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This project was aimed at determining the extent to which the metabolism of trichloroethylene (TCE) to trichloroacetate (TCA) was responsible for its hepatotoxic effects in rodents. Originally ethanol coadministration was to be used to selectively decrease the production of TCA. Although the basic tenants of the hypothesis driving this study have been confirmed, the interaction produces variable internal exposures to TCA. Consequently, the project has shifted focus to more quantitative identification of the effects of TCA and dichloroacetate (DCA) in quantitative terms and use the metabolism studies to determine whether sufficient amounts of these metabolites are produced to account for the hepatotoxic and hepatocarcinogenic effects of TCE. It has been established that both TCA and DCA are capable of rapidly producing hepatic tumors in B6C3F1 mouse. They are much more potent than TCE in this regard. Although closely (cont.)

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16. Acid (DCA) and Trichloroacetic Acid (TCA) Induced DNA Strand Breaks Occur before Peroxisome Proliferation.

19. related, these metabolites differ significantly in their hepatotoxic effects. DCA induces a severe hypertrophic effect on the liver that is associated with focal necrotic lesions. an effect not observed with TCA. This effect does greatly enhance tumor formation at high doses of DCA, but at lower doses TCA appears to be the more potent carcinogen. TCA had previously been shown the more potent inducer of single strand breaks in hepatic DNA in mice.

INTRODUCTION

Trichloroethylene (TCE) has been associated with ground water contamination near military bases where it has been used in degreasing operations. Since this solvent is widely used in industry as well, it is perhaps the most pervasive contaminant of ground water impacted by hazardous waste sites.

Compared to alternatives, TCE is relatively non-toxic. It is recognized as a hepatotoxin, but only at doses which approximate hypnotic and lethal doses. Compared to its predecessors (e.g. carbon tetrachloride chloroform and Stoddard solvent) its use represents a considerable improvement in worker safety. However, there is evidence that TCE possesses hepatocarcinogenic activity. Unlike other toxicological risks, carcinogenic risks are considered to be without population thresholds. Thus, any non-zero dose, no matter how small, is thought to carry a finite probability of producing cancer.

There are a number of reasons for suspecting that the activity of TCE as a hepatocarcinogen does not conform to the notion that carcinogenic effects have no thresholds. The present work is directed at identifying the metabolites of TCE that are responsible for its hepatotoxic effects and to determine whether these effects are in turn responsible for the hepatocarcinogenicity of the compound.

Some of the data coming from this project has been published or submitted for publication. Where this has been the case, reference will be made to the appropriate manuscript. These manuscripts are included as Appendix to this report. Included are:

Reprint of paper "Induction of strand breaks in DNA by trichloroethylene and metabolites in rat and mouse liver *in vivo*." authored by M.A. Nelson and R.J. Bull and published in Toxicology and Applied Pharmacology 94:45-54 1088.

Copy of manuscript "Dichloroacetic acid (DCA) and Trichloroacetic Acid (TCA) induced DNA strand breaks occur before peroxisome proliferation." Authored by M.A. Nelson, A.J. Lansing, R.J. Bull and D.L. Springer and submitted to Toxicology and Applied Pharmacology.

METHODS

Several experiments have been conducted with trichloroethylene and its metabolites, dichloroacetate (DCA) and trichloroacetate (TCA). The method of administration differs somewhat depending upon the duration of the study. In acute exposures and in our first short-term studies, these compounds were administered by gavage dissolved or suspended in a 1% Tween 80 in water vehicle. In this case all animals were administered a constant volume of drug and the control animals treated with an equal volume of the vehicle. In subsequent repeated dose experiments, including the chronic (12 month) study animals have been treated with the chemicals dissolved in drinking water. These experiments have been confined to dichloroacetic and trichloroacetic acids at concentrations of 1 or 2 g/L and neutralized to pH 7.0 - 7.5 with NaOH. Control animals received distilled water as drinking water.

Animals used in these experiments were male and female B6C3F1 mice

or male Sprague-Dawley rats. In the chronic study, these animals were begun on treatment between 30 and 40 days of age. In acute and shorter term experiments the animals ranged in age from 50 to 90 days of age at the start of their treatments.

To avoid confusion of the various experiments that have been done other methodological details are dealt with as the results of each experiment is presented.

RESULTS and DISCUSSION

Metabolism of TCE in mice and rats.

During the past year we have completed studies of the influence of alcohol on the metabolism of TCE in the rat. The data showing the time course of TCE absorption and elimination from the blood compartment and the appearance and disappearance of the two ot TCE's metabolites from this compartment is shown in Figure 1. Each group contained either 5 or 6 animals.

As expected, the concentrations of TCE in the blood of rats increases with dose. Ethanol coadministration at equimolar doses has little effect on this time course at low doses, but significantly ($P < 0.05$) decreases the peak concentrations of TCE achieved at the high doses. Consistent with this result was a tendency towards a prolonged residence of TCE within the blood. The reason for the decreased peak concentration is not clear, but the result was consistent with an effect of ethanol on TCE absorption. It should be pointed out that the high dose of TCE produces profound CNS depression. It is likely

that ethanol increases the depth of this depression (no attempt was made to quantitate this effect). It is entirely possible that the decrease in absorption could be secondary to pharmacological effects of ethanol on intestinal motility and/or blood flow to the small intestine.

Coadministration of ethanol did not affect blood trichloroethanol (TCEtOH) concentrations signficantly. However, there was a tendency for the highest dose of ethanol to increase the concentration of TCEtOH 24 hours after the administration of the chemicals. This more or less parallels the somewhat greater residence time of TCE in the body when administered with 22.8 mMol ethanol/kg. It is important to note that only free TCEtOH was measured in blood. Since TCEtOH is efficiently conjugated and excreted, this figure should not be used to judge the relative conversions of TCE to TCEtOH and TCA. The conjugation accounts in part for the relative insensitivity of TCEtOH concentrations to ethanol coadministration.

At high doses of TCE and ethanol, ethanol clearly modifies the early production of trichloroacetate (TCA). The concentrations of TCA in blood at 1, 2, 4, 8 and 12 hours after TCE administration are significantly ($P < 0.05$) depressed relative to the concentrations observed with TCE alone. By 24 hours the concentrations of TCA in blood were almost identical with or without ethanol coadministration. At lower doses, ethanol had no consistent effects on TCA production as measured by blood concentrations of this metabolite.

Consistent with the observations in blood, ethanol had no significant effects on TCEtOH recovered in the urine over a 72 hour period (Figure 2). In terms of TCEtOH production, saturation of

metabolism with increasing doses of TCE was not evident until after the dose exceeds 4.6 mMol/kg. However, at a dose of 22.8 mMol/kg the fraction of the TCE dose that is recovered as TCEtOH is reduced to about 10%, compared to more than 20% at the lower doses.

Ethanol consistently depressed the amounts of TCA that was recovered in the urine of rats administered TCE (Figure 3). This effect was observed at all doses, but was somewhat less apparent at the intermediate dose than at the low and high doses. It is notable that the fraction of the TCE dose that is recovered as TCA progressively decreases throughout the dose range that was utilized in these studies. Despite the fact that TCEtOH and TCA are derived from a common intermediate (trichloroacetaldehyde), there appears to be a differential modification of their production with high doses of TCE.

Before leaving Figure 1, it should be noted that the time to peak concentrations of TCEtOH and TCA was progressively delayed as the dose of TCE increases. Peak concentrations of TCEtOH was reached between 1 to 2 hours, 8 hours and 12 hours at 1.5, 4.6 and 22.8 mMol TCE/kg, respectively. Peak concentrations of TCA in blood were achieved somewhat later; between 4 to 8 hours, 12 hours and 24 hours at the same respective doses.

A better appreciation of the dynamics of TCE metabolism at high doses can be obtained by observing the effects of increasing dose on peak concentrations and area under the blood concentration-time curves (AUC) for TCE and TCA. TCEtOH is eliminated from this table because only free concentrations were measured in blood. This comparison shows that the peak concentration of TCE in blood increases linearly with dose. However, the AUC increases by two-fold when the dose of

TCE is increased from 1.5 to 4.6 mMol/kg and 1.64 times when increased from 4.6 to 22.8 mMol/kg. On the other hand, peak concentrations of TCA were increased to about one-half the expected value with each increase in dose, but the AUC was not affected by the increase between 1.5 and 4.6 mMol/kg. This illustrates that even when metabolism is beginning to become saturated (as indicated by the increased AUC for TCE), that the increased residence time of the chemical in the body results in virtually the same integrated measure of exposure for TCA. This does not hold as dose continues to increase, however, because at the transition between 4.6 and 22.8 mMol/kg the AUC for TCA falls short of that would be expected from the increase in dose.

The effects of ethanol on the peak concentration of TCE was clearly apparent with the increase in dose from 4.6 to 22.8 mMol/kg. It decreased the peak concentration by 50%, but the AUC was still increased almost two-fold. A somewhat attennuated increase in peak concentration when the dose was increased from 1.5 to 4.6 mMol/kg seems to have resulted primarily from an elevated, but not significant, level of TCE observed at the lowest dose rather than a geniune effect by the highest dose (see figure 1). Nevertheless, ethanol had only a modest effect on the AUC for TCA.

Table 2 indicates that the TCEtOH/TCA ratios observed in the urine were clearly modified by ethanol coadministration. Since total TCEtOH was measured in urine, this is the best measure applied to determining whether ethanol coadministration shifted metabolism of TCE away from TCA and to TCEtOH as hypothesized in our original proposal. Ethanol increased this ratio at all doses and the effect was statistically significant by ANOVA ($P < 0.05$). These data demonstrate that the

notion that the coadministration of ethanol could divert metabolism of TCE away from TCA to TCEtOH is correct. Unfortunately, other variations in the metabolism of TCE appear to prevent predictable modification of the systemic exposure of the rat to TCA. Only at extremely high doses, where many other factors are at play, was a clear decrease in the systemic exposure to TCA demonstrated apparent in the blood-concentration curves. This modification was transient and would necessitate repeated administrations of very high doses of alcohol to modify the AUC for TCA. Consequently, we have abandoned the original notion of the grant that we could use ethanol-induced modifications of TCE metabolism to demonstrate the importance of TCA to the hepatotoxic and hepatocarcinogenic effects of TCE. Instead we have taken the approach of identifying dose-response relationships involved in toxic effects produced by dichloroacetic acid (DCA) and TCA to see if their effects can account for the effects of TCE qualitatively and quantitatively.

One part of the effort to determine the importance of DCA and TCA in the production of hepatotoxic effects will be to develop an integrated estimate (i.e. AUC curves) of the systemic doses DCA and TCA that are achieved with the administration of TCE. Our data in this area are preliminary, as yet only involving the appearance of DCA and TCA in the urine following varying doses of TCE (Table 3). These data clearly show that a larger fraction of the TCE administered is converted to TCEtOH and TCA in mice than in rats (Table 3 vs. Figures 2 & 3). At low doses of TCE, mice converted about 35% of the dose to TCEtOH whereas only 20% was observed in the rat. The conversion to TCA is more or less equivalent in the two species at low doses. Thus,

the difference in the extent to which the two species metabolize TCE appears to be somewhat less than reported by other investigators. We attribute this to the fact that most often prior studies collected urine for only a 24 hour period. While this is adequate at low doses, the longer residence time of TCE and its metabolites in the blood of rats makes urine collection over a 72 hour period necessary to obtain complete recovery of the metabolites, particularly TCA.

Contrary to the reports of other investigators there is evidence of saturation of TCE metabolism in mice at similar doses as observed with rats. While the production of TCEtOH is relatively insensitive to increasing the doses of TCE from 1.5 to 4.6 mMol/kg, the fraction of the dose converted to TCA is already showing signs of diminishing at this dose.

We intend to move rapidly in documenting the AUC for these metabolites in blood in the final year of the grant. New support from NIH will make it possible to determine the extent to which DCA and TCA are further metabolized in mice and rats. This will allow a more precise determination the dose of each metabolite that is encountered by each species following exposure to TCE and will aid in determining whether these metabolites account for all of the hepatotoxic and hepatocarcinogenic effects of TCE. Currently available evidence suggests that DCA is extensively metabolized, a fact that may account for not detecting it in the blood of rats in this study. However, DCA or its metabolites may well be produced in significant quantities in the liver.

Hepatotoxic and Hepatocarcinogenic Effects of Chronic TCA and DCA Treatments.

In Tables 4 & 5 the effects of DCA and TCA on liver weights of male and female B6C3F1 mice after intervals of treatment that vary between 15 weeks and 52 weeks are shown. It is clear that both sexes respond to DCA by liver enlargement. The effects of DCA are much more severe than those of TCA, with liver size approaching and eventually exceeding twice the size observed in the control animals. Although partially reversed by 12 months if treatment is terminated after 9 months in male mice, the enlarged liver produced by DCA is still evident. The effect of both compounds on liver weight is clearly dose related and exceeds the effect of phenobarbital, included in the experiment as a positive control.

Prior reports on this project have detailed the histological effects that accompany the enlarged livers observed with DCA administration in mice. Parenchymal cells become greatly enlarged and engorged with glycogen. DCA also produces numerous necrotic lesions that occur locally within or on the surface of the liver. Liver enlargement following TCA treatments is associated with only modest increases in liver glycogen content and cells that appear only marginally swollen. There may be occasional necrotic lesions, but they are of such low frequency it is difficult to determine whether they are treatment related.

A small experiment was started early in our project for the purposes of developing techniques for identifying enzyme-altered foci in liver. It was conducted in the rat utilizing the

diethylnitrosamine/phenobarbital initiation/promotion used by many other investigators. This experiment was never intended for an evaluation of TCA and DCA hepatotoxicity in the rat, however, a few rats of each sex were treated with DCA and TCA alone at concentrations of 5 g/L of drinking water. Data concerning the liver/body weight and kidney/body weight ratios in this limited experiment are provided in Table 6. Even from this limited amount of data it is relatively clear that the results obtained in rats more or less parallel the results obtained in mice in terms of the hypertrophy produced. DCA induces a greatly enlarged liver, whereas the effect of TCA is much more modest. In the case of TCA, the effect was not statistically different from the ratio observed in control animals. The effect of DCA was observed as an absolute increase in liver weight, but the number of animals of each sex included in each treatment group is too small to make a valid statistical comparison (liver weights of males and females are quite different although the ratios to body weight are similar). It is notable that the kidney/body weight ratio is also significantly increased by treatment with DCA. This was not reflected in the absolute kidney weights.

The ability of these compounds to induce single strand breaks (SSB) in hepatic DNA following single oral doses has been previously reported. Although we have demonstrated that both DCA and TCA are capable of inducing peroxisome synthesis in the liver of B6C3F1 mice, the induction of single strand breaks in DNA was found to be independent of this phenomena. The results of these experiments are detailed in the two papers attached as an appendix to this report.

As previously reported the examination of the livers of mice for

enzyme altered foci at 15 and 24 weeks of exposure made clear that a high frequency of such lesions were not going to be apparent in DCA and TCA-treated mice. Complete sectioning of the left lateral lobe of mice treated with 2 g/L DCA revealed the presence of 4 basophilic and glycogen-poor foci in 5 mice at 24 weeks of treatment and 8 in 5 mice at 37 weeks. Such foci were not observed in control animals, animals treated with TCA at 2 g/L or phenobarbital at 0.5 g/L. Given this observation it was decided to continue the experiments with each compound until 12 months to examine tumor development under three circumstances:

11 animals to be treated continuously at a dose of 1 g/L
24 animals to be treated continuously with a dose of 2 g/L
11 animals to be treated for 36 weeks at a dose of 2 g/L and treatment suspended for the remainder of the 12 months.

The rationale behind this experiment was that if tumorigenesis had progressed to the point of irreversibility, the tumorigenic response observed at 12 months should be directly related to the total dose received. If the response is still at a reversible stage the response in the group treated for only 9 months should be less than that predicted by the two doses continued for 12 months.

Table 7 summarizes the results of the experiment testing the tumorigenicity of DCA and TCA. Control animals were found to have a relatively low incidence of hepatic tumors, 5 animals having one tumor each in a population of 35 animals. DCA induced 2 tumors in 11 animals at 1 g/L. This response was sharply increased by simply

doubling the dose to 2 g/L where 23 of 24 animals were found to have hepatic tumors. At this higher dose the multiplicity of tumors in each animal greatly increased such that the average tumor yield in this group was 3.8 tumors per animal. Seventy-three percent of the mice that were treated with DCA at 2 g/L for 9 months developed hepatic tumors with an average of 2.2 tumors per animal. While TCA produced a 45% incidence of tumors at a dose of 1 g/L, the increased tumor yield at 2 g/L was considerably less than observed with DCA, averaging only 1.2 tumors per animal. Tumor yields when TCA was administered at 2 g/L for 9 months were less than observed at a dose of 1 g/L for 12 months. Therefore, the tumor yield is considerably lower than would be predicted if the effects of TCA had progressed to an irreversible stage after 9 months of treatment. Phenobarbital, an established hepatic tumor promoter, did not increase tumor yield relative to control with 12 months of continuous treatment at 0.5 g/L.

In sharp contrast with results obtained in male B6C3F1 mice, treatment of females with 2 g/L TCA or DCA for a period of 12 months did not increase the incidence of hepatic tumors (Table 8).

Hepatic responses to short-term repeated exposures to DCA and TCA

The results obtained from the chronic study has prompted us to examine the effects DCA and TCA have on the liver in shorter term experiments. Establishing a shorter term model will facilitate investigation of the mechanisms that might be responsible for the hepatotoxic and hepatocarcinogenic effects of these compounds.

A 10-day experiment in which TCA and DCA were administered by

gavage in a 1% Tween 80 in water vehicle was used to establish that both DCA and TCA were capable of stimulating the synthesis of hepatic peroxisomes. We also found that this response occured much after the induction of single strand breaks in hepatic DNA in B6C3F1 mice. The results of these experiments are detailed in the manuscript included in the appendix.

Table 9 provides data on liver weights and liver to body weight ratios of two additional experiments involving administration of varying doses of DCA and TCA in drinking water. The first experiment was a 10 day experiment conducted only with DCA, whereas the second experiment was continued for a 14 day period. Water consumption varied between the two experiments, so the calculated doses per unit body weight are provided to allow comparison of the results. In the case of DCA dose-related increases in liver size were observed at doses of 181 mg/Kg and above. In the two experiments liver weight was increased by 33 to 50% at doses approximating 300 mg/kg. While there is a tendency for liver weight to be increased in animals treated with TCA, this increase was not statistically significant with the limited number of animals utilized. In fact, the greater liver weight observed at the 300 mg/L dose was strictly attributable to one animal with a very large liver as is apparent from the large SEM. In the 14 day experiment it was necessary to eliminate one control animal from the table because it had been injected directly in the liver with ^{3}H-thymidine (animals in this experiment are being examined for evidence of increased cell division in the liver following DCA and TCA treatments). An animal in the group receiving the high dose of TCA was eliminated because of its very small size before the experiment

started.

Histological sections of the livers from the animals in the above experiments have been examined (data not shown). In both experiments the enlarged cell size that was associated with increased glycogen content in the chronic study was already evident in animals treated with 1 or 2 g DCA/L. However, the intensity of the effect was not as severe as was observed in the chronic study. Mice receiving these treatments also displayed a lesion that was frequently apparent grossly as a white streaking on the surface of the liver. In cross-section the streaks corresponded to multiple focal necrotic areas on the borders of liver sections. Such lesions were also observed in the interior of the liver. The frequency of these lesions is such at every sacrifice time that has been made during the course of both short and long term studies that there is little doubt that the production and healing these lesions is a continuous process in animals treated with doses of DCA above 180 mg/kg. These lesions have not yet been observed in TCA-treated animals, but a systematic examination of slides has yet to be intiated. Thus, it is not possible to exclude the possibility that a low frequency of such lesions might be observed. It is nevertheless clear that the frequency and severity of these lesions is much greater in DCA-treated animals than TCA-treated animals. These lesions were not apparent in control animals.

Comparison of Dose-Response Data for Strand Break Induction, Liver Hypertrophy and Tumorigenic Responses.

Figure 4 provides a comparison of the dose-response relationships for the induction of single strand breaks (SSB) in hepatic DNA of male B6C3F1 mice following a single oral doses of DCA or TCA with the calculated daily doses required for tumor induction. This comparison showed that the induction of SSB occurs at doses much lower than required for tumor induction. TCA is the more potent inducer of strand breaks and at the low dose appears to be the more potent carcinogen. However, a simple doubling of the dose of DCA sharply increased the yield of hepatic tumors, while increasing the dose of TCA accordingly resulted in a more modest increase in tumor yield. This difference in the dose-response characteristics of the tumorigenic responses to these two closely related compounds make it apparent that there are real differences in the mechanisms by which they produce hepatic tumors in mice.

One possible basis for this difference is seen in Figure 5 where the dose-response relationship of liver hypertrophy is compared to the tumorigenic responses. The range of doses of DCA which produce the hypertrophic response and necrotic lesions in the 14 day study previously described correspond closely to the daily doses in which the sharp upturn in the tumorigenic response is observed in chronic studies.

The difference in the reversibility of the tumorigenic responses referred to earlier is graphically presented in Figure 6 as a reminder of differences in the tumorigenic responses to these compounds. In

the case of DCA the tumorigenic response was directly proportional to the total dose consumed despite the fact that the intermediate dose was given over a 9 month rather than a 12 month period. Conversely, the response to TCA was less than would be expected by the total dose consumed in this 9 month period. As mentioned previously, by nine months a low frequency of basophilic and glycogen-poor foci are apparent in animals treated with 2 g DCA/L and such lesions are absent from TCA-treated animals. The staining properties of the tumors produced match those of the foci. Therefore, these lesions have a very high probability of progressing to tumors and have properties similar to the late hepatic nodules described by Farber. The reversibility of TCA's effects after 9 months may be related to the fact that these foci had not yet appeared.

It is also remarkable that both DCA and TCA failed to induce hepatic tumors in female B6C3F1 mice after receiving the same doses as male animals. Liver hypertrophy and necrotic lesions are produced to a similar extent in female mice as in male mice. This difference in response between the sexes appears directly related to the substantial differences in the spontaneous rate of hepatic tumor development in this strain. Female B6C3F1 mice have had approximately 5% background incidence in NTP experience whereas the male incidence usually exceeds 25%. This observation supports the notion that DCA and TCA simply stimulate the growth of spontaneously initiated tumors in this hybrid mouse.

Based on the appearance of basophilic and glycogen-poor foci as early as 24 months after beginning treatment with DCA and the apparent irreversibility of the tumorigenic effect after 9 months we propose

that DCA acts by accelerating tumor progression. The ability of DCA to induce localized necrosis and a severe liver hypertrophy at high doses appears to be at least partially responsible for the more rapid progression to tumor relative to TCA.

WORK PLANNED FOR THE THIRD YEAR

The final year of the grant will be spent on the following activities:

1. Complete histological diagnosis of hepatic tumors induced in the chronic study.
2. Characterize the non-tumor histology of livers from animals removed from treatments of DCA and TCA at 9 months.
3. Prepare and submit a manuscript on the results of the chronic study.
4. Complete study of TCE metabolism in B6C3F1 mice to quantify the systemic exposure to DCA and TCA. These estimates will be used to determine whether sufficient quantities of these metabolites are formed to account for the hepatotoxic and hepatocarcinogenic effects of TCE. These estimates will be facilitated by the purchase of ^{14}C-labeled DCA and TCA made possible by receipt of a grant from NIH.
5. Characterize the toxic effects resulting from shorter term treatments (i.e. 14 day) with DCA and TCA. These experiments will include:
   a. Establish dose-response relationships for induction of

cellular hypertrophy, induction of cellular hypertrophy and induction of necrotic lesions.

b. Establish the pattern and extent of DCA and TCA-induced cell division in the liver at varying doses using autoradiographic detection of ^{3}H-thymidine incorporation as a measure.

c. Design and conduct experimetns to differentiate a generalized mitogenic effect and localized cell division resulting from reparative hyperplasia associated with focal necrotic lesions.

6. Determine whether mitogenic effects of DCA and TCA can be associated with increased expression of protooncogenes (ras, myc and fos).

7. Determine whether DCA or TCA is capable of acting as a clastogenic agent in CHO cells and confirming, if indicated in primary hepatocyte cultures.

8. Determine if SSB induced by DCA and TCA result from direct or indirect interactions with DNA.

9. Prepare and submit at least one manuscript describing the short-term effects of DCA and TCA on the liver.

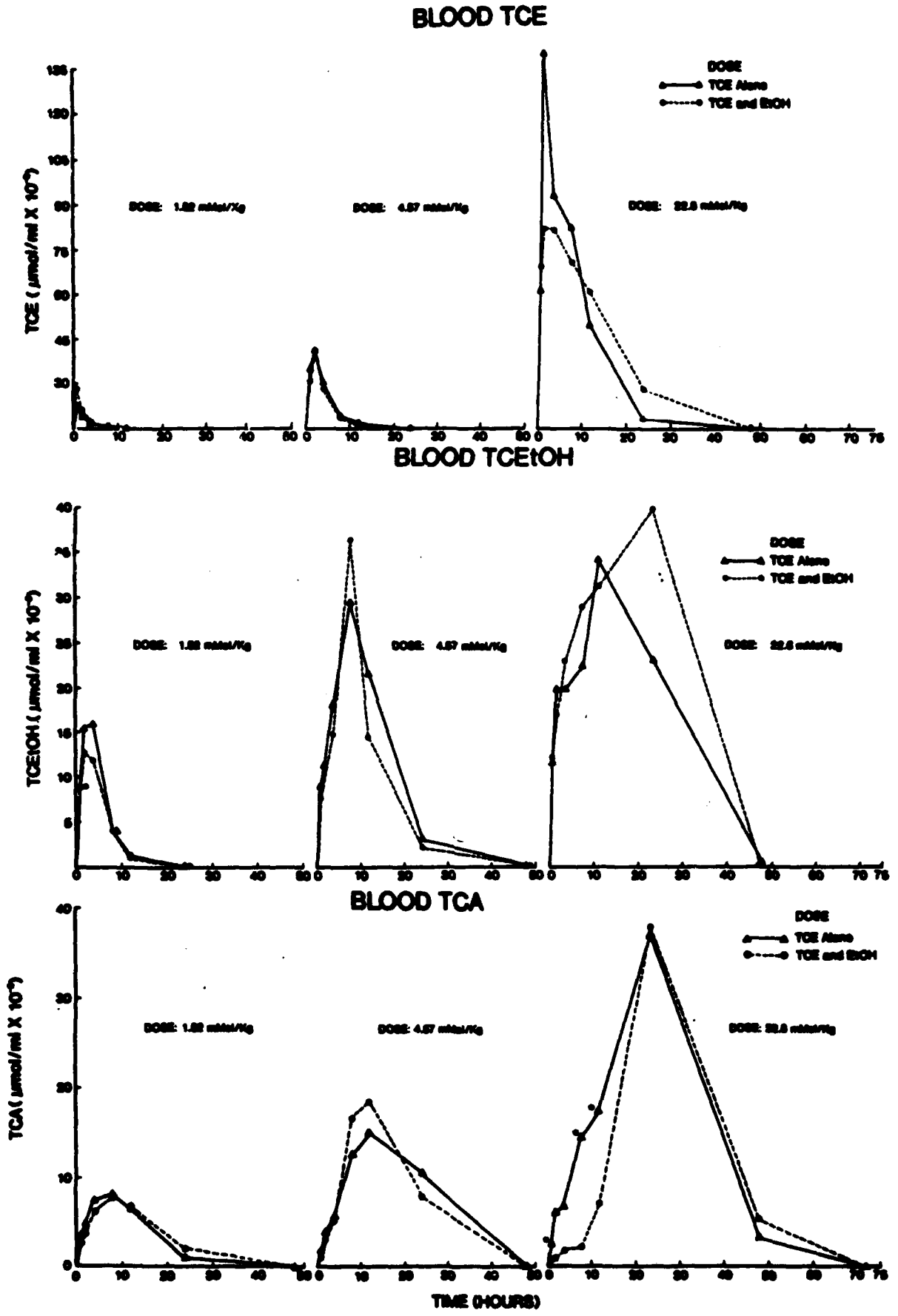

Figure 1. Concentrations of trichloroethylene (TCE), free trichloroethanol (TCEtOH) and trichloroacetate (TCA) in blood of male Sprague-Dawley rats administered varying doses of TCE with and without coadministration of equimolar doses of ethanol.

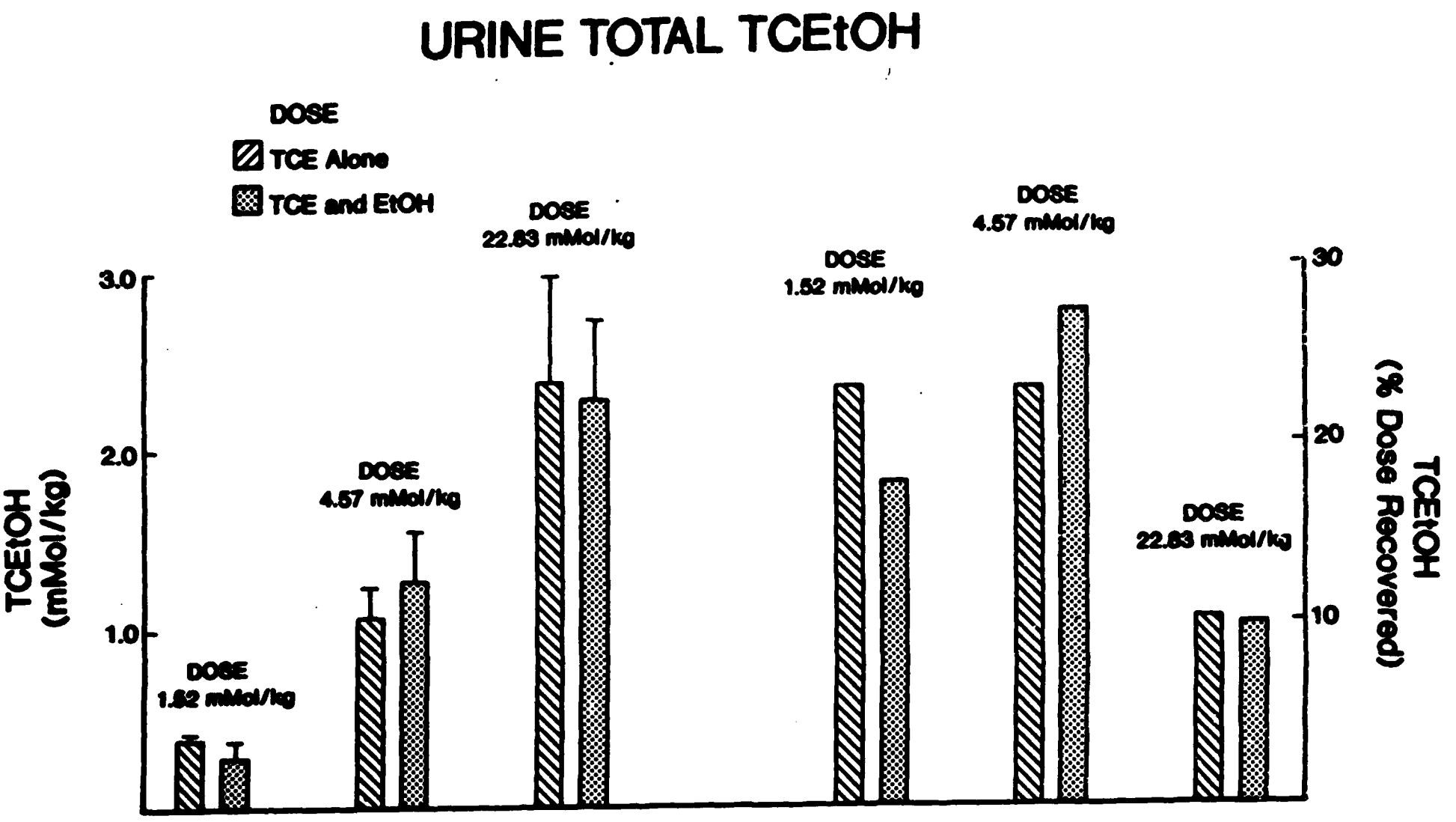

Figure 2. Effect of the dose of trichloroethylene (TCE) and ethanol coadministration on the recovery of total trichloroethanol (TCEtOH) in the urine of male Sprague-Dawley rats.

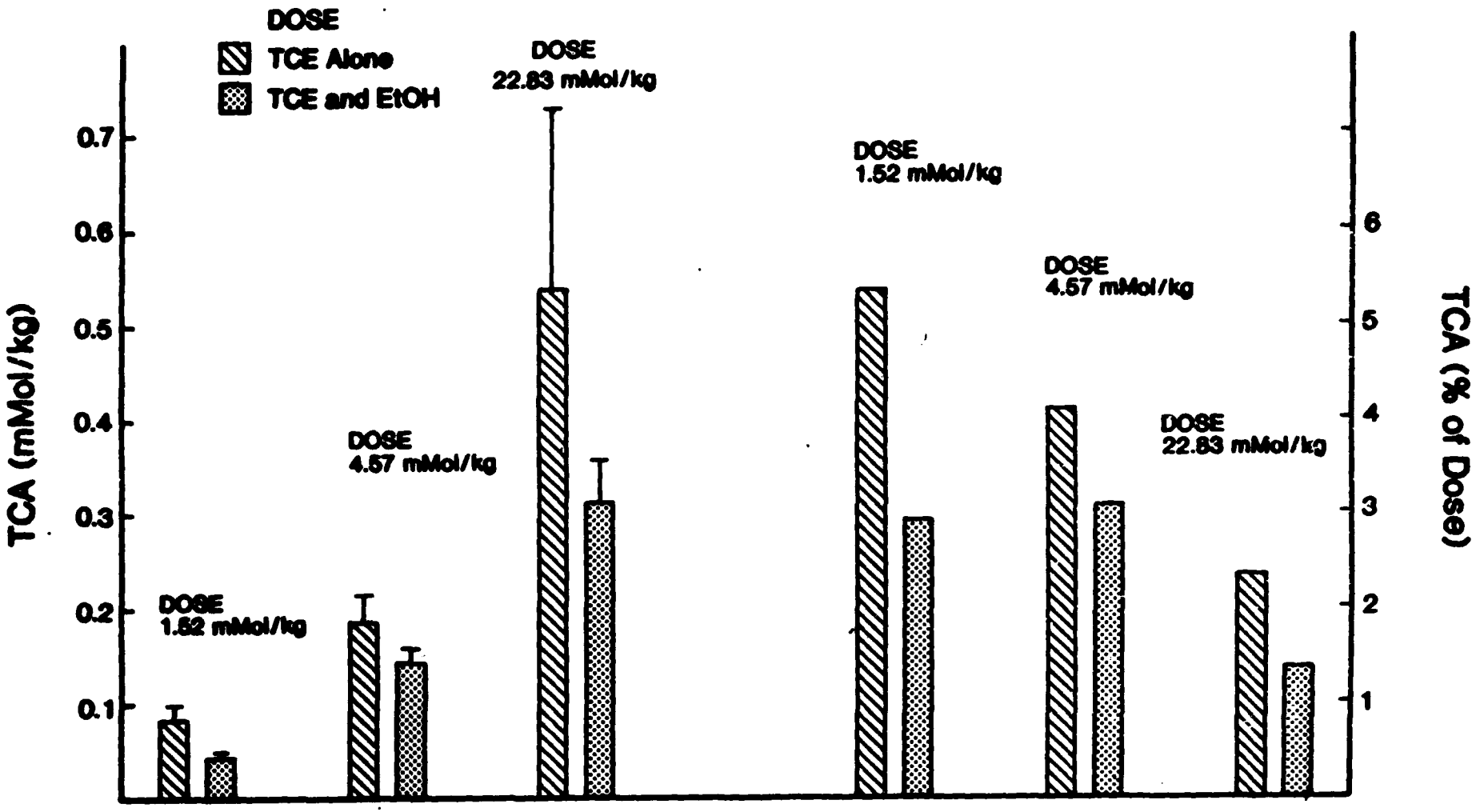

Figure 3. Effect of the dose of trichloroethylene (TCE) and ethanol coadministration on the recovery of trichloroacetate (TCA) in the urine of male Sprague-Dawley rats.

Table 1. The effect of dose and ethanol coadministration on the amounts of trichloroethylene (TCE) and trichloroacetate (TCA) in the blood of male Sprague-Dawley rats.

		TCE		TCA	
Dose range mMol/kg	Increase in TCE Dose	Increase in peak Conc.	Increase in AUC	Increase in peak Conc.	Increase in AUC
1.5 to 4.6 TCE	3.0	3.2	6.4	1.7	2.8
4.5 to 22.8 TCE	5.0	4.7	8.2	2.4	2.8
1.5 to 4.6 TCE + Ethanol	3.0	2.0	4.9	2.4	2.6
4.6 to 22.8 TCE + Ethanol	5.0	2.5	9.1	2.0	2.6

Table 2. The effect of TCE dose and ethanol coadministration on the ratio of total trichloroethanol (TCEtOH) and trichloroacetate (TCA) recovered in urine.

	TCEtOH/TCA	
Dose of TCE mMol/kg	Without Ethanol	With Ethanol
1.5	4.8 ± 0.5	6.5 ± 1.0
4.6	6.2 ± 1.1	7.3 ± 1.1
22.8	4.9 ± 0.4	7.2 ± 0.5

Table 3. Recovery of total trichloroethanol (TCEtOH) and trichloroacetic (TCA) in 72 hour urines of B6C3F1 mice treated with varying doses of trichloroethylene (TCE).

Dose of TCE mMol/kg	Total TCEtOH	Fraction of TCE dose	TCA	Fraction of TCE dose
1.5	0.49 ± 0.01[a]	0.32 ± 0.01	0.10 ± 0.05[a]	0.057 ± 0.017
4.6	1.74 ± 0.34	0.35 ± 0.07	0.17 ± 0.03	0.038 ± 0.003
15.2	3.60 ± 0.82	0.24 ± 0.05	0.35 ± 0.14	0.023 ± 0.009

[a] values expressed in mMol/kg body weight ± SEM of 3 or 5 animals/group

Table 4. Effects of dichloroacetic and trichloroacetic acids on liver weight of male B6C3F1 mice.

Compound	Liver weight g	Liver weight % of body weight
15 weeks		
Control (Dist. H_2O)	1.06 ± 0.05	3.2 ± 0.1
Phenobarbital 0.5 g/L	1.34 ± 0.16*	4.2 ± 0.4*
Dichloroacetate 2 g/L	1.80 ± 0.15*	5.6 ± 0.3*
Trichloroacetate 2 g/L	1.34 ± 0.09*	4.1 ± 0.1*
24 Weeks		
Control	1.06 ± 0.04	2.8 ± 0.2
Phenobarbital 0.5 g/L	1.24 ± 0.04*	3.7 ± 0.1*
Dichloroacetate 2 g/L	1.78 ± 0.07*	5.4 ± 0.1*
Trichloroacetate 2 g/L	1.44 ± 0.05*	4.2 ± 0.1*
37 weeks		
Control	1.56 ± 0.06	4.1 ± 0.3
Phenobarbital 0.5 g/L	2.14 ± 0.15*	6.0 ± 0.4*
Dichloroacetate 2 g/L	2.50 ± 0.11*	7.3 ± 0.2*
Trichloroacetate 2 g/L	1.90 ± 0.05*	5.1 ± 0.1*
52 weeks		
Control	1.7 ± 0.1	4.6 ± 0.1
Phenobarbital 0.5 g/L	2.0 ± 0.1*	5.6 ± 0.2*
Dichloroacetate 1 g/L 12 months	2.5 ± 0.1*	6.5 ± 0.2*
2 g/L 12 months	5.1 ± 0.1*	10.5 ± 0.4*
2 g/L 9 months	2.2 ± 0.1*	5.7 ± 0.3*
Trichloroacetate 1 g/L 12 months	2.2 ± 0.1*	6.0 ± 0.3*
2 g/L 12 months	2.7 ± 0.1*	7.5 ± 0.5*
2 g/L 9 months	1.9 ± 0.1	5.4 ± 0.2

* Statistically different from corresponding negative control $P \leq 0.05$, by Student's t-test. N = 5 at 15, 24 and 37 weeks and is either 11 or 24 at 52 weeks.

Table 5. Effect of treating female B6C3F1 mice with dichloroacetate (DCA) and trichloroacetate (TCA) for one year on liver weight.

Treatment	N	Liver weight	% of body weight
Distilled Water	10	1.31 ± 0.06	4.8 ± 0.3
Dichloroacetate 2 g/L	10	2.61 ± 0.05	9.0 ± 0.2
Trichloroacetate 2 g/L	10	1.69 ± 0.06	6.0 ± 0.3
Phenobarbital 0.5 g/L	10	1.43 ± 0.03	5.4 ± 0.2

Table 6. Effect of chronic treatment (12 months) with dichloroacetate (DCA) and trichloroacetate (TCA) in drinking water on the ratios of liver/body weight and kidney/body weight ratios in Sprague-Dawley rats.

Treatment	N	$\frac{\text{liver wt.}}{\text{body wt}} \times 100$	$\frac{\text{kidney wt.}}{\text{body wt.}} \times 100$
Control	4[a]	4.0 ± 0.4	0.91 ± 0.01
DCA 5000 mg/L	5	7.2 ± 0.4	1.12 ± 0.04
TCA 5000 mg/L	6	4.2 ± 0.3	0.94 ± 0.06

[a] Data includes both male and female rats in each group. Split is 2 and 2 in control, 2 males and 3 females in DCA-treated and 4 males and 2 females in TCA-treated animals.

Table 7. Induction of hepatic tumors in the male B6C3F1 mice by dichloroacetate and trichloroacetate in drinking water.

Treatment	24 weeks	37 weeks	52 weeks
Control (Dist. H_2O)			
# mice with tumors (%)	0/5 (0)	0/5 (0)	5/35 (14)
Avg. # tumors/mouse	0	0	0.14
Dichloroacetate			
1 g/L			
# mice with tumors	ND	ND	2/11 (18)
Avg. # tumors/mouse			0.27
2 g/L			
# mice with tumors	0/5	0/5	23/24 (96)
Avg. # tumors/mouse	0	0	3.83
2 g/L 9 months			
# mice with tumors	ND	ND	8/11 (73)
Avg. # tumors/mouse			2.18
Trichloroacetate 2 g/L			
1 g/L			
# mice with tumors	ND	ND	5/11 (45)
Avg. # tumors/mouse			0.64
2 g/L			
# mice with tumors	0/5	1/5	19/24 (79)
Avg. # tumors/mouse	0	0.2	1.25
2 g/L 9 months			
# mice with tumors	ND	ND	4/11 (36)
Avg. # tumors/mouse			0.45
Phenobarbital 0.5 g/L			
# mice with tumors	0/5	0/5	1/24 (4)
Avg. # tumors/mouse	0	0	0.04

Table 8. Incidence of neoplasms in female B6C3F1 mice treated with dichloroacetate or trichloroacetate in drinking water for 1 year.

Treatment	N	Animals with hepatic tumors	No. neoplasms per animal.
Distilled water	10	2	0.2
Dichloroacetate 2 g/L	10	0	0
Trichloroacetate 2 g/L	10	0	0
Phenobarbital 0.5 g/L	10	1	0.1

Table 9. Effect of short term (10 or 14 day) exposures to dichloroacetate (DCA) or trichloroacetate (TCA) in drinking water on liver weight in male B6C3F1 mice.

Treatment	mg/kg	N	Liver Weight	Liver weight as % body wt.
10 day experiment				
Control	0	4	1.5 ± 0.03	5.7 ± 0.2
DCA 100 mg/L	31	4	1.5 ± 0.14	5.4 ± 0.3
300	87	4	1.6 ± 0.10	6.1 ± 0.3
1000	289	4	1.8 ± 0.03*	6.7 ± 0.1*
2000	370	4	2.0 ± 0.09*	7.5 ± 0.1*
14 day experiment				
Control	0	7	1.3 ± 0.07	4.9 ± 0.2
DCA 300 mg/L	55	4	1.3 ± 0.08	4.9 ± 0.3
1000	181	4	1.7 ± 0.16*	5.9 ± 0.4*
2000	321	4	1.9 ± 0.05*	7.6 ± 0.1*
TCA 300 mg/L	67	4	1.5 ± 0.17	5.8 ± 0.7
1000	207	4	1.4 ± 0.06	5.4 ± 0.3
2000	356	3	1.4 ± 0.05	5.5 ± 0.2

* Significantly different from concurrent control @ $P < 0.05$

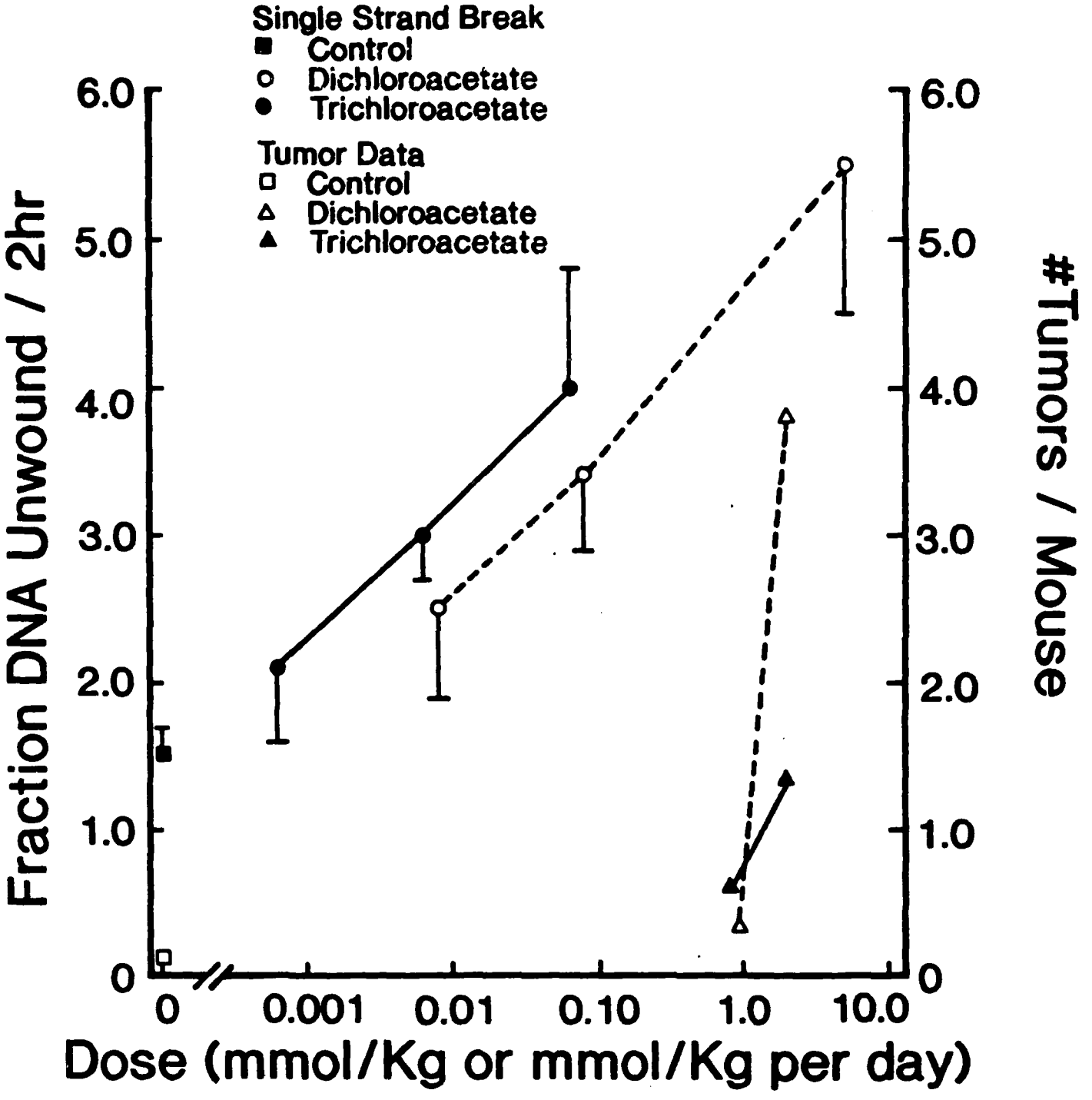

Figure 4. The relationship between the single doses of TCA and DCA required to induce single strand breaks in the hepatic DNA of male B6C3F1 mice in vivo and the daily doses giving rise to hepatic tumors in a 12 month study. The effects on single strand breaks was observed 4 hours after a single dose administered by gavage in a 1% Tween 80 in water vehicle. DCA and TCA were administered in the drinking water for the chronic study. The doses indicated are corrected for water consumption and normalized to body weight.

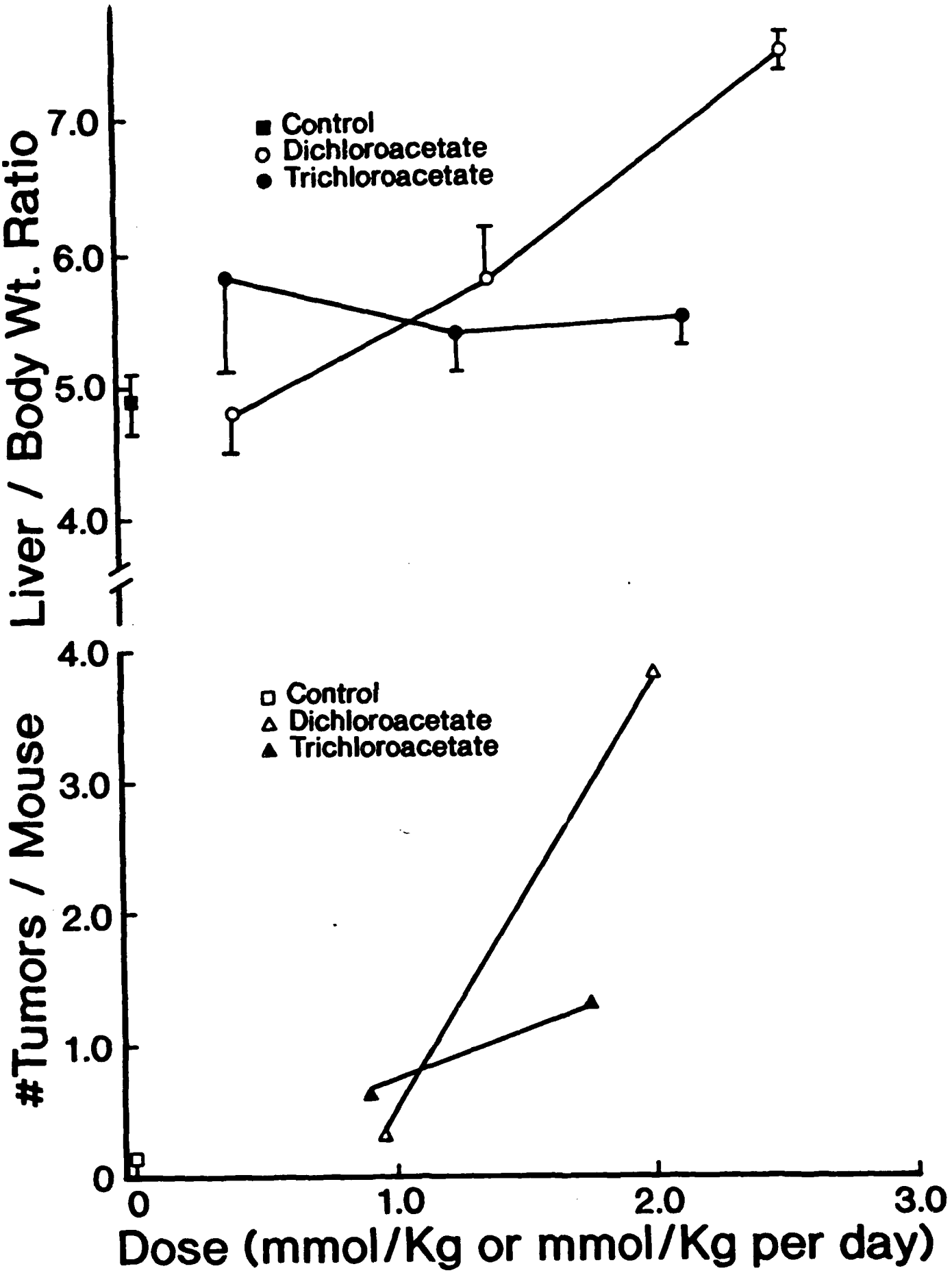

Figure 5. The relationship of tumor induction by DCA and TCA in male B6C3F1 mice in a 12 month study and the production of liver hypertrophy in a 14 day study. In both cases the compounds were administered in drinking water at concentrations of 1 and 2 g/L. The doses indicated are corrected for drinking water consumption and are normalized to body weight.

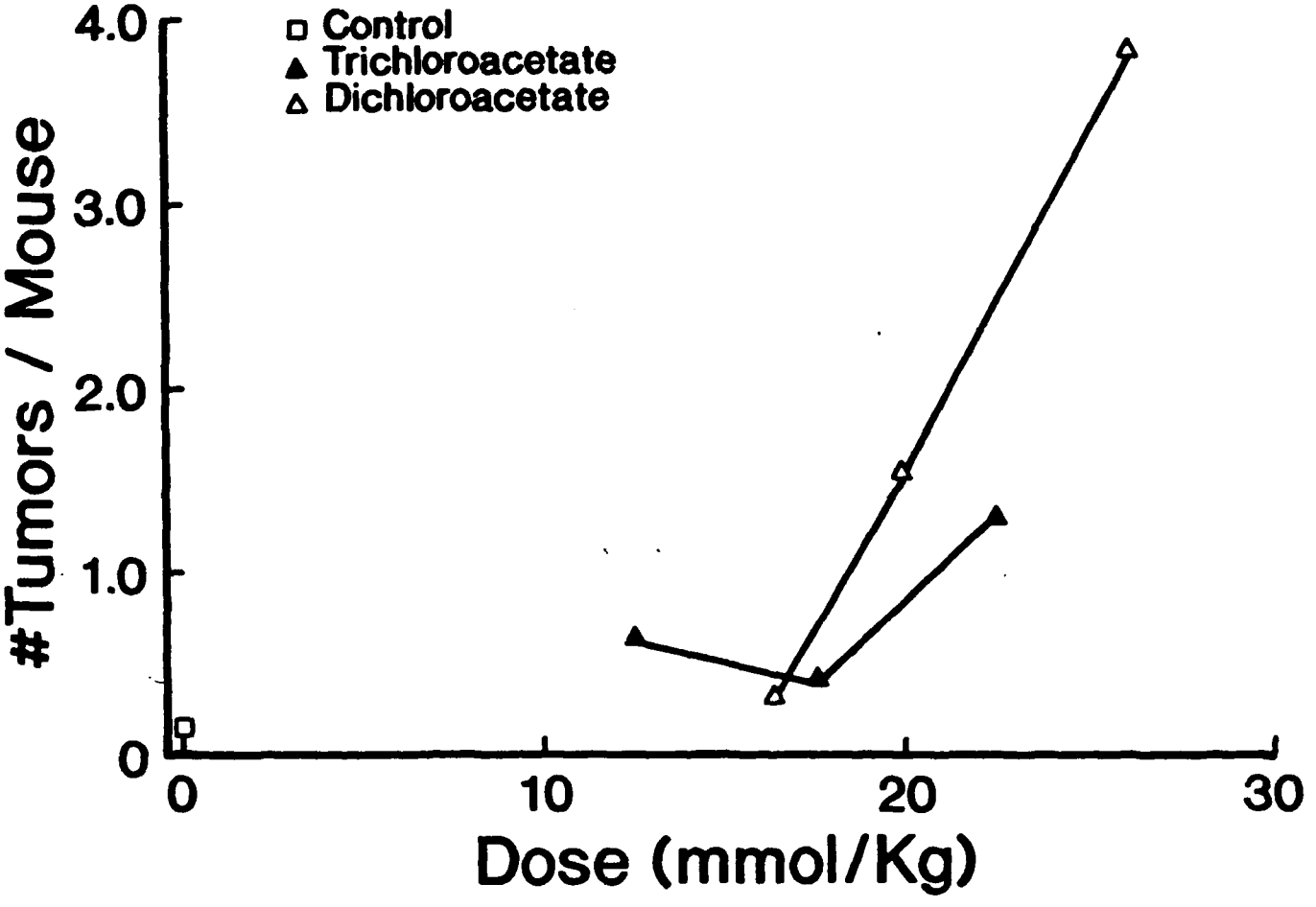

Figure 6. The reversibility of the tumorigenic effects of DCA and TCA in male B6C3F1 mice. Doses are expressed as the total dose consumed by the animals in each group. In each curve the lowest and highest dose resulted from consumption of drinking water containing 1 or 2 g/L of drinking water, respectively, for a period of 12 months. The intermediate point represents the tumor yield obtained when 2 g/L of each compound was administered for 9 months and the animals placed on distilled water for the remaining 3 months. Numerical data is the same as that provided in Table 7.

DICHLOROACETIC ACID (DCA) AND TRICHLOROACETIC ACID (TCA) INDUCED DNA STRAND BREAKS OCCUR BEFORE PEROXISOME PROLIFERATION

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Running Title: Strand breaks, peroxisomes, and DCA/TCA

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Dichloroacetic Acid and Trichloroacetic Acid Induced DNA Strand Breaks Occur Before Peroxisome Proliferation. M.A. Nelson, A.J. Lansing, R.J. Bull, and *D.L. Springer (1988). Toxicol. Appl. Pharmacol. 00, 000-000. This study examined whether the induction of single strand breaks in hepatic DNA by dichloroacetic acid (DCA) and trichloroacetic acid (TCA) depends upon peroxisome proliferation. Male B6C3F1 mice were given a single oral dose of either DCA or TCA. At varing times, between 1 and 24 hr, after administration of the compounds breaks in DNA were measured using an alkaline unwinding assay. Peroxisome proliferation was monitored at the same time intervals in a parallel experiment by measuring peroxisomal B-oxidation of [^{14}C]palmitoyl-CoA in liver homogenates. Both DCA and TCA significantly increased breaks in DNA at 1, 2, and 4 hr post-treatment, with a return to control levels after 8 hr. No evidence for an increase in peroxisomal B-oxidation was produced by either chemical up to 24 hr after administration. In a separate experiment, mice were treated with DCA or TCA for 10 days and their livers examined for evidence of peroxisome proliferation. An increase in liver weight was observed, particularly with DCA. Both TCA and DCA increased peroxisomal B-oxidation in liver homogenates, with TCA-treated animals showing more activity than those treated with DCA. Electron microscopy revealed that the number of peroxisomes were approximately the same in DCA- and TCA-treated animals. However, peroxisomes induced by DCA treatment frequently lacked nucleoid cores. These data indicate that peroxisomes induced by these compounds differ in their concentration of peroxisomal enzymes. Except for a slight hypertrophy, repeated doses of TCA doesn't produce significant degenerative changes in the liver of mice. Repeated doses of DCA produce multifocal, subcapsular necrotic regions. DCA also caused a marked hypertrophic response.

Dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are major nonvolatile chlorinated by-products formed during the chlorination of water containing organic material (Johnson et al., 1982; Coleman et al., 1984; Miller and Uden, 1983). These compounds are also metabolites of a number of chlorinated hydrocarbons (Hathway, 1980; Costa and Ivanetich, 1980; Yllner, 1971). Recently, DCA and TCA have been shown to increase the incidence of hepatocellular carcinoma and adenomas in male B6C3F1 mice (Herren-Freund et al., 1987).

The mechanism by which these compounds induce neoplasia remains to be established. Although DCA and TCA are not mutagenic in the Salmonella/microsome assay (Waskel, 1978; Rapson et al., 1980; Crabb et al., 1981), they do produce single strand breaks (SSBs) in hepatic DNA in vivo (Nelson and Bull, 1988). These compounds also induce hepatic peroxisome proliferation (Elcombe, 1965; DeAngelo et al., 1986; Goldsworthy and Popp, 1987). Hepatic peroxisome proliferation is thought to cause cancer by producing imbalanced increases in catalase and peroxisomal oxidases, resulting in increased steady-state concentration of intracellular H_2O_2 (Reddy and Lalwani, 1983). Increased levels of H_2O_2 , or other reactive oxygen species (i.e. OH^-, O^-_2, or lipid peroxides) may lead to SSBs in DNA and eventually result in neoplasia. H_2O_2 and OH^- are known to be mutagenic and cause strand breaks in DNA in vitro (Moody and Hassen, 1982; Fahl et al., 1984; Lesko et al., 1980). In the present study, we have investigated whether or not SSB induced in hepatic DNA of mice by DCA or TCA are secondary to the proliferation of peroxisomes. We also present data on the extent of peroxisome proliferation and discuss histopathology associated with repeated doses of DCA or TCA.

METHODS

Chemicals. [1-^{14}C]palmitoyl-CoA was purchased from New England Nuclear Products (Boston, MA); clofibrate, dichloroacetic acid (DCA) and tricholoroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO). The purity of DCA and TCA

was 99+% as measured by gas chromatographic methods described by Prout et al., (1985).

Animals. Male B6C3F1 mice (Simonsen Laboratories Inc., Gilroy, CA.) weighing 25-30 g were housed in temperature controlled rooms with a 12 hr light/dark cycle and provided with food (Purina Laboratory Rodent Chow, Ralston-Purina Co., St. Louis, MO.) and water ad libitum.

Experiment 1. Animals were gavaged (0.1 ml/g body weight) with a single dose of DCA (3.9 mmol/kg) or TCA (3.1 mmol/kg) dissolved in 1% aqueous Tween 80. Controls received an appropriate volume of 1% Tween 80 alone. Animals were killed by cervical dislocation 1, 2, 4, 8, and 24 hr after administration of the compounds and the livers removed. Single strand breaks in hepatic DNA were determined using an alkaline unwinding assay (Morris and Shertzer, 1985). The fraction of DNA unwound was calculated as:

$$\frac{[\text{Total DNA} - \text{DS DNA}]_2}{[\text{Total DNA}]} - \frac{[\text{Total DNA} - \text{DS DNA}]_0}{[\text{Total DNA}]}$$

where the subscript indicates the amount of single-stranded DNA at time "0" and "2 hr" in alkaline solution.

Experiment 2. Mice were treated with a single oral dose of DCA (3.9 mmol/kg), TCA (3.1 mmol/kg), or vehicle control as in Experiment #1. Peroxisome proliferation was determined by measuring palmitoyl-CoA oxidation (PCO) in liver homogenates prepared from animals killed at the same time intervals described above. At appropriate times, the animals were killed, the livers

removed, frozen in liquid nitrogen, and stored at -70 °C until assayed. Livers were thawed, weighed, and homogenized in 10 volumes of ice-cold 0.25 M sucrose using a Brinkmann Polytron Homogenizer. The ability of the homogenates to oxidize palmitoyl-CoA was measured using a radioisotopic method described by Lazarow (1981). This assay measures the extent of peroxisomal B-oxidation of fatty acids by following the conversion of acid insoluble [^{14}C]palmitoyl-CoA to acid soluble [^{14}C]acetyl-CoA. The lack of carnitine, the addition of Triton x-100 to the reaction mixture, and freeze/thawing make this assay peroxisomal dependent. Enzyme activity is expressed as umols/min/g liver.

Experiment 3. Animals were given DCA (3.9 mmol/kg), TCA (3.1 mmol/kg), or vehicle by gavage daily for 10 consecutive days. Clofibrate, at doses of 250 mg/kg/day, was included in this experiment as a positive control. Twenty-four hours after the last dose the animals were killed and the livers excised and weighed. The left lateral lobe was used for light and electron microscopic studies, and the remaining liver was used for palmitoyl-CoA oxidation (PCO) determinations, as described above.

Electron microscopy. Tissue for electron microscopy was fixed in 2.5% glutaraldehyde in 100 mM phosphate buffer, pH 7.2 for 18 hr at 4°C. Following dehydration through an acetone series and embedment in Spurr's resin (Spurr, 1960), 60 nm sections of liver were cut with glass knives, and stained with uranyl acetate and lead citrate. From two animals from each vehicle, DCA, and TCA treatments, six micrographic fields of 184

um^2 each, were randomly chosen and recorded from an total area of 10^4 um^2. The number of peroxisomes profiles present in each sample field were recorded for each animal.

Light microscopy. Portions of liver tissue were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections (6 um thick) were cut and stained with both hematoxylin and eosin and periodic acid/Schiff's reagent (Humason, 1967).

Statistical Analysis. To simplify construction of Figure 1, data from all control groups were combined and expressed as a single mean $\pm$ SE for each time point. However, statistical comparisons were made only with concurrently run controls and treatments using Student's t test. Rates of peroxisome palmitoyl-CoA oxidation from single oral dose experiments was also analyzed using Student's t test.

Palmitoyl-CoA oxidation data and liver weight data from the repeated dose experiment were analyzed by analysis of variance (ANOVA) with Duncan's New Multiple Range test for mean separation.

For morphometry, the electron micrographs taken from each animal were treated as subsamples and statistical comparisons conducted using ANOVA and Duncan's New Multiple Range test (Steel and Torrie, 1980). In both the ANOVA and t test, a P value $\leq$ 0.05 was considered significant.

RESULTS

Time course of the induction of hepatic DNA strand breaks in vivo. Both DCA and TCA significantly increased the rate of

alkaline unwinding of hepatic DNA at 1, 2, and 4 hr after administration. The rate returned to the range observed with control animals 8 hr after exposure (Fig 1). These data deomostrate that the induction of SSB by DCA or TCA was maximal at 1 hr and that the majority of breaks were repaired within 8 hr.

Time course of hepatic palmitoyl-CoA oxidation. No evidence of increased peroxisomal PCO activity was observed within 24 hr with either DCA or TCA (Table 1).

Hepatic peroxisomal proliferation after repeated doses of DCA or TCA. Repeated doses of either DCA or TCA had no effect upon the body weight gain of the animals relative to vehicle control animals (data not shown). Both compounds significantly increased liver weight as well as the liver to body weight ratio (Table 2). DCA treatment caused a substantially greater increase in both the absolute and relative liver weights than TCA.

The repeated dosing of DCA and TCA did induce peroxisomal synthesis as measured by the PCO activity of liver homogenates (Table 3). The TCA-induced PCO activity was significantly higher than that seen following DCA administration. Neither treatment increased PCO activity to the extent observed with clofibrate.

Counts of peroxisome profiles were significantly higher in DCA- and TCA-treated livers than in control livers (Table 3). There were no discernible difference in the peroxisome counts between animals treated with either DCA or TCA. Peroxisomes in DCA-treated livers generally lacked the pronounced nucleoid core

that was usually observed in peroxisomes from tissue taken TCA-treated and control animals.

Light microscopy. There was no evidence of gross hepatotoxicity in vehicle- and TCA-treated mice. Periodic acid-Schiff's reagent (PAS) produced approximately the same intensity of staining. Amylase digestion revealed that the vast majority of PAS-positive material was glycogen.

Hematoxylin- and eosin-stained liver sections from control and TCA-treated animals showed no abnormalities. In both treatment groups the architecture and tissue pattern of the liver was intact. The hepatocytes from TCA-treated mice were slightly larger than hepatocytes from control animals. This slight hypertrophy could be seen throughout the liver section.

The histopathology seen after repeated doses of DCA was markedly different than that observed with either vehicle or TCA treatments. The most pronounced change was in the size of the hepatocytes. DCA produced a marked cellular hypertrophy uniformly throughout the liver sections. The hepatocytes were approximately 1.4X larger in diameter than control liver cells. This hypertrophy was accompanied by an increase in PAS staining; indicating greater glycogen deposition than in TCA-treated and control liver tissue.

Multiple white streaks were grossly visible on the surface of liver of DCA-treated mice. The white areas corresponded with subcapsular foci of coagulative necrosis. These localized necrotic areas were not encapsulated and varied in size. The

largest necrotic foci occupied the area of a single lobule. These necrotic areas showed a change in staining characteristics. Often this change consisted of increased eosinophilia and decreased PAS staining of the hepatocytes. A slight inflammatory response, characterized by heterophil infiltration, was present. These changes were evident in all DCA-treated mice.

DISCUSSION

The present study confirms our previous observation that DCA and TCA can induce SSB in hepatic DNA in vivo (Nelson and Bull, 1988). We further demonstrate that SSB are rapidly induced by these compounds, with significant levels apparent within 1 hr after dosing. Repair of SSB also proceeds rapidly, with a return to control levels within 8 hr after dosing.

Within the first 24 hr after dosing neither DCA or TCA increased PCO activity in mouse liver. These results demonstrate that induction of SSB by these compounds occurs too early to be accounted for by increased peroxisomal fatty acid oxidation.

This study confirms the observations of Elcombe (1985) and Goldsworthy and Popp (1987) that TCA induces synthesis of hepatic peroxisomes in rats and in mice. In addition, we found that DCA induces hepatic peroxisomes in mice consistent with the preliminary observations of DeAngelo et al. (1986). Our morphometric data indicate that DCA and TCA cause approximately equivalent increases in the number of peroxisomes. Assays of PCO activity indicate that these peroxisomes differ in their ability

to metabolize palmitoyl-CoA. DCA-treated mouse livers frequently lacked a visible nucleoid core, a structure thought to contain high concentrations of the peroxisomal B-oxidation enzymes (Rothman, 1983).

The administration of repeated doses of DCA or TCA increased liver weights and liver to body weight ratios in mice. Similar findings have been reported by Goldsworthy and Popp (1987) with TCA and by Herren-Freund et al. (1987) in a chronic study with DCA and TCA. The marked cellular hypertrophy seen with DCA appears to account for the much greater liver size increase observed in DCA- versus TCA- treated mice.

Hematoxylin and eosin and PAS staining showed no detectable differences between livers of vehicle- and TCA-treated mice. However in DCA treated mouse livers, an increase in glycogen concentration was apparent. Furthermore, DCA produced a noticeable cellular hypertrophy and multifocal, subcapsular necrotic areas. The extent to which these additonal activities of DCA influence the carcinogenic response is currently under study.

In summary, the time course of induction of SSB in hepatic DNA by TCA or DCA occurs much too rapidly to be accounted for by increases in PCO activity, indicating that peroxisome proliferation cannot account for DCA- and TCA-induced breaks. Therefore, the mechanism by which DCA and TCA produce SSB in hepatic DNA remains to be established.

Repeated doses of both DCA and TCA do increase peroxisomal

numbers as well as PCO activity relative to controls. Peroxisomes induced by DCA generally lack a nucleoid core and are associated with smaller increases in hepatic PCO activity. These differences indicate that the enzymes involved in B-oxidation are smaller in peroxisomes of DCA-treated mice relative to those of TCA-treated mice.

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Fig. 1. Time course for dichloroacetic acid (DCA) and trichloroacetic acid (TCA) induced single strand breaks in hepatic DNA *in vivo*. Mice were given a single oral dose of either DCA (3.9 mmol/kg) or TCA (3.1 mmol/kg). Controls received an equivalent volume of 1% aqueous Tween 80 vehicle. At the indicated time points, animals were killed and SSB determined. For simplicity, control animals were combined at a given time point (n=9). Each experimental point represents the mean of at least 6 animals ± SE. *Different from concurrent control, $p \leq 0.05$, by Student's t test.

Fig. 1. Time course for dichloroacetic acid (DCA) and trichloroacetic acid (TCA) induced single strand breaks in hepatic DNA in vivo. Mice were given a single oral dose of either DCA (3.9 mmol/kg) or TCA (3.1 mmol/kg). Controls received an equivalent volume of 1% aqueous Tween 80 vehicle. At the indicated time points, animals were killed and SSB determined. For simplicity, control animals were combined at a given time point (n=9). Each experimental point represents the mean of at least 6 animals ± SE. *Different from concurrent control, $p \leq 0.05$, by Student's t test.

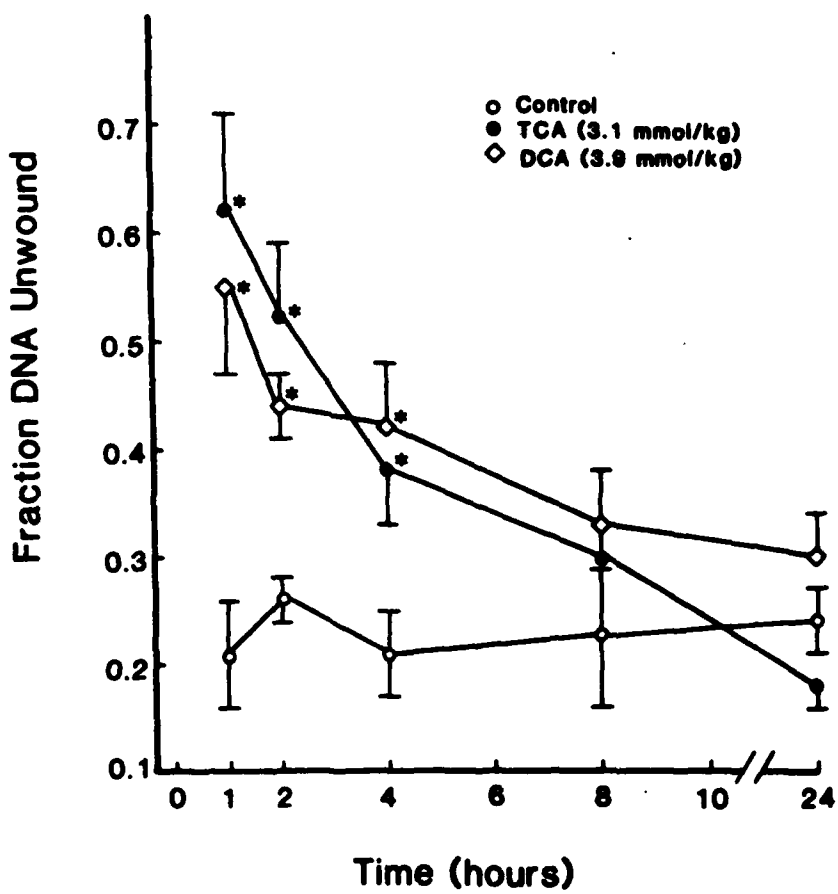

TABLE 3

EFFECTS OF REPEATED ORAL DOSES DICHLOROACETIC ACID (DCA) AND TRICHLOROACETIC ACID (TCA) ON HEPATIC PEROXISOME INDUCTION IN B6C3F1 MICE[a]

Treatment	Palmitoyl-CoA oxidation	Peroxisomes/unit area
Tween	0.63 ± 0.07[b]	9.8 ± 1.2[c]
DCA	1.03 ± 0.09[d]	25.4 ± 2.9[d]
TCA	1.70 ± 0.08[de]	23.6 ± 1.3[d]
Clofibrate	3.26 ± 0.05[def]	----------[g]

[a]Male mice were administered DCA (3.9 mmol/kg) or TCA by gavage for 10 consecutive days. Controls received an equivalent volume of 1% aqueous Tween 80 vehicle. Twenty four hours after the last dose, animals were killed and palmitoyl-CoA oxidation measured in liver homogenates.

[b]Mean value expressed umols/min/g liver ± SE of 6 animals.

[c]Liver tissue from 2 animals per treatment group was processed for electron microscopy. Morphometry was performed on 6 randomly selected photomicrographs from each animal from a total area of 10^4 um^2.

[d]Different from Tween control, $p < 0.05$, by ANOVA and Duncan's new multiple range test.

[f]Different from DCA, $p < 0.05$, by ANOVA and Duncan's multiple range test.

[e]Different from TCA, $p < 0.05$, by ANOVA and Duncan's multiple range test.

[g]Not determined

TABLE 2

EFFECTS OF REPEATED ORAL DOSES OF DICHLOROACETIC ACID (DCA) AND TRICHLOROACETIC ACID (TCA) ON BODY AND LIVER WEIGHTS OF B6C3F1 MICE[a]

Treatment	N	Liver wt[b] (g)	Liver wt/Body wt[b] Ratio (%)
Tween	6	1.3 ± 0.05	4.9 ± 0.14
DCA	6	2.1 ± 0.10[c]	7.5 ± 0.18[c]
TCA	6	1.7 ± 0.09[cd]	5.7 ± 0.14[cd]

[a]Male mice were administered DCA (3.9 mmol/kg) or TCA (3.1 mmol/kg) by gavage for 10 consecutive days. Controls received an equivalent volume of 1% aqueous Tween 80 vehicle.

[b]Mean ± SE of N animals

[c]Different from Tween control, $p < 0.05$, by ANOVA and Duncan's multiple range test.

[d]Different from DCA, $p < 0.05$, by ANOVA and Duncan's multiple range test.

TABLE 1
EFFECTS OF A SINGLE ORAL DOSE OF DICHLOROACETIC ACID (DCA) OR TRICHLOROACETIC ACID (TCA) ON HEPATIC PALMITOYL-CoA OXIDATION ACTIVITY OF MALE B6C3F1 MICE[a]

Sacrifice Time (hr)	N	Control	N	Treated
		DCA EXPERIMENT		
1	6	0.78 ± 0.11[bc]	6	0.68 ± 0.06[bc]
2	6	0.79 ± 0.25	6	0.79 ± 0.18
4	6	0.82 ± 0.22	6	0.50 ± 0.04
8	6	1.04 ± 0.28	6	0.94 ± 0.14
24	6	0.77 ± 0.21	6	0.67 ± 0.19
		TCA EXPERIMENT		
1	6	0.74 ± 0.12[bc]	6	0.72 ± 0.12[bc]
2	6	0.51 ± 0.07	6	0.44 ± 0.07
4	6	0.61 ± 0.05	6	0.51 ± 0.05
8	6	0.38 ± 0.07	6	0.44 ± 0.02
24	6	0.73 ± 0.08	6	0.72 ± 0.08

[a]Mice were given a single oral dose of 3.9 mmol/kg DCA or 3.1 mmol/kg TCA. Control animals received 1% aqueous Tween 80 vehicle. At the indicated time points animals were killed and palmitoyl-CoA oxidation determined in liver homogenates.

[b]Mean values expressed as umols/min/g liver ± SE of N animals.

[c]No differences found, $p \leq 0.05$, by Student's t test.