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Induction of Strand Breaks in DNA by Trichloroethylene and Metabolites in Rat and Mouse Liver *in Vivo*

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Induction of Strand Breaks in DNA by Trichloroethylene and Metabolites in Rat and Mouse Liver *in Vivo*. NELSON, M. A., AND BULL, R. J. (1988). *Toxicol. Appl. Pharmacol.* **94**, 45–54. The ability of trichloroethylene (TCE) and selected metabolites to induce single-strand breaks in hepatic DNA of male B6C3F1 mice and Sprague–Dawley rats *in vivo* was evaluated using an alkaline unwinding assay. Doses of TCE of 22–30 mmol/kg were required to produce strand breaks in DNA in rats, whereas a dose of 11.4 mmol/kg was sufficient to increase the rate of alkaline unwinding in mice. To assess the importance of TCE metabolism to this response, rats were subjected to pretreatments of ethanol, phenobarbital, TCE, or the appropriate vehicle for 4 days prior to challenge doses of TCE. Phenobarbital and TCE, but not ethanol pretreatments, reduced the dose of TCE required to produce significant increases in single-strand breaks. In another series of experiments, mice and rats were treated with metabolites of TCE. Trichloroacetate, dichloroacetate, and chloral hydrate induced strand breaks in hepatic DNA in a dose-dependent manner in both species. Strand breaks in DNA were observed at doses that produced no observable hepatotoxic effects as measured by serum aspartate aminotransferase and alanine aminotransferase levels. The slopes of the dose–response curves and the order of potency of these metabolites differed significantly between rats and mice, suggesting that different mechanisms of single-strand break induction may be involved in the two species. These data provide a potential explanation for the different sensitivity of mice and rats to the hepatocarcinogenic effects of TCE.

Trichloroethylene (TCE) is used extensively as an industrial solvent and as a household cleaning fluid (Waters *et al.*, 1977). It is commonly found in waste disposal sites and is a frequent contaminant of both surface and ground waters (Westrick *et al.*, 1982).

TCE produces an increase in the incidence of liver neoplasms in B6C3F1 mice. This mouse strain has a high background incidence of hepatic tumors, particularly in males. As a consequence, the mechanisms by which such tumors arise are a source of controversy. TCE also produces a low (4–8%) but significant increase in renal (but not hepatic) neoplasms in Fischer-344 rats (NCI, 1976; NTP, 1983).

A number of explanations have been offered for the difference in tumorigenic responses between the two species. First, the rate of TCE metabolism is much greater in the mouse than in the rat. This is particularly noticeable at high doses (>7.6 mmol/kg) where the proportion of unchanged TCE excreted markedly increases in the rat but less so in the mouse (Prout *et al.*, 1985). Second, TCE is also more effective in inducing liver peroxisomes in mice as compared to rats (Elcombe, 1985), a property that has been associated with hepatic carcinogenesis (Reddy and Lalwani, 1983). Trichloroacetic acid (TCA) is postulated as the metabolite of TCE responsible for peroxisome induction (El-

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combe, 1985; Goldsworthy and Popp, 1987). Third, the induction of overt liver damage by TCE has been reported to be greater in mice than in rats (Schumann *et al.*, 1980). This activity could result in regenerative hyperplasia that could stimulate the outgrowth of spontaneously initiated cells into neoplasms.

TCE covalently binds to DNA when incubated in the presence of hepatic microsomes or the S9 fraction *in vitro* (Banerjee and Van Duuren, 1978; Cunningham *et al.*, 1981; DiRenzo *et al.*, 1982). However, the level of binding of ^{14}C-labeled TCE to hepatic DNA *in vivo* is so low that binding to protein contaminants in the isolated DNA cannot be excluded (Parchman and Magee, 1982; Bergman, 1983). This low level of covalent interaction of TCE with DNA *in vivo* has lead to the suggestion that TCE is an epigenetic carcinogen (Stott *et al.*, 1982).

The induction of single-strand breakage (SSB) in DNA has been associated with both initiation and promotion events in chemically induced carcinogenesis (Walles and Erixson, 1984; Hartley *et al.*, 1985; Rushmore *et al.*, 1986). SSBs are known to arise from increases in intracellular calcium (Hartley *et al.*, 1985), from increased levels of cellular H_2O_2 (Cantoni *et al.*, 1986), through modification of poly(ADP)-ribosylation of DNA-associated proteins (Fujiwara, 1987), topoisomerase-II activity (Markovits *et al.*, 1987), and through the activity of a variety of endonucleases (Cleaver and Morgan, 1985). Therefore, measurement of SSB can detect effects upon DNA by mechanisms that do not require direct interaction of the compound or its metabolites with DNA. For these reasons, we compared the ability of TCE and its metabolites to induce SSB in hepatic DNA of mice and rats *in vivo*.

METHODS

Chemicals. TCE (epichlorohydrin free) was obtained from Fisher Scientific. Chloral hydrate (CH), dichloroacetate (DCA), trichloroethanol (TCEOH), and trichloroacetate (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO). Purity of administered chemicals was found to be 99+% using gas–liquid chromatography methods described by Prout *et al.* (1985).

Animals and exposures. Male Sprague–Dawley rats weighing 300–400 g (from Washington State University Laboratory Animal Resource Center) and male B6C3F1 mice (from Simonsen Labs, CA) weighing 25–30 g were used. The animals were housed in temperature-controlled rooms with a 12-hr. light/dark cycle. Food (Purina Laboratory Rodent Chow, Ralston-Purina Co., St. Louis, MO.) and water were provided *ad libitum*. Each experiment involved four groups containing three animals each, with one group always as a vehicle control. Animals were fasted overnight before dosing. The occasional animals that died or suffered respiratory distress due to intubation errors were excluded from the experiments.

In the first set of experiments, single oral doses of TCE or its metabolites were administered in a total volume of 1 ml of 1% aqueous of Tween 80/kg body wt. Control animals received an equivalent volume of vehicle alone. Four hours after treatment, the animals were killed and 10% liver suspensions prepared, and SSB was determined as described below. The timing of the animal deaths was based upon the time course of DCA accumulation in the liver following oral intubation (Evans, 1982).

In a second series of experiments, rats were subjected to daily treatments of ethanol (0 or 3.0 g/kg, po), phenobarbital (0 or 50 mg/kg, ip), TCE (0 or 3.8 or 11.4 mmol/kg, po), or the appropriate vehicle for 4 days prior to the administration of challenge doses of TCE ranging from 3.8 to 30 mmol/kg. Animals were killed 4 hr after administration of the TCE challenge dose and SSB in DNA was determined.

To be certain that alkaline unwinding was responsible for the different fractions of single-stranded DNA seen at a given time, the rate of unwinding was followed at intervals of 30, 60, and 120 min of incubation. The animals received a dose of 3.9 mmol/kg DCA and controls were given an appropriate volume of vehicle. At the indicated time intervals DNA unwinding was stopped and the amount converted to single-stranded DNA determined.

Alkaline unwinding assay. To measure SSB in DNA, the alkaline unwinding assay of Morris and Shertzer (1985) was used. This assay measures the rate of transition of double-stranded DNA to single-stranded DNA during alkaline denaturation. DNA strand breaks serve as points at which unwinding is initiated (Rydberg, 1975).

Termination of animals and preparation of liver samples were conducted in subdued, indirect incandescent lighting to avoid the introduction of strand breaks by uv irradiation. DNA was assayed using Setaro and Morley's (1976) modification of Kissane and Robins' (1958) diaminobenzoic acid flourometric assay. The fraction of DNA unwound was calculated as

$$\frac{(\text{Total DNA} - \text{DS DNA})_t}{(\text{Total DNA})} - \frac{(\text{Total DNA} - \text{DS DNA})_0}{(\text{Total DNA})},$$

where the subscript indicates the amount of double-stranded DNA at time "0" and "t" of incubation in alkaline solution.

Serum enzyme determinations. Animals, treated in a parallel fashion to those used in the experiments described above, were killed 24 hr after a challenge dose of TCE or its metabolites to determine the extent of hepatic injury, indicated by increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme levels. The animals were killed at 24 hr, rather than 4 hr, to provide full opportunity for expression of the enzymes's increase (Pappas, 1986).

Serum was separated by centrifugation from blood drawn from the inferior vena cava and assayed for serum ALT and AST levels using diagnostic kits purchased from Sigma. ALT and AST serum determinations are expressed in international units/liter. One international unit (U) of enzyme activity is defined as the amount of enzyme that produces 1 μmol of NAD/min (Sigma procedure No. 59-UV, 1987).

Statistical analysis. All values are expressed as means ± SE. To simplify construction of dose–response curves, data from the multiple control groups employed were combined and expressed as a single mean ± SE. However, when testing the various treatments for significance the statistical analyses were performed using the concurrent control. Log dose–response curves were constructed and regression analysis was used to determine the slopes. Student's t test was used to test whether differences in slopes were statistically significant (Goldstein, 1964). Analysis of variance was used to determine significant differences among treatment groups (a level of $p \leq 0.05$ being considered significant), with Duncan's multiple range test used for mean separation.

TABLE 1

THE INDUCTION OF STRAND BREAKS IN RAT AND MOUSE HEPATIC DNA BY TCE *IN VIVO*[a]

Dose	N	Species	Fraction DNA unwound
0	7	SD-rats	0.22 ± 0.03[b]
3.9	5	SD-rats	0.25 ± 0.06
11.4	5	SD-rats	0.22 ± 0.03
22.9	7	SD-rats	0.34 ± 0.05[c]
30.4	7	SD-rats	0.47 ± 0.05[c]
0	5	B6C3F1-mice	0.13 ± 0.04
0.76	4	B6C3F1-mice	0.20 ± 0.03
5.8	6	B6C3F1-mice	0.22 ± 0.03
11.4	6	B6C3F1-mice	0.29 ± 0.03[c]
22.9	6	B6C3F1-mice	0.32 ± 0.04[c]

[a] Male Sprague–Dawley rats and male B6C3F1 mice were given single oral doses of TCE suspended in 1% Tween 80 in distilled water. Control animals received an equal volume of vehicle alone.

[b] Values are means ± SE of N animals.

[c] Significantly different from vehicle control, $p \leq 0.05$, by ANOVA and Duncan's multiple range test.

RESULTS

The induction of strand breaks in rat and mouse hepatic DNA by TCE in vivo. For simplicity, increases in the rate of DNA unwinding under alkaline conditions will be referred to as increases in single-strand breaks (SSBs) in DNA. SSBs have been demonstrated to be the major factor involved in increased rates of alkaline unwinding (Rydberg, 1975).

TCE induces SSB in hepatic DNA of both rats and mice *in vivo* (Table 1). However, the characteristics of the dose–response curves vary between the two species. The slopes of the dose–response curves (change in fraction unwound/log dose) were significantly different ($p \leq 0.05$). Slopes of 0.22 ± 0.07 (95% confidence interval) and 0.08 ± 0.03 were found in the rat and mouse, respectively. In rats, single oral doses of 22.9 and 30 mmol/kg TCE were required to significantly increase SSB relative to controls in the rats. In mice, TCE produced increased rates of DNA unwinding following the administration of a dose of 11.4 mmol/kg (1.5 g/kg). Therefore, mice are more sensitive to the induction of SSB in hepatic DNA than rats. Doses of TCE above 22.9 mmol/kg (i.e., 30.0 mmol/kg) were lethal to mice, preventing valid experimentation in this range and accounting for the smaller maximum response observed relative to rats.

The effects of phenobarbital, ethanol, and TCE pretreatments on the induction of single-strand breaks in hepatic DNA of the rat by TCE. Phenobarbital pretreatment is known to increase the metabolism of TCE (Leibman and McAllister, 1967; Miller and Guenger-

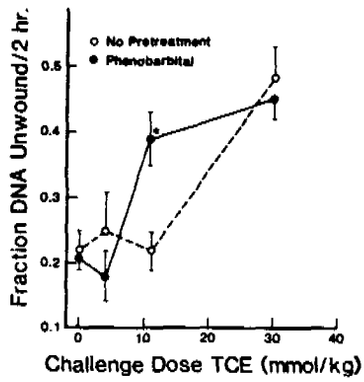

FIG. 1. Modification of the induction of SSB in rat hepatic DNA by TCE with phenobarbital pretreatment. Phenobarbital was administered ip at a dose of 50 mg/kg for 4 consecutive days. On the fifth day animals received the indicated doses of TCE and were killed 4 hr later. For simplicity, control animals receiving either phenobarbital or vehicle pretreatments were combined (n = 20, 17, respectively). Each experimental point represents the mean of at least five animals ± SE. *Different from vehicle-pretreated animals administered the same dose of TCE (11.4 mmol/kg) and concurrent controls, $p \leq 0.05$, by ANOVA and Duncan's multiple range test.

ich, 1983). To investigate the role that metabolism of TCE might play in the induction of SSB, rats were pretreated with 50 mg/kg phenobarbital (ip) for 4 days. On the fifth day, the challenge doses of TCE were administered. Under these conditions, the dose of TCE required to produce significant increases in SSB was lowered to 11.4 mmol/kg (1.5 g/kg) (Fig. 1).

Ethanol pretreatment was used as a second means of modifying the cytochrome *P*-450 activities of the liver. Ethanol induces *P*-450_{et}, a form much less effective in metabolizing TCE than *P*-450_{pb} (Koop *et al.*, 1982; Ryan *et al.*, 1984). No significant difference in the mean rate of alkaline unwinding was observed between ethanol-treated and control animals (Fig. 2).

The biotransformation of TCE to CH by rat liver microsomal preparations has been shown to increase when rats were pretreated with TCE (Leibman and McAllister, 1967). Therefore, we investigated the influence of prior doses of TCE on the induction of SSB. Previous experiments demonstrated that single doses of 3.8 (0.5 g/kg) and 11.4 mmol/kg (1.5 g/kg) TCE failed to produce SSB, thus, these doses were chosen for the pretreatment. In animals pretreated with either 3.8 or 11.4 mmol/kg TCE, a challenge dose of 11.4 mmol/kg of TCE increased the number of SSB in DNA (Fig. 3). If one assumes that the fourth dose of 11.4 mmol/kg TCE was as effective as the fifth, it would appear from the control data that the SSB produced by the fourth pretreatment dose were repaired by the time of measurement (i.e., 28 hr after the last pretreatment dose). This has been confirmed in subsequent experiments (data not shown).

The induction of strand breaks in rat hepatic DNA by metabolites of TCE. It was found that several of the stable metabolites of TCE were able to cause SSB in rat hepatic DNA at much lower doses than TCE (Fig. 4). DCA was clearly the most potent metabolite, with doses as low as 0.23 mmol/kg producing significant increases in the rate of alkaline un-

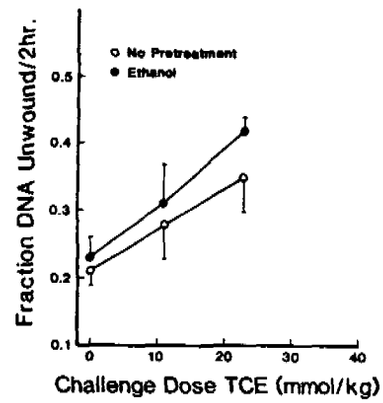

FIG. 2. The lack of effect of ethanol pretreatment on the induction of SSB in rat hepatic DNA. Ethanol was administered by gavage at a dose of 3 g/kg for 4 consecutive days. On the fifth day animals were given the indicated doses of TCE and killed 4 hr later. Each experimental point represents the mean of at least five animals ± SE. No differences were found between ethanol- and vehicle-pretreated animals, p = 0.05, by ANOVA and Duncan's multiple range test.

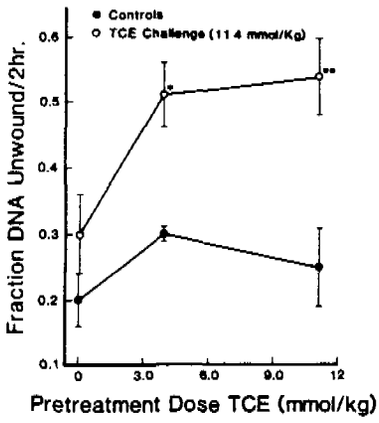

FIG. 3. The induction of SSB in rat hepatic DNA by CE and alteration of this response by TCE pretreatments. Pretreatment with doses of TCE were given by avage for 4 consecutive days. On the fifth day the animals were given a dose of TCE (11.4 mmol/kg) by gavage nd killed 4 hr later. For simplicity, Tween/Tween (n = 18) and TCE/Tween (n = 18) controls have been combined. Values for Tween/TCE and TCE/TCE treatments are the means from at least five animals ± SE. *Different from concurrent controls; Tween/Tween, Tween/TCE (11.4 mmol/kg), and TCE (3.8 mmol/kg)/Tween group, $p \leq 0.05$, by ANOVA and Duncan's multiple range test. **Different from concurrent controls: Tween/Tween, Tween/TCE (11.4 mmol/kg) and TCE (11.4 mmol/kg)/Tween group, $p \leq 0.05$, by ANOVA and Duncan's multiple range test.

winding. The lowest dose of TCA that produced significant SSB in hepatic DNA was 0.6 mmol/kg. The dose–response curve of chloral hydrate closely approximated that of TCA, but the lowest dose to cause significant increases in SSB was 1.8 mmol/kg. Trichloroethanol did not induce significant SSB at any dose tested.

The dose–response curves obtained from rats were uniformly steep. As mentioned earlier, a slope of 0.22 was observed for TCE. The corresponding slopes and 95% confidence intervals for DCA, TCA, and CH were 0.29 ± 0.02, 0.31 ± 0.06, and 0.37 ± 0.06, respectively.

The time course of DNA unwinding under alkaline conditions was consistent with the assumption that the proportion of single-stranded DNA after 2 hr incubation is associated with the induction of SSB (Fig. 5). Dichloroacetate treatment accelerated the alkaline unwinding of DNA, a pattern of response similar to that observed by Morris and Shertzer (1985) with *N*-nitrosodimethylamine.

The induction of strand breaks in mouse liver DNA by TCE metabolites. As with rats, a dose-dependent increase in SSB was observed with DCA, TCA, and CH (Fig. 6). Unlike the rat, the most potent metabolite in inducing SSB in mouse liver DNA was TCA. A dose of only 0.006 mmol/kg was required to produce a significant increase in the rate of alkaline unwinding. A similar dose of DCA also increased the rate of alkaline unwinding, but the effect was less than that observed with TCA. CH was considerably less potent than the other metabolites, requiring a dose of 0.6 mmol/kg before a significant increase in the rate of alkaline unwinding was observed.

The slopes of the log dose–response curves for DCA, TCA, and CH in the mouse were

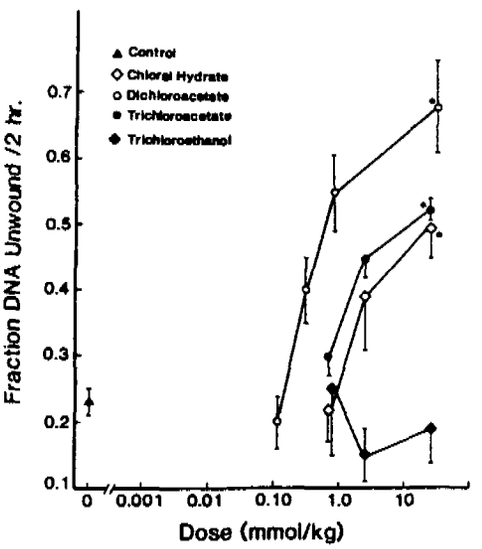

FIG. 4. The induction of SSB in rat hepatic DNA after exposure to DCA, TCA, CH, and TCEOH. The indicated doses of these metabolites were given by gavage and 4 hr later the animals were killed. For simplicity, control data were combined (n = 27). Each experimental point represents the mean from at least five animals ± SE. *Different from concurrent vehicle control, $p \leq 0.05$, by ANOVA and Duncan's multiple range test.

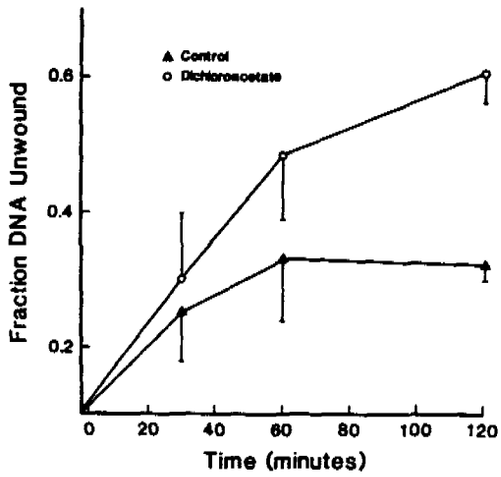

FIG. 5. The effect of DCA on the rate of alkaline unwinding of rat liver DNA. Values given are the means from at least four animals ± SE. Rates of unwinding between DCA and vehicle control are significantly different ($p \leq 0.05$) by regression analysis and Student's t test.

not as steep as they were for these compounds in the rat. In the mouse, the slopes and 95% confidence intervals for DCA, TCA, and CH were 0.11 ± 0.02, 0.13 ± 0.01, and 0.09 ± 0.04, respectively. These slopes are significantly different from the slopes in the rat ($p = 0.05$).

Serum AST and ALT determinations. Serum ALT and AST levels were monitored at 24 hr to determine whether hepatic injury occurred with the doses used in the various SSB experiments. In the rat, TCE increased the serum ALT and AST levels only after phenobarbital pretreatment and at the high dose of 30 mmol/kg at (Table 2). Neither ethanol nor TCE pretreatment resulted in any increases in these serum enzymes following challenge doses of TCE (Table 3). CH, DCA, and TCA also failed to increase serum ALT and AST levels (Table 4).

Similar evaluation of serum ALT and AST levels were conducted in mice using doses of TCE, CH, DCA, and TCA that produced a significantly increased rate of alkaline DNA unwinding. Serum samples, collected from mice 24 hr after the acute administration of these compounds, did not have increased ALT or AST levels relative to vehicle control treated animals (Table 5).

DISCUSSION

The present study demonstrates that TCE is capable of inducing SSB in hepatic DNA of both mice and rats *in vivo*. In both species, the induction of SSB by TCE required massive doses. The doses required for SSB induction in mice correspond to those used to induce liver tumors in chronic bioassays (NCI, 1976; NTP, 1983). The doses required to induce SSB in the hepatic DNA of rats were higher than the maximum tolerated dose in chronic studies (NCI, 1976). Walles (1986) also demonstrated that there was a linear increase in the level of SSB in mouse liver DNA following ip administration of TCE, reporting that the strand breaks are repaired within 24 hr.

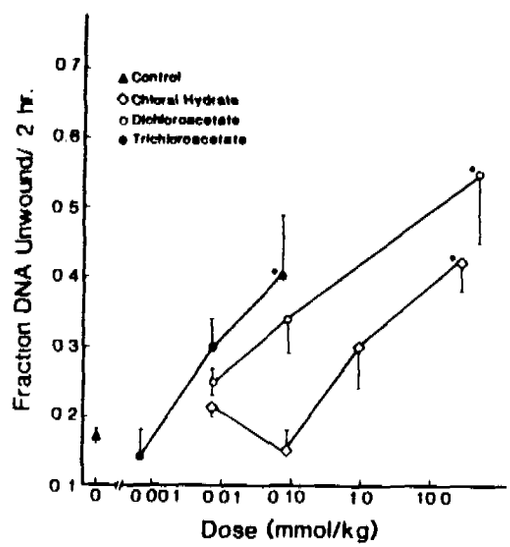

FIG. 6. The induction of SSB in mouse hepatic DNA after exposure to DCA, TCA, and CH. The indicated doses of these metabolites were given by gavage and 4 hr later the animals were killed. For simplicity, control data were combined ($n = 28$). Each experimental point represents the mean from at least five animals ± SE. *Different from concurrent vehicle control, $p \leq 0.05$, by ANOVA and Duncan's multiple range test.

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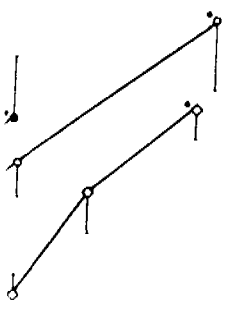

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TABLE 2

EFFECTS OF TCE ON ASPARTATE AMINOTRANSFERASE (AST) AND ALANINE AMINOTRANSFERASE (ALT) ACTIVITIES IN SERUM OF RATS FOLLOWING PRETREATMENT WITH PHENOBARBITAL OR TCE[a]

Pretreatment/ Challenge Dose	*N*	AST (U/liter)	ALT (U/liter)
Saline/Tween	5	30 ± 6[b]	15 ± 6[b]
Phenobarbital/Tween	5	33 ± 2	14 ± 1
Tween/Tween	4	22 ± 2	3 ± 1
Saline/TCE			
11.4 mmol/kg	5	36 ± 9	12 ± 2
30.5 mmol/kg	5	24 ± 3	9 ± 1
Phenobarbital/TCE			
11.4 mmol/kg	5	30 ± 4	13 ± 2
30.5 mmol/kg	4	136 ± 3[c]	47 ± 16[c]
TCE/TCE			
11.4 mmol/kg	5	27 ± 2	7 ± 1

[a] Male Sprague–Dawley rats were given ip injections of 50 mg/kg (in saline) or 11.4 mmol/kg TCE (in 1% aqueous Tween 80)/day for 4 days. Control animals received an equivalent volume of vehicle alone. Challenge doses of TCE were administered 24 hr after the last pretreatment dose. AST and ALT levels were determined 24 hr after the challenge dose of TCE.

[b] Values are expressed as means ± SE of *N* animals.

[c] Significantly different from Saline/Tween and Phenobarbital/Tween groups, $p \leq 0.05$, by ANOVA and Duncan's multiple range test.

This is consistent with observations made in the rat in the present study.

The increased number of SSB produced by TCE following phenobarbital pretreatment suggests that metabolic activation is necessary for DNA damage to occur. Metabolism of TCE has been shown to be saturated at doses of this magnitude in the rat (Prout *et al.*, 1985). Apparently, phenobarbital pretreatment increased the enzymatic activity of the rat mixed-function oxidase system such that a comparatively larger fraction of the 11.4 mmol/kg dose was metabolized. Metabolism studies from our laboratory show that peak concentrations of TCE in blood only slightly increase with doses of TCE in this range. This limits the concentrations of metabolites measured in blood (Larson and Bull, 1988), which could account for the failure of higher doses of TCE (30 mmol/kg) to further increase the response in animals pretreated with phenobarbital.

Unlike pretreatments with phenobarbital or TCE, ethanol did not increase the ability of TCE to cause SSB in DNA. The relative ineffectiveness of ethanol is probably related to the fact that it induces different isozymes of cytochrome *P*-450 than does phenobarbital. Ethanol administration is known to induce a unique cytochrome *P*-450 isozyme with specific catalytic properties (Ko *et al.*, 1987; Ryan *et al.*, 1984; Koop *et al.*, 1982). Miller and Guengerich (1983) have shown that cytochrome $P\text{-}450_{pb\text{-}b}$ is particularly efficient in converting TCE to CH, which in turn is converted to TCA and TCEOH. Our data suggest that TCE pretreatment also activates this pathway.

Several studies have reported that covalent binding of TCE to exogenous DNA takes place in the presence of liver microsomes

TABLE 3

EFFECTS OF TCE ON ASPARTATE AMINOTRANSFERASE (AST) AND ALANINE AMINOTRANSFERASE (ALT) ACTIVITIES IN SERUM OF RATS FOLLOWING PRETREATMENT WITH ETHANOL[a]

Pretreatment/ challenge Dose	*N*	AST (U/liter)	ALT (U/liter)
Water/Tween	5	30 ± 2[b,c]	8 ± 1[b,c]
EtOH/Tween	5	26 ± 6	7 ± 1
EtOH/TCE			
11.4 mmol/kg	5	23 ± 2	8 ± 1
22.7 mmol/kg	5	22 ± 1	9 ± 1
Water/TCE			
11.4 mmol/kg	5	24 ± 2	10 ± 1
22.7 mmol/kg	5	22 ± 1	9 ± 1

[a] Male Sprague–Dawley rats received oral doses of ethanol 3.0 g/kg (in water)/day for 4 days. Challenge doses of TCE were administered in a 1% Tween 80 in distilled water 24 hr after the last pretreatment dose. Control animals received an equivalent volume of vehicle alone. AST and ALT levels were determined 24 hr after the challenge dose of TCE.

[b] Values expressed as means ± SE of *N* animals.

[c] No significant differences found, $p \leq 0.05$, by ANOVA and Duncan's multiple range test.

TABLE 4

EFFECTS OF DCA, TCA, TCEOH, AND CH ON ASPARTATE AMINOTRANSFERASE (AST) AND ALANINE AMINOTRANSFERASE (ALT) ACTIVITIES IN THE SERUM OF RATS[a]

Challenge dose (mmol/kg)	N	AST (U/liter)	ALT (U/liter)
Tween	5	16 ± 2[b,c]	5 ± 1[b,c]
DCA (3.9)	4	16 ± 1	4 ± 1
TCA (3.1)	4	24 ± 7	6 ± 2
TCEOH (3.3)	4	18 ± 2	3 ± 1
CH (3.4)	4	17 ± 0	2 ± 1

[a] Male Sprague–Dawley rats were administered agents by gavage in 1% Tween 80 in distilled water solution. Control animals received an equivalent volume of vehicle alone. AST and ALT levels were determined 24 hr after the agents were administered.

[b] Values expressed as means ± SE of N animals.

[c] No significant differences found, $p \leq 0.05$, by ANOVA and Duncan's multiple range test.

(Cunningham *et al.*, 1981; DiRenzo *et al.*, 1982; Bergman, 1983). The low level of DNA binding *in vivo* suggests that the DNA binding observed in the *in vitro* studies is not directly related to the induction of SSB reported here. It is presumed that such binding would involve the epoxide or acid chloride intermediates of TCE metabolism, whereas the present data indicate that the more stable metabolites can produce the effect at low doses. The mechanism by which SSBs are induced by these agents requires further study.

The most interesting result of this study was the substantial differences in sensitivity of mice and rats to TCE and its metabolites. Significantly different responses were observed at much lower doses in mice than in rats. This was due to the large differences in the slopes of the dose–response curves for the two species.

The differences in slopes of the dose–response curves constructed for the two species also have mechanistic implications. The steep dose–response curves for the rat are consistent with a process requiring more steps than would be involved in producing the shallower slopes observed for mice (Goldstein *et al.*, 1974). We do not believe this difference can be explained on pharmacokinetic grounds, since metabolism studies show blood concentrations of the metabolites are limited at high doses of TCE (Larson and Bull, 1988). One explanation that may be responsible for the differing slopes could be inefficient mechanism(s) for recognition and repair of strand breaks. However, there have been no studies that identify such aberrations in the B6C3F1 mouse. While the mechanisms responsible for the response are not clear at present, the observation is interesting, considering the difference in the sensitivity of these species to the hepatocarcinogenic effects of TCE.

While there was a tendency for mice to be more sensitive to the metabolites of TCE tested, the dose of TCA required to increase the rate of alkaline unwinding in mice was 1/100 of that required in the rat. These results parallel the sensitivity of rats and mice to the induction of hepatic peroxisomes by TCA (Elcombe, 1985; Goldsworthy and Popp, 1987).

TABLE 5

EFFECTS OF CH, DCA, TCA, AND TCE ON ASPARTATE AMINOTRANSFERASE (AST) AND ALANINE AMINOTRANSFERASE (ALT) ACTIVITIES IN THE SERUM OF MICE[a]

Challenge dose (mmol/kg)	N	AST (U/liter)	ALT (U/liter)
Tween	5	23 ± 6[b,c]	16 ± 4[b,c]
CH (3.4)	5	18 ± 1	16 ± 4
DCA (3.9)	4	20 ± 3	15 ± 2
TCA (3.1)	5	24 ± 2	11 ± 1
TCE (11.4)	5	24 ± 3	24 ± 6

[a] Male B6C3F1 mice were administered agents by gavage in 1% Tween 80 in distilled water. Control animals were given equivalent volumes of vehicle. AST and ALT levels were determined 24 hr after the agents were administered.

[b] Values expressed as means ± SE of N animals.

[c] No significant differences found, $p \leq 0.05$, by ANOVA and Duncan's multiple range test.

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AST (U/liter)	ALT (U/liter)
$\pm 6^{b,c}$	$16 \pm 4^{b,c}$
± 1	16 ± 4
± 3	15 ± 2
± 2	11 ± 1
± 3	24 ± 6

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Preliminary data (Nelson and Bull, 1988) dicate that the induction of SSB with these emicals in mice occurs long before there is y evidence of peroxisome proliferation. ierefore, the increased β oxidation of lipids peroxisomes could not account for the B observed in the present study. Neverthe- s, it is interesting to see that these two re- onses parallel one another in their respon- eness to TCA, suggesting a less obvious ıkage between the two responses.

In summary, we have demonstrated that CE is capable of inducing SSB in hepatic NA of mice and rats *in vivo*. Pretreatment ıth phenobarbital or low doses of TCE, but ot with ethanol, decreases the dose of TCE equired to induce SSB. This suggests the in- olvement of a metabolite(s) of TCE, pro- luced by pathways inducible by phenobarbi- al or TCE. The induction of SSB occurred at much lower doses of the stable metabolites of TCE (DCA, TCA, and CH), suggesting that one or more of these compounds are respon- sible. Variation in the relative potency of TCA in mice and rats parallels the species specificity of the hepatocarcinogenic effects of TCE. More importantly, the dose–re- sponse relationships observed suggest differ- ences in the mechanisms by which strand breaks are induced in the hepatic DNA of the two species. The induction of SSB appears to be independent of the hepatotoxicity of these chemicals, since increased damage occurred in the absence of significant elevations in se- rum AST and ALT levels.

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