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TRICHLOROETHYLENE: ITS INTERACTION WITH HEPATIC MICROSOMAL CYTOCHROME P-450 *IN VITRO**

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Abstract—The effects of inducing agents on the binding and metabolism of trichloroethylene by hepatic microsomal cytochrome P-450 are reported. The binding constant (K_s) for the interaction of trichloroethylene with hepatic microsomal cytochrome P-450 was not altered by induction with phenobarbital, 3-methylcholanthrene or spironolactone, while the maximum extent of binding (ΔA_{max}) was increased only following phenobarbital induction. The K_s values (*ca.* 1 mM) obtained for the binding of trichloroethylene to cytochrome P-450 were similar whether the enzyme was partially purified or an integral part of hepatic microsomes. The Michaelis constant (K_m) for the production of chloral hydrate from trichloroethylene by hepatic microsomal cytochrome P-450 was not altered by induction of different forms of cytochrome P-450. V_{max} for the production of chloral hydrate and the rate of hepatic microsomal NADPH oxidation in the presence of excess trichloroethylene were increased by phenobarbital induction, but not by spironolactone or 3-methylcholanthrene induction. The artificial electron donors $NaClO_2$ and H_2O_2, but not $NaIO_4$, supported the metabolism of trichloroethylene by partially purified cytochrome P-450 from phenobarbital-induced rat liver microsomes. Incubation of hepatic microsomes with NADPH and trichloroethylene resulted in decreased levels of cytochrome P-450 and heme, but did not alter the levels of NADPH-cytochrome *c* reductase, cytochrome b_5 or glucose-6-phosphatase. The degradation of the heme moiety of cytochrome P-450 by trichloroethylene was not supported by NADH and was not inhibited by reduced glutathione (GSH). The inhibitors of cytochrome P-450—SKF 525-A, metyrapone and CO—inhibited the binding and metabolism of trichloroethylene and the trichloroethylene-mediated degradation of cytochrome P-450. It is concluded that the form of cytochrome P-450 which is induced by phenobarbital, binds and metabolizes trichloroethylene, whereas other forms of the enzyme, such as cytochrome P-448, do not. Trichloroethylene appears to be activated by the phenobarbital-induced form of cytochrome P-450 to a reactive species which can then chemically alter the heme moiety of cytochrome P-450.

Trichloroethylene is extensively used in industry, primarily as a dry cleaning solvent and a metal degreasing agent. It is also in use as a general anesthetic agent and analgesic. In addition, trichloroethylene has been utilized to decaffeinate tea and coffee [1, 2].

Exposure to trichloroethylene has been reported to result in a variety of disorders, including central nervous system depression, hepatotoxicity and nephrotoxicity [1, 2]. Large doses of trichloroethylene are fatal to laboratory rodents (LD_{50} = 2 ml/kg) [3] and have occasionally proved fatal to humans [1, 2, 4]. In addition, trichloroethylene becomes weakly mutagenic in the presence of liver activating enzymes and is carcinogenic in mice, but not in rats [1 and 5, but see 6]. The deleterious effects of trichloroethylene may result from reactive metabolites of trichloroethylene, since there is a direct correlation between the extent of metabolism of trichloroethylene and its hepatotoxicity [7–9]. The reactive metabolite presumed to be responsible for these effects is trichloroethylene oxide [7, 9, 10]. Interestingly, the existence of an epoxide metabolite of trichloroethylene was proposed by Powell [11] as early as 1945 to explain the observation that urinary trichloroacetate was produced from trichloroethylene *in vivo*.

The enzyme system which catalyzes the primary oxidation of trichloroethylene to its first readily isolable metabolite, chloral hydrate, appears to be the hepatic microsomal cytochrome P-450 mixed-function oxidase system [9, 12, 13]. This reaction is presumed to proceed via an initial enzymic conversion of trichloroethylene to trichloroethylene oxide and a subsequent non-enzymic internal rearrangement of the epoxide to chloral hydrate [7, 12, 14]. The epoxide can undergo alternative reactions with cellular macromolecules, which might lead to toxic effects, or with small molecules, such as glutathione or water, in a detoxification reaction [7, 9].

We have attempted to clarify the roles of different forms of hepatic microsomal cytochrome P-450 in the metabolism and metabolic activation of trichloroethylene. This investigation represents a por-

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tion of our ongoing studies of the hepatic metabolism and toxicity of chlorinated ethylenes.

EXPERIMENTAL

Materials. Trichloroethylene, 2,2,2-trichloroethanol and trichloroacetic acid were reagent grade from Merck, Darmstadt, Germany. Chloral hydrate was from B.D.H. Ltd., Poole, England. SKF 525-A (β-diethylaminoethyl-2,2-diphenylvalerate) and metyrapone (2-methyl-1,2-bis-(3-pyridyl)-1-propane) were gifts from Smith, Kline & French Ltd., Isando, Transvaal, S.A., and from Ciba–Geigy Ltd., Basle, Switzerland, respectively. Partially purified cytochrome P-450 was isolated from the hepatic microsomes of phenobarbital-induced rats, as described by van der Hoeven and Coon [15].

Treatment of animals. Male Long–Evans rats (180–200 g) were used for all experiments. Animals were induced with 3-methylcholanthrene and phenobarbital, as described earlier [16], and with spironolactone, as described by Menard *et al.* [17].

Preparation of hepatic microsomes. Hepatic microsomes were isolated by differential ultracentrifugation [18] and were suspended at a concentration of 2 mg protein/ml of 0.02 M Tris–HCl (pH 7.4) for all experiments.

Spectral assays. The binding of trichloroethylene to partially purified cytochrome P-450 (1.5–1.8 μM) was monitored in 0.02 M Tris–HCl (pH 7.4) or in 0.1 M Tris-acetate–KCl (pH 7.4) containing 20% glycerol. Difference spectra with partially purified cytochrome P-450 and with hepatic microsomal cytochrome P-450 were measured at 25°, as described elsewhere [19]. The rates of NADPH oxidation by hepatic microsomes were measured at 30° in the presence of 7.5 mM trichloroethylene, as described earlier [19]. Incubations for assaying the metabolism of trichloroethylene or the effect of trichloroethylene on hepatic microsomal components were performed at 30° with shaking in a Gallenkampstat shaking water bath, as described earlier [41].

Cytochrome P-450 concentrations were determined from measurements of the difference spectrum of CO–ferrocytochrome P-450 vs ferrocytochrome P-450, according to the method of Omura and Sato [20]. An extinction coefficient of 91 cm^{-1} mM^{-1} for the difference in absorbance between 450 and 490 nm was utilized [20]. The levels of cytochrome b_5 were measured spectrally as the difference in absorbance at 424 and 409 nm of ferrocytochrome b_5 vs ferricytochrome b_5 [20]. The activity of NADPH-cytochrome *c* reductase was determined by monitoring the increase in absorbance of ferrocytochrome *c* at 550 nm ($\varepsilon = 21.1\ cm^{-1}\ mM^{-1}$), according to the method of Omura and Takesue [21]. Microsomal heme was determined spectrally as the reduced pyridine hemochrome ($\varepsilon_{557\ nm-575\ nm} = 32.4\ cm^{-1}\ mM^{-1}$), according to Omura and Sato [20]. Glucose-6-phosphatase was assayed by the method of Nordlie and Arion [22]. Inorganic phosphate was determined by the method of King [23].

Chloral hydrate, trichloroacetic acid and 2,2,2-trichloroethanol were routinely assayed by the modified Fujiwara assay of Leibman and Hindman [24]. Chloral hydrate was identified and measured quantitively by gas–liquid chromatography on a Packard model 428 gas–liquid chromatograph with electron capture detector, using a 3 m × 3 mm column of 10% carbowax 20 M on chromosorb W (80/100 mesh). Column, injector and detector temperatures were 160°, 220° and 230°, respectively. Peak areas were calculated by a Pye Unicam DP88 computing integrator. Standard solutions of halogenated compounds were routinely prepared in the presence of hepatic microsomes.

The ability of artificial electron donors to support the metabolism of trichloroethylene by partially purified cytochrome P-450 was assessed in incubation mixtures (3 ml) containing (where indicated) trichloroethylene (7.5 mM), partially purified cytochrome P-450 from phenobarbital-induced rat liver microsomes (*ca.* 2 μM), $NaClO_2$ (5 mM), H_2O_2 (10 mM) and $NaIO_4$ (7.5 mM) in 0.02 M Tris–HCl, pH 7.4. Incubations were carried out at 30° with shaking. Reaction mixtures were assayed at time zero and at 10 min by method A of Leibman and Hindman [24].

Calculations and statistical analysis. Binding (K_s) and Michaelis (K_m) constants, maximal extents of binding (ΔA_{max}) and maximal rates of metabolism (V_{max}) were calculated from computerized Hanes and Eadie–Hofstee plots using linear regression analysis. Student's *t*-test was utilized to calculate significant differences between means. A significant difference was taken as $P < 0.01$ with $P < 0.05$ being probably significant. All reported values are means ± standard deviations.

RESULTS

Binding of trichloroethylene to hepatic microsomal cytochrome P-450 in vitro. Trichloroethylene bound to hepatic cytochrome P-450 in uninduced and induced microsomes, resulting in the production of a type I difference spectrum (λ_{max} = 386 nm; λ_{min} = 416 nm), confirming an earlier report by Pelkonen and Vainio [25]. Trichloroethylene also bound to partially purified cytochrome P-450 from phenobarbital-induced rats with the production of a type I difference spectrum (λ_{max} = 388 nm; λ_{min} = 419 nm).

The effects of inducing agents for different forms of cytochrome P-450 on the binding constants (K_s) and the maximum extents of binding (ΔA_{max}) for the binding of trichloroethylene to hepatic microsomal cytochrome P-450 are shown in Table 1. The K_s values for the binding of trichloroethylene to hepatic microsomal cytochrome P-450 were not altered by prior induction with 3-methylcholanthrene, spironolactone or phenobarbital. The K_s values calculated for the binding of trichloroethylene to phenobarbital-induced partially purified cytochrome P-450 were 1.5 ± 0.1 and 0.82 ± 0.11 mM for the enzyme suspended in 0.02 M Tris–HCl (pH 7.4) and for the glycerol solubilized enzyme respectively. The ΔA_{max} values for the binding of trichloroethylene to hepatic microsomal cytochrome P-450 were increased following phenobarbital induction ($P < 0.01$), but not following 3-methylcholanthrene or spironolactone induction ($P > 0.1$).

Table 1. Effects of induction on the binding of trichloroethylene with hepatic microsomal cytochrome P-450*

Induction	Cytochrome P-450 (nmoles/mg microsomal protein)	K_s (mM)	A_{max} (A_{386}–A_{416})
None	0.93 ± 0.10	0.69 ± 0.39	0.034 ± 0.008
3-Methylcholanthrene	1.40 ± 0.04	0.86 ± 0.36	0.039 ± 0.007
Spironolactone	0.78 ± 0.12	0.54 ± 0.28	0.032 ± 0.012
Phenobarbital	2.54 ± 0.69	0.63 ± 0.14	0.180 ± 0.043†

* Values reported are means ± S.D. for experiments performed in triplicate with three or more different preparations of hepatic microsomes. Experimental conditions are described in the text.

† Differs from uninduced microsomes, $P < 0.01$.

Metabolism of trichloroethylene by hepatic microsomal cytochrome P-450. In the absence of an NADPH-generating system or trichloroethylene, measurable amounts of Fujiwara positive material were not formed in hepatic microsomal incubation mixtures. In the presence of trichloroethylene, an NADPH-generating system, EDTA and hepatic microsomes, the production of chlorinated metabolites, as assayed by the modified Fujiwara method [24], was linear for 5 min for microsomes from uninduced or induced rats. This incubation time was utilized in all further experiments in which the metabolism of trichloroethylene was monitored by this method. Measurable amounts of trichloroacetic acid or of 2,2,2-trichloroethanol were not produced from trichloroethylene in any incubation mixtures.

Chloral hydrate was identified as the sole volatile metabolite of trichloroethylene by gas–liquid chromatography (see Experimental). The amounts of chloral hydrate produced by microsomes from phenobarbital-induced rats (6.2 ± 0.3 nmoles chloral hydrate/min/mg of microsomal protein) are identical to the amounts of chloral hydrate produced from trichloroethylene, as measured by the modified Fujiwara assay (Table 2). The levels of chloral hydrate were assayed routinely using the latter method.

The K_m values for the conversion of trichloroethylene to chloral hydrate were unaffected by the induction of different forms of cytochrome P-450. For each type of induction, the K_m value was within experimental error of the corresponding K_s value ($P > 0.05$) (Tables 1 and 2). The V_{max} value for trichloroethylene was unaffected by induction with spironolactone or 3-methylcholanthrene ($P > 0.1$) but was increased following phenobarbital induction (Table 2).

NADH (1 mM) was found to support the metabolism of trichloroethylene by phenobarbital-induced hepatic microsomes to the extent of 0.39 nmole chloral hydrate formed/mg of microsomal protein/min, which represents approximately 13 per cent of the rate of production of chloral hydrate seen in the presence of trichloroethylene and an NADPH-generating system.

The rate of NADPH oxidation of hepatic microsomes was stimulated by trichloroethylene, and presumably to some extent reflects the oxidation of NADPH which accompanies the metabolism of trichloroethylene by hepatic microsomal cytochrome P-450. Induction with phenobarbital resulted in significantly increased rates of trichloroethylene-stimulated NADPH oxidation, while induction with spironolactone and 3-methylcholanthrene did not ($P > 0.1$) (Table 2). For each type of induction, the rate of NADPH oxidation in the presence of trichloroethylene did not differ significantly from the rate of production of chloral hydrate ($P > 0.05$ for phenobarbital induction, $P > 0.1$ for all other types of induction) (Table 2). Neither NADPH oxidation nor chloral hydrate production was corrected for $CO:O_2$ (80:20) background rate, inasmuch as CO affected the two processes to different extents (see Table 4).

Metabolism of trichloroethylene by partially purified cytochrome P-450. Incubation of trichloroethylene for 10 min, with either H_2O_2, $NaIO_4$, $NaClO_2$ or cytochrome P-450, did not result in an appreciable increase of absorbance (0.00 ± 0.04) in the modified Fujiwara assay, relative to zero time samples. Following incubation of trichloroethylene, cytochrome P-450 and $NaClO_2$ or H_2O_2, but not $NaIO_4$, appreciable conversion of trichloroethylene to a Fujiwara positive product was observed. Absorbances at 530 nm of 0.12, 0.18 and −0.02, respectively, were obtained for these artificial electron donors, the values of which are equivalent to 1.8, 2.8 and 0 nmoles chloral hydrate/10 min/nmole of cytochrome P-450.

Effect of trichloroethylene on the levels of hepatic microsomal enzymes. The effects of trichloroethylene on the levels of hepatic microsomal cytochrome P-450, heme, cytochrome b_5 and NADPH–cytochrome *c* reductase are shown in Table 3. Incubation of trichloroethylene, hepatic microsomes, EDTA and an NADPH-generating system resulted in decreases in the levels of cytochrome P-450 and heme for each type of induction. No appreciable degradation of cytochrome P-450 or heme was observed in the absence of trichloroethylene or an NADPH-generating system. The losses of cytochrome P-450 were approximately equivalent to the losses of heme for each type of induction. Reduced glutathione plus liver post-microsomal supernatant fraction—which contains a mixture of glutathione transferases—did not inhibit the degradation of cytochrome P-450 in uninduced or phenobarbital-induced microsomes. NADH did not measurably support the degradation of cytochrome P-450 in phenobarbital-induced microsomes (Table 3).

The levels of cytochrome b_5 and NADPH-cyto-

Table 2. Effects of induction on the metabolism of trichloroethylene by hepatic microsomal cytochrome P-450*

Induction	Cytochrome P-450 (nmoles/ mg microsomal protein)	K_m (mM)	V_{max} (nmoles chloral hydrate/min/mg microsomal protein)	NADPH oxidation† (nmoles/min/mg microsomal protein)
None	0.98 ± 0.10	0.62 ± 0.45	1.55 ± 0.65	1.70 ± 0.59
3-Methylcholanthrene	1.40 ± 0.04	0.45 ± 0.02	2.00 ± 0.57	2.25 ± 0.10
Spironolactone	0.78 ± 0.12	0.71 ± 0.02	2.00 ± 0.20	1.73 ± 0.45
Phenobarbital	2.54 ± 0.69	0.48 ± 0.30	5.80 ± 0.70‡	4.57 ± 1.28‡

* Values reported are means ± S.D. for experiments performed in triplicate with three or more different preparations of hepatic microsomes. Experimental conditions are as described in the text.

† In the presence of 7.5 mM trichloroethylene.

‡ Differs from uninduced microsomes, $P < 0.01$.

chrome *c* reductase were not altered following incubation of uninduced or induced microsomes in the presence of trichloroethylene and an NADPH-generating system (Table 3). The activity of glucose-6-phosphatase in phenobarbital-induced microsomes was not significantly affected by incubation of hepatic microsomes (2 mg protein/ml), an isocitrate dehydrogenase NADPH-generating system [26] and trichloroethylene (7.5 mM). The activity of glucose-6-phosphatase was 0.15 ± 0.049 and 0.13 ± 0.055 μmoles P_i/min/mg of protein at time zero and after 15 min of incubation, whether or not trichloroethylene was present in the incubation mixture.

Effects of inhibitors on the interation of trichloroethlene with hepatic microsomal cytochrome P-450. The effects of inhibitors of cytochrome P-450 on the interaction of trichloroethylene with this group of enzymes are shown in Table 4. Metyrapone and SKF 525-A were equivalent as inhibitors of the binding and metabolism of trichloroethylene, and they inhibited each of these processes to a similar extent. Metyrapone and SKF 525-A inhibited the degradation of cytochrome P-450 to a greater extent than the binding and metabolism of trichloroethylene. In contrast, CO inhibited the metabolism of trichloroethylene and the degradation of cytochrome P-450 to about the same extent.

DISCUSSION

That the hepatic mixed-function oxidase of the post-mitochondrial supernatant fraction catalyzes the metabolism of trichloroethylene to chloral hydrate was first demonstrated by Leibman *et al.* [12, 13, 27]. Evidence later accrued which suggested that the enzyme system involved in this reaction is the cytochrome P-450 mixed-function oxidase system [8, 28]. The ability of hepatic microsomal cytochrome P-450 to metabolize trichloroethylene is supported further by results presented herein. The ability of inhibitors of cytochrome P-450—metyrapone, CO and SKF 525-A [29]—to inhibit the binding and metabolism of trichloroethylene (Table 4), and the ability of phenobarbital, an inducing agent for cytochrome P-450, to stimulate these processes (Tables 1 and 2) support this proposal. Conclusive evidence for the ability of cytochrome P-450 to bind and metabolize trichloroethylene is provided by the demonstration that trichloroethylene binds to the substrate binding site of partially purified cytochrome P-450 and that the artificial active oxygen donors $NaClO_2$ and H_2O_2 support the metabolism of trichloroethylene by partially purified cytochrome P-450 (see Results).

It appears that the form of cytochrome P-450 induced by phenobarbital is the only form of the enzyme which binds and metabolizes trichloroethylene. This conclusion is drawn from the observation that the binding constants (K_s) and Michaelis constants (K_m) for the interaction of trichloroethylene with hepatic microsomal cytochrome P-450 are not altered by induction of different forms of cytochrome P-450, while the maximum extents of binding and metabolism (ΔA_{max}, V_{max}) of trichloroethylene are

Table 3. Effects of trichloroethylene on the levels of hepatic microsomal enzymes *in vitro**

Induction	TCE	Other additions	Cytochrome P-450†,‡ (loss/initial level)	Heme†,§ (loss/initial level)	Loss heme†,‖ / Loss cyt P-450	Cytochrome b_5† (30 min/0 min)	NADPH-cytochrome *c* reductase¶ (30 min/0 min)
None	−	—	0.05/0.92	0.13/1.66		0.44 ± 0.04/0.40 ± 0.06	0.056 ± 0.004/0.058 ± 0.005
	+	—	0.21/0.88	0.29/1.58	0.19/0.16	0.42 ± 0.05/0.37 ± 0.03	0.058 ± 0.006/0.057 ± 0.005
	−	GSH/SN	0.09/0.99				
	+	GSH/SN	0.20/0.92				
3-MC	−	—	0.00/1.35	0.08/2.16		0.57 ± 0.05/0.54 ± 0.07	0.044 ± 0.003/0.045 ± 0.002
	+	—	0.21/1.32	0.29/2.10	0.25/0.21	0.53 ± 0.05/0.50 ± 0.06	0.046 ± 0.005/0.043 ± 0.004
	+	—**	0.03/1.33				
SP	−	—	0.05/0.67	0.03/1.57		0.36 ± 0.01/0.33 ± 0.01	0.076 ± 0.009/0.075 ± 0.003
	+	—	0.18/0.59	0.24/1.50	0.21/0.13	0.39 ± 0.02/0.32 ± 0.01	0.076 ± 0.004/0.076 ± 0.005
PB	−	—	0.13/1.87	0.23/2.35		0.54 ± 0.02/0.47 ± 0.03	0.085 ± 0.016/0.083 ± 0.012
	+	—	0.38/1.76	0.62/2.31	0.39/0.25	0.53 ± 0.05/0.42 ± 0.04	0.078 ± 0.017/0.080 ± 0.014
	+	—**	0.00/1.77				
	+	NADH**	0.05/1.64				
	−	GSH/SN	0.01/1.90				
	+	GSH/SN	0.41/2.03				

* Values reported are means ± S.D. for experiments performed in triplicate with at least three different preparations of hepatic microsomes. Incubation mixtures (3 ml) contained: hepatic microsomes (2 mg protein/ml), an NADPH-generating system [35], 0.2 mM EDTA and, where indicated, 7.5 mM trichloroethylene, 1 mM glutathione plus 10 μl of rat liver post-microsomal supernatant fraction, or 0.6 mM NADH. Losses are reported for samples incubated for 30 min at 30° with shaking relative to unincubated samples. Abbreviations used are: TCE. trichloroethylene; 3-MC, 3-methylcholanthrene; SP, spironolactone; PB, phenobarbital; GSH, reduced glutathione; SN, rat liver post-microsomal supernatant fraction; and cyt, cytochrome.

† In nmoles/mg of microsomal protein.

‡ S.D. ± 0.10.

§ S.D. ± 0.21.

‖ Corrected for losses of heme and cytochrome P-450 following incubation of hepatic microsomes, NADPH-generating system and EDTA.

¶ In units/mg of microsomal protein, S.D. ± 0.008.

** NADPH-generating system was omitted from incubation mixture.

Table 4. Effects of inhibitors on the interaction of trichloroethylene with hepatic microsomal cytochrome P-450*

Additions	Binding (%)	NADPH oxidation (%)	Metabolite production (%)	Degradation of cytochrome P-450 (%)
None	100	100	100	22
SKF 525-A (200 mM)	33	ND†	29	0
Metyrapone (2.33 mM)	23	33	31	0
$CO:O_2$ (80:20 v/v)	ND	31	14	5

* Means ± S.D. are reported for experiments performed in triplicate with 2–3 different preparations of hepatic microsomes. Experiments were performed with phenobarbital-induced microsomes as described in the Experimental section and Table 2. In all cases, the inhibitors were added before the addition of the trichloroethylene (7.5 mM, final concentration). For binding studies, the inhibitors were added to both the sample and the reference cuvettes. Values in the absence of inhibitors were as follows: binding, ΔA = 0.069/nmole of cytochrome P-450; NADPH oxidation, 1.75 nmoles/min/nmoles of cytochrome P-450; metabolite production, 2.52 nmoles/min/nmoles of cytochrome P-450; degradation of cytochrome P-450, 0.48 nmoles/mg of protein/15 min from initial levels of 2.36 nmoles/mg of protein.

† ND, not determined.

increased following phenobarbital induction but not following 3-methylcholanthrene or spironolactone induction (Tables 1 and 2). The lack of effect of induction with 3-methylcholanthrene on the parameters for the binding and metabolism of trichloroethylene by hepatic microsomes (Tables 1 and 2) indicates that cytochrome P-448 may not interact with trichloroethylene.

The K_s and K_m values reported here for the interaction of trichloroethylene with hepatic microsomal cytochrome P-450 are considerably greater (by approximately 10 to 90-fold) than the K_m values reported for the conversion of trichloroethylene to chloral hydrate by the post-mitochondrial supernatant fraction [13, 30]. This discrepancy may reflect the different liver preparations used or perhaps reflects the further metabolism of chloral hydrate to trichloroethanol and trichloroacetate which occurs with the post-mitochondrial supernatant fraction [13, 30], while chloral hydrate was the sole chlorinated metabolite of trichloroethylene with isolated hepatic microsomes (see Results).

The K_m and V_{max} values reported in this paper for the metabolism of trichloroethylene to chloral hydrate (Table 2) are similar to those reported by Traylor *et al.* [31] for the production of CO from trichloroethylene by hepatic microsomal cytochrome P-450, but a more recent report suggests that CO is not produced from the metabolism of trichloroethylene by hepatic microsomes [32].

That the K_s value for the binding of trichloroethylene is equivalent to the K_m value for the metabolism of trichloroethylene for each type of induction indicates that the binding of trichloroethylene is rapid, compared to the rate-limiting step of the reaction. The inability of spironolactone, which elevates the levels of NADPH-cytochrome P-450 reductase/mg of microsomal protein [33], to increase significantly the rate of metabolism of trichloroethylene (Table 2) ($P > 0.1$) indicates that the rate-limiting step in the metabolism of trichloroethylene by cytochrome P-450 is subsequent to the reduction of the cytochrome P-450–substrate complex.

It appears that trichloroethylene is, indirectly, capable of degrading hepatic microsomal cytochrome P-450 *in vitro*, without affecting the levels of other hepatic microsomal enzymes, viz. cytochrome b_5, NADPH-cytochrome *c* reductase (Table 3) and glucose-6-phosphatase (see Results). Inasmuch as the levels of microsomal heme and cytochrome P-450 are decreased to similar extents by trichloroethylene (Table 3), it would appear that trichloroethylene is modifying the heme moiety of cytochrome P-450.

The degradation of the heme of cytochrome P-450 by trichloroethylene *in vitro* appears to require the metabolic activation of trichloroethylene by cytochrome P-450 since the reaction requires NADPH and is not supported by NADH (Table 3). Furthermore, metyrapone, SKF 525-A and CO, which inhibit the metabolism of trichloroethylene, also inhibit the degradation of cytochrome P-450 by trichloroethylene (Table 4). In addition, each type of induction affects the metabolism of trichloroethylene by hepatic microsomal cytochrome P-450 and the degradation of the heme of cytochrome P-450 by trichloroethylene to similar extents (Tables 2 and 3). This is in contrast to the situation with fluroxene, a compound which is also known to degrade the heme moiety of cytochrome P-450 *in vitro* [34]. For fluroxene, inducing agents for different forms of cytochrome P-450 differentially affect its metabolism by cytochrome P-450 relative to its degradation of cytochrome P-450 [35]. In addition to trichloroethylene and fluroxene, several other unsaturated compounds appear to require metabolic activation by cytochrome P-450 in order to degrade the heme moiety of the enzyme, viz. vinyl chloride [36], allyl-*iso*-propylacetamide and other allyl compounds [37, 38, 41]. The most obvious reactive metabolites of these unsaturated compounds and of trichloroethylene which could mediate the degradation of cytochrome P-450 are epoxides. The epoxide of trichloroethylene—trichloroethylene oxide—has been proposed to be produced from trichloroethylene by hepatic microsomal cytochrome P-450.

The effects of inducing agents on the metabolism and metabolic activation of trichloroethylene by

hepatic microsomal cytochrome P-450 *in vitro* compare well with their effects on the metabolism and hepatotoxicity of trichloroethylene *in vivo*. Phenobarbital increases the metabolism of trichloroethylene, the degradation of cytochrome P-450 by trichloroethylene [13, 30] (Tables 2 and 3) and the binding of metabolites of trichloroethylene to macromolecules *in vitro* [7], as well as the metabolism and toxicity of trichloroethylene *in vivo* and the degradation of cytochrome P-450 by trichloroethylene *in vivo* [7–9, 39, 40]. Induction by spironolactone or 3-methylcholanthrene has no effect, or only slightly increases the metabolism of trichloroethylene and the trichloroethylene-mediated degradation of cytochrome P-450 *in vitro* (Tables 2 and 3) and similarly affects the metabolism and hepatotoxicity of trichloroethylene *in vivo* [8, 40]. The excellent correlation between the effects of inducing agents on the metabolism and the metabolic activation of trichloroethylene *in vitro* and *in vivo* indicates that the metabolism of trichloroethylene by the phenobarbital inducible form of cytochrome P-450 is the first and possibly the rate-limiting step in the metabolism of trichloroethylene *in vivo* and in the production of the toxic effects observed following exposure to trichloroethylene.

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