 ppm level and only within the promotion protocol. This finding is in contrast to the promoting activity seen at all three concentrations of TCA. The lack of hepatomegaly associated with TCA administration is further evidence of TCA's weak ability to stimulate hepatic peroxisomes. Known hepatic peroxisomal proliferators have been shown to induce an associated hepatomegaly (*40*).

This study provides evidence that TCA is a possible weak, epigenetic carcinogen. It should be pointed out that no hepatocellular carcinomas or other hepatic tumors were found in any of the experimental animals used in this study. Further research is needed to verify possible carcinogenic effects of TCA in other bioassay systems.

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