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TETRACHLOROETHYLENE METABOLISM BY THE HEPATIC MICROSOMAL CYTOCHROME P-450 SYSTEM*

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Abstract—The interaction of tetrachloroethylene with hepatic microsomal cytochromes P-450 has been investigated using male Long–Evans rats. The spectral binding of tetrachloroethylene to cytochromes P-450 in hepatic microsomes from uninduced rats was characterized by a K_s of 0.4 mM. The K_s was not affected by phenobarbital induction, but was increased following pregnenolone-16α-carbonitrile induction. The K_M of 1.1 mM, calculated for the conversion of tetrachloroethylene to total chlorinated metabolites by the hepatic microsomal cytochrome P-450 system, was decreased by phenobarbital induction and increased by pregnenolone-16α-carbonitrile induction. The maximum extents of binding (ΔA_{max}) and metabolism (V_{max}) of tetrachloroethylene were increased by both phenobarbital and pregnenolone-16α-carbonitrile induction. Induction with β-naphthoflavone was without effect on any of the above parameters. The effects of the inducing agents on tetrachloroethylene-stimulated CO-inhibitable hepatic microsomal NADPH oxidation followed the same trend as their effects on V_{max} for the metabolism of tetrachloroethylene, although in all cases the extent of NADPH oxidation was 5- to 25-fold greater than the extent of metabolite production. The inhibitors of cytochromes P-450, viz. metyrapone, SKF 525-A, and CO, inhibited the hepatic microsomal binding and metabolism of tetrachloroethylene. Free trichloroacetic acid was found to be the major metabolite of tetrachloroethylene from the hepatic microsomal cytochrome P-450 system. Neither 2,2,2-trichloroethanol nor chloral hydrate was produced in measurable amounts from tetrachloroethylene. A minor but significant metabolite of tetrachloroethylene by cytochrome P-450 was the trichloroacetyl moiety covalently bound to components of the hepatic microsomes. Incubation of tetrachloroethylene, an NADPH-generating system, EDTA and hepatic microsomes was without effect on the levels of microsomal cytochromes P-450, cytochrome b_5, heme, and NADPH-cytochrome *c* reductase. It is concluded that hepatic microsomal cytochromes P-450 bind and metabolize tetrachloroethylene. The major product of this interaction is trichloroacetic acid, which is also the major urinary metabolite of tetrachloroethylene *in vivo*. The forms of cytochrome P-450 that bind and metabolize tetrachloroethylene include those induced by pregnenolone-16α-carbonitrile and by phenobarbital. Cytochrome P-448, which was induced in rat liver by β-naphthoflavone, does not appear to spectrally bind or metabolize tetrachloroethylene. The metabolism and toxicity of tetrachloroethylene are considered in relation to other chlorinated ethylenes.

Tetrachloroethylene ($Cl_2C{=}CCl_2$) is widely used industrially, primarily as a dry-cleaning, fabric finishing, and metal degreasing agent [1, 2]. As a consequence of its non-inflammability and lower vapor pressure, tetrachloroethylene is often utilized industrially in preference to trichloroethylene [2, 3]. A further advantage of tetrachloroethylene is its apparent lack of mutagenic potential, in contrast to trichloroethylene, which is mutagenic in the modified Ames' test [4, 5]. Although tetrachloroethylene is generally regarded as being of low toxicity, toxic effects such as liver impairment, central nervous system depression, and disturbances of the peripheral nervous system have been reported in humans following exposure to tetrachloroethylene [1, 2]. Tetrachloroethylene has also been found to be weakly carcinogenic in some mammalian species [1, 6].

Tetrachloroethylene is retained unchanged within the body for relatively long periods of time: the half-life of tetrachloroethylene in humans is approximately 144 hr, whereas that of trichloroethylene is 44 hr [7]. The longer retention time of tetrachloroethylene in the body presumably arises, in part, from the inability of mammalian species to extensively and rapidly metabolize this lipophilic compound; in rats, 98 per cent of the dose of tetrachloroethylene is excreted unchanged, whereas only 72–85 per cent of the dosage of trichloroethylene administered is excreted unchanged [8]. Similarly, in isolated, perfused rat liver, only 10–15 per cent of the total tetrachloroethylene taken up by the liver is metabolized, whereas 82–100 per cent of the trichloroethylene taken up is metabolized, even though the uptake of trichloroethylene is 7-fold greater than that of tetrachloroethylene [9].

The major urinary metabolites of tetrachloroethylene *in vivo* have been shown to be trichloroacetic acid and chloride, with 2,2,2-trichloroethanol being a minor metabolite [3, 8, 10, 11]. Free trichloroacetate is also the major metabolite of tetrachloroethylene in perfused liver, with the minor metabolites (*ca.* 25 per cent) being the trichloroacetyl moiety covalently bound to cellular constituents [9].

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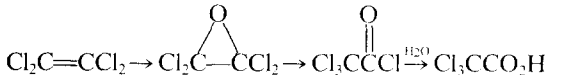
The currently accepted pathway for the production of trichloroacetic acid from tetrachloroethylene is shown in Scheme 1. This pathway was initially proposed by Powell in 1945 [12] and was subsequently supported by the results of Daniel [8] and others [4, 9]:



Scheme 1. Proposed pathway for the conversion of tetrachloroethylene to trichloroacetic acid.

The first step in this pathway appears to be catalyzed by the hepatic cytochrome P-450 system. Tetrachloroethylene is known to bind to the substrate binding site of hepatic microsomal cytochromes P-450 *in vitro*, and a direct relation has been demonstrated between the level of hepatic microsomal cytochromes P-450 and the extent of metabolism of tetrachloroethylene *in vivo* [11, 13]. Also suggestive of a role for hepatic cytochromes P-450 in the metabolism of tetrachloroethylene is the ability of this enzyme system to metabolize analogues of tetrachloroethylene, viz. vinyl chloride and trichloroethylene [14–19].

In the present investigation we have attempted to determine whether hepatic microsomal cytochromes P-450 bind and metabolize tetrachloroethylene and to examine in detail the interaction of tetrachloroethylene with several forms of cytochromes P-450, namely, those induced by phenobarbital, β-naphthoflavone and pregnenolone-16α-carbonitrile. This study is one avenue in an ongoing investigation of the metabolism and toxicity of the chlorinated ethylenes.

METHODS

Materials. Sodium phenobarbital and β-naphthoflavone were obtained from Maybaker, Port Elizabeth, R.S.A., and the Aldrich Chemical Co., Milwaukee, WI, U.S.A., respectively. NADPH and NADH were purchased from Miles Laboratories, Cape Town, R.S.A. Tetrachloroethylene, trichloroacetic acid, and 2,2,2-trichloroethanol were purchased from Merck Chemicals, Darmstadt, F.R.G. Horse liver alcohol dehydrogenase was from the Sigma Chemical Co., St. Louis, MO, U.S.A. Cylinders of CO and O_2 were supplied by Afrox Ltd., Cape Town, R.S.A. Pregnenolone-16α-carbonitrile was a gift from Searle Laboratories, Chicago, IL, U.S.A. SKF-525A (2-diethylaminoethyl-2,2-diphenylvalerate) and metyrapone [2-methyl-1,2-bis(3-pyridyl)-1-propanone] were gifts from Smith, Klein & French Ltd., Isando, Transvaal, R.S.A., and from Ciba-Geigy Ltd., Basle, Switzerland, respectively. All other chemicals were analytical grade reagents. Water was distilled and deionized.

Animals. Male Long–Evans rats weighing 180–200 g (obtained from the University of Cape Town Medical School animal house) were used in all experiments. Animals were permitted free access to Epol laboratory chow (protein min. 20%, fat 2.5%, fibre max. 6%, calcium 1.4%, phosphorus 0.7%; obtained from Epol Ltd., Goodwood, C.P., R.S.A.) and water, unless otherwise indicated. β-Naphthoflavone was given as a single injection of 80 mg/kg in corn oil 36 hr prior to killing the animals [20]. Pregnenolone-16α-carbonitrile and phenobarbital were administered i.p. at doses of 50 mg/kg in corn oil and of 80 mg/kg in 0.9% sterile saline, respectively, on each of 3 consecutive days [21]. The rats were starved overnight after the last injection and were killed the following morning. Hepatic microsomes were isolated by differential centrifugation [22]. For all experiments, microsomal suspensions of 2 mg protein/ml in 0.02 M Tris–HCl, pH 7.4, were used.

Difference spectra. Three milliliters of hepatic microsomal suspension (2 mg protein/ml) was placed in each of two cuvettes of 1 cm pathlength. Tetrachloroethylene was delivered to the sample cuvette below the surface of the microsomal suspension with a Hamilton Syringe. The cuvette was immediately stoppered and vortex mixed for 90 sec. Vortex mixing for shorter time intervals did not result in the full production of the difference spectrum. Reference cuvettes contained microsomal suspension only and were not vortex mixed. The magnitude of the resultant difference spectrum for tetrachloroethylene was measured as the difference in absorbance between the peak at 386 nm and the trough at 418 nm. The reported magnitude of the difference spectrum has been corrected for any intrinsic difference in absorbance at these wavelengths between the reference cuvette that contained microsomal suspension and the sample cuvette that was vortex mixed for 90 sec, but with no added tetrachloroethylene. In no case was any sample vortex mixed for more than 90 sec.

NADPH oxidation. An estimation of the rate of metabolism of tetrachloroethylene by the hepatic microsomal cytochrome P-450 system was obtained by monitoring the oxidation of NADPH at 340 nm in the presence of hepatic microsomes and tetrachloroethylene, with and without $CO:O_2$ (80:20; v/v) as described earlier [23], except that tetrachloroethylene was dispersed by vortex mixing for 90 sec.

Identification of the major metabolite of tetrachloroethylene. Probable metabolites of tetrachloroethylene were assayed in, and isolated from, incubation mixtures constituted of tetrachloroethylene (3.3 mM), an NADPH-generating system [24], EDTA (0.2 mM), and hepatic microsomes (2 mg protein/ml), in 0.02 M Tris–HCl, pH 7.4, unless otherwise indicated. Incubations were at 30° with shaking at 60 cycles/min for 30 min. The levels of the chlorinated metabolites of tetrachloroethylene in these reaction mixtures were determined spectrally using the modified Fujiwara assay of Leibman and Hindman [25], except that after centrifugation the supernatant fraction was bubbled with medical air for 10 min to displace residual tetrachloroethylene. The presence of trichloroacetic acid in incubation mixtures was ascertained using procedures A and B of this method. 2,2,2-Trichloroethanol was measured by the method of Friedman and Cooper [26] and by gas–liquid chromatography on a Pye-Unicam GCV gas–liquid chromatograph with a flame ionization detector, using a 2 m × 6 mm copper column of 10% di-iso-decylphthalate on acid-washed Chromosorb P. Column, detector and injector temperatures were 130°, 230° and 250°, respectively.

Tetrachloroethylene and 2,2,2-trichloroethanol eluted from the column at 150 and 580 sec, respectively.

Two methods were used for the isolation of trichloroacetic acid from incubation mixtures. In the first method, H_2SO_4 plus Na_2WO_4 [25] was added to precipitate protein, and the precipitant was removed by centrifugation at 1000 *g* for 10 min. The resulting supernatant fraction was thrice extracted with diethyl ether. In the second method, protein was precipitated with HCl (2 N, final concentration), and the precipitate was removed as in the first method. The supernatant fraction was extracted thrice with diethyl ether. The ether layer was subsequently extracted with 0.1 N NaOH. The aqueous alkaline extracts were pooled, acidified to pH 2 with 7.5 M HCl, and extracted repeatedly with diethyl ether. The ether was evaporated at 25° under a stream of N_2.

The isolated reaction products and authentic samples of trichloroacetic acid that had been treated exactly as were the reaction products either were dissolved in CCl_4 and analyzed by infrared spectroscopy on a Perkin–Elmer model 700 infrared spectrometer or were methylated with diazomethane at room temperature. The resulting methyl trichloroacetate was determined quantitatively on a Packard 428 gas–liquid chromatograph with an electron capture detector, using a 2 m × 6 mm glass column of Chromosorb 101 [27]. Column, detector and injector temperatures were 200, 250 and 220°, respectively. The retention time of methyl trichloroacetate under these conditions was 800 sec. The areas of peaks on the chromatograms were calculated by a Pye-unicam DP 88 mini-integrator.

Assays for microsomal components. Incubations for determining the effects of tetrachloroethylene on the levels of hepatic microsomal components were performed with hepatic microsomes from variously pretreated rats as described above, with an incubation time of 30 min. Assays on hepatic microsomal incubation mixtures were performed as follows. The levels of hepatic microsomal cytochromes P-450 were determined from the difference spectrum of CO-ferrocytochrome P-450 vs ferrocytochrome P-450 according to the method of Omura and Sato [28]. An extinction coefficient of 91 mM^{-1} cm^{-1} for the difference in absorbance between 450 and 490 nm was used [28]. 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 ($\varepsilon_{424-409\,nm}$ 185 mM^{-1} cm^{-1}) [29]. The activity of NADPH-cytochrome *c* reductase was determined from the increase in absorbance of ferrocytochrome *c* at 550 nm ($\varepsilon_{550\,nm}$ 21.1 mM^{-1} cm^{-1}) according to the method of Omura and Takesue [29]. Microsomal heme was determined spectrally as the reduced pyridine hemochrome ($\varepsilon_{557-575\,nm}$ 32.4 mM^{-1} cm^{-1}) according to Omura and Sato [28]. Glucose-6-phosphatase was assayed by the method of Nordlie and Arion [30]. Inorganic phosphate was determined by the method of King [31], using a Gilford single beam spectrophotometer.

For all spectral studies, unless otherwise indicated, a Pye-Unicam SP 1800 spectrophotometer was used. The thermostatically controlled compartment adjacent to the photomultiplier, designed to accommodate turbid samples, was used for all microsomal samples.

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 Hanes and Eadie–Hofstee plots. Student's *t*-test for unpaired data was utilized to calculate significant differences between means. A difference was considered significant when $P < 0.01$. Reported values are means ± standard deviations.

RESULTS

Spectral binding of tetrachloroethylene. Tetrachloroethylene bound to hepatic microsomal cytochromes P-450 resulting in the production of a Type I difference spectrum (λ_{max} 386 nm; λ_{min} 418 nm), as described earlier by Pelkonen and Vainio [13]. The effect of induction of different forms of cytochrome P-450 on the spectral binding constant (K_S) and the maximum extents of binding (ΔA_{max}) of tetrachloroethylene are presented in Table 1. It can be seen that induction of different forms of cytochrome P-450 by β-naphthoflavone and phenobarbital did not affect K_S, whereas induction with pregnenolone-16α-carbonitrile significantly increased K_S. The maximum extent of spectral binding of tetrachloroethylene was increased by both pregnenolone-16α-carbonitrile and phenobarbital, with the latter compound having the greater effect.

Table 1. Effect of induction on the binding of tetrachloroethylene by hepatic microsomal cytochromes P-450*

Inducing agent	Cytochromes P-450 (nmoles/mg microsomal protein)	K_S (mM)	A_{max} ($A_{386-418\,nm}$/nmole cytochrome P-450)
None	0.75 ± 0.05	0.43 ± 0.05	0.058 ± 0.008
β-Naphthoflavone	1.01 ± 0.03	0.39 ± 0.03	0.045 ± 0.006
Pregnenolone-16α-carbonitrile	1.56 ± 0.16	1.03 ± 0.10†	0.077 ± 0.006
Phenobarbital	2.53 ± 0.28	0.43 ± 0.03	0.114 ± 0.009†

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

† Differs significantly from value for uninduced microsomes, $P < 0.01$.

Table 2. Effect of induction on the metabolism of tetrachloroethylene by the hepatic microsomal cytochrome P-450 system*

Inducing agent	Cytochromes P-450 (nmoles/mg microsomal protein)	K_M (mM)	V_{max} [nmoles TCA . min^{-1} . (nmole cytochrome P-450)$^{-1}$]	NADPH oxidation† [nmoles . min^{-1} . nmole cytochrome P-450)$^{-1}$]
None	0.89 ± 0.06	1.1 ± 0.8	0.046 ± 0.004	1.2 ± 0.1
β-Naphthoflavone	1.03 ± 0.01	0.5 ± 0.5	0.055 ± 0.014	0.8 ± 0.2
Pregnenolone-16α-carbonitrile	1.50 ± 0.11	2.4 ± 0.8‡	0.16 ± 0.09‡	0.8 ± 0.2
Phenobarbital	2.32 ± 0.32	0.2 ± 0.1‡	0.19 ± 0.02‡	2.5 ± 0.5‡

* Values are means ± S.D. for experiments performed in triplicate with three or more different preparations of hepatic microsomes. Experimental conditions are described in Methods. TCA, trichloroacetic acid.

† Measured in the presence of 3.3 mM perchloroethylene.

‡ Differs significantly from value for uninduced microsomes, $P < 0.01$.

Hepatic microsomal NADPH oxidation and metabolism of tetrachloroethylene. The carbon monoxide inhibitable oxidation of NADPH by hepatic microsomes was stimulated by tetrachloroethylene (3.3 mM). The rates of tetrachloroethylene-stimulated NADPH oxidation reported in Table 2 have been corrected for the background rates of NADPH oxidation observed in the presence of tetrachloroethylene plus CO : O_2 (80 : 20; v/v), in an attempt to correct for non-cytochrome P-450 dependent oxidation of NADPH by hepatic microsomes [30]. Induction with phenobarbital resulted in a marked increase in tetrachloroethylene-stimulated CO-inhibitable NADPH oxidation, whereas induction with β-naphthoflavone or pregnenolone-16α-carbonitrile had no effect on this process.

Using procedure A of the modified Fujiwara assay of Leibman and Hindman [25], the conversion of tetrachloroethylene to chlorinated metabolites was linear for 30 min for microsomes from uninduced rats. Using microsomes from β-naphthoflavone-, pregnenolone-16α-carbonitrile- and phenobarbital-induced rats, the metabolism of tetrachloroethylene was linear for 15, 20 and 7 min, respectively (Fig. 1). These incubation times were used for all subsequent experiments in which the metabolism of tetrachloroethylene was assessed by the method of Leibman and Hindman [25].

The effects of agents that induce different forms of cytochrome P-450 on the Michaelis constants (K_M) and the maximum rates of metabolism (V_{max}) of tetrachloroethylene as assessed by the modified Fujiwara assay, are shown in Table 2. The K_M for tetrachloroethylene was decreased following phenobarbital induction of the cytochrome P-450 system and was increased following induction with pregnenolone-16α-carbonitrile. Neither K_M nor V_{max} changed significantly following induction by β-naphthoflavone. The V_{max} was increased following induction of the system by pregnenolone-16α-carbonitrile and phenobarbital. For all types of induction, V_{max} for the metabolism of tetrachloroethylene was 5- to 25-fold lower than the rate of tetrachloroethylene-stimulated CO-inhibitable NADPH

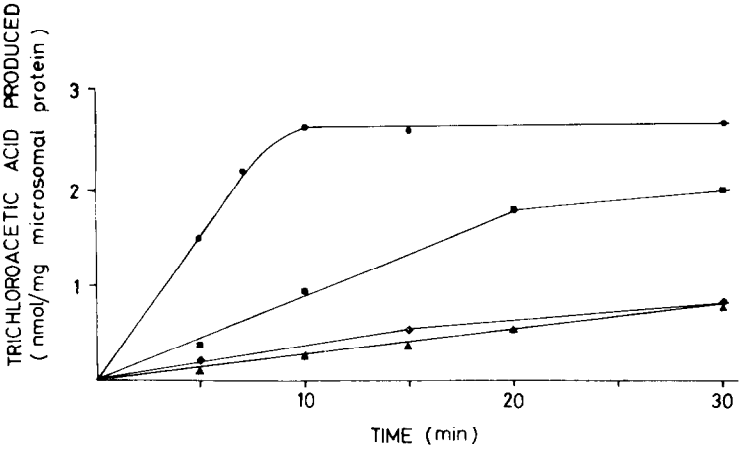


Fig. 1. Production of trichloroacetic acid from tetrachloroethylene by hepatic microsomes from differently pretreated rats as a function of time. Incubation mixtures contained in 0.02 M Tris–HCl (pH 7.4), tetrachloroethylene (3.3 mM), NADPH-generating systems, EDTA (0.2 mM), and hepatic microsomes (2 mg protein/ml) from uninduced (▲), β-naphthoflavone-induced (◆), pregnenolone-16α-carbonitrile (■) and phenobarbital-induced (●) male Long–Evans rats. Reaction mixtures were shaken at 60 cycles/min at 30°.

oxidation (Table 2), indicating that tetrachloroethylene may be a partial uncoupler of microsomal electron transfer.

Identification of the major metabolite of tetrachloroethylene. No appreciable amounts of 2,2,2-trichloroethanol were detected in incubation mixtures by the method of Friedman and Cooper [26] (limit of detection at 440 nm, *ca.* 0.5 nmole/ml). Furthermore, assaying by gas–liquid chromatography failed to demonstrate measurable levels of volatile metabolites, including 2,2,2-trichloroethanol, with the limit of detection of the latter compound being below 0.5 nmole/ml.

To assay for chloral hydrate, reaction mixtures were incubated for 30 min as described in Methods. At the end of the incubation period, horse liver alcohol dehydrogenase (0.33 unit/ml) and NADH (1 mM) were added to the reaction mixture, which was then incubated for a further 30 min. Final incubation mixtures were assayed by gas–liquid chromatography for 2,2,2-trichloroethanol, which would have been formed from the reduction of chloral hydrate by alcohol dehydrogenase. Under these conditions, no appreciable trichloroethanol (< 0.5 nmole/ml) was found.

Trichloroacetic acid, assayed by method B of Leibman and Hindman, appeared not to be present in the incubation mixtures. It was subsequently observed that the NADPH-generating system interfered with method B (but not with method A) of this assay with incubation mixtures or with authentic samples of trichloroacetic acid (data not shown).

The major product of tetrachloroethylene was shown to be trichloroacetic acid by infrared spectroscopy and gas–liquid chromatography. The infrared spectrum of the reaction product isolated from incubation mixtures was identical to that of an authentic sample of trichloroacetic acid that had been taken through the same extraction procedure. Notably, the distinctive peaks characteristic of carboxylic acids (3300 cm^{-1}) and of the carbonyl group (1740 cm^{-1}) were present in both spectra.

Methyl trichloroacetate was found to be present in methylated extracts of incubation mixtures by gas–liquid chromatography. The amounts of methyl trichloroacetate produced from phenobarbital-induced microsomes of 0.26 ± 0.01 nmole $\cdot$ min^{-1} $\cdot$ (mg microsomal protein)$^{-1}$ compare well with the value of 0.19 ± 0.05 nmole trichloroacetic acid $\cdot$ min^{-1}(mg microsomal protein)$^{-1}$ produced in incubation mixtures using the modified Fujiwara assay of Leibman and Hindman [25].

Assay for the covalently bound trichloroacetyl moiety. The treatment of reaction mixtures (containing tetrachloroethylene, NADPH-generating system, EDTA, and hepatic microsomes from phenobarbital-induced rats) that had been incubated previously at 30° for 30 min with H_2SO_4 (20% H_2SO_4 at 80° for 6 hr) [9] resulted in significantly ($P < 0.001$) increased levels of trichloroacetic acid. The levels of trichloroacetate before and after incubation with H_2SO_4 were 1.01 ± 0.04 and 1.81 ± 0.19 nmoles trichloroacetic acid $\cdot$ 30 min^{-1} $\cdot$ (nmole cytochrome P-450)$^{-1}$.

Effects of inhibitors on the binding and metabolism of tetrachloroethylene. The effects of three inhibitors of the cytochrome P-450 system on the binding and metabolism of tetrachloroethylene are presented in Table 3. Metyrapone, CO, and SKF-525A inhibited the binding, NADPH oxidation, and metabolism of tetrachloroethylene in the presence of hepatic microsomes. Metyrapone and CO were equivalent as inhibitors of these processes, whereas SKF 525A was much less effective. Each inhibitor inhibited the three processes to approximately the same extent.

Effects of tetrachloroethylene on hepatic microsomal enzymes and heme. Incubation of the NADPH-generating system, EDTA and tetrachloroethylene and hepatic microsomes from rats treated, or not, with pregnenolone-16α-carbonitrile or phenobarbital, did not result in appreciable alteration in the levels of hepatic microsomal cytochromes P-450, cytochrome b_5, heme, NADPH-cytochrome *c* reductase, or glucose-6-phosphatase (data not shown).

DISCUSSION

The results presented here indicate that tetrachloroethylene is metabolized by the hepatic micro-

Table 3. Effect of inhibitors on the interaction of tetrachloroethylene with the hepatic microsomal cytochrome P-450 system*

Additions	Spectral binding (%)	NADPH oxidation (%)	Trichloroacetate production (%)
None	100	100	100
SKF 525A (200 mM)	81 ± 3	ND†	67 ± 8
Metyrapone (2.33 mM)	20 ± 4	35 ± 6	25 ± 7
$CO:O_2$ (80:20; v/v)	ND	25 ± 12	25 ± 10

* Means ± S. D. are reported for experiments performed in triplicate with two to three different preparations of hepatic microsomes from phenobarbital-treated rats. Experiments were performed as described in Methods and in Table 2. In each case, the inhibitor was added before the tetrachloroethylene (3.3 mM, final concentration). For binding studies, the inhibitor was added to both the sample and the reference cuvettes. Values in the absence of inhibitors were as follows: binding, $A_{386nm}-A_{418nm}$, 0.10/nmole cytochrome P-450; NADPH oxidation, 2.04 nmoles $\cdot$ min^{-1} $\cdot$ (nmole cytochrome P-450)$^{-1}$; trichloroacetate production, 0.113 nmole $\cdot$ min^{-1} $\cdot$ (nmole cytochrome P-450)$^{-1}$.

† ND, not determined.

somal cytochrome P-450 system *in vitro*. First it was confirmed that tetrachloroethylene binds to the active site of the enzyme system, as evidenced by the production of a Type I difference spectrum (Table 1) [13]. Tetrachloroethylene also stimulated CO-inhibitable NADPH oxidation by hepatic microsomes (Table 2), which is characteristic of cytochrome P-450 dependent reactions [32]. Furthermore, chlorinated metabolites of tetrachloroethylene were produced during the aerobic incubation of hepatic microsomes, NADPH and tetrachloroethylene, with all components having been required for the reaction to proceed (see Results). Finally, the inhibitors of cytochrome P-450, viz. metyrapone, SKF 525-A and CO [33, 34], inhibited the hepatic microsomal spectral binding and metabolism of tetrachloroethylene (Table 3).

The different forms of cytochrome P-450 appear to bind and metabolize tetrachloroethylene to differing extents. Cytochrome P-448 does not appear to bind or metabolize tetrachloroethylene measurably, inasmuch as induction with β-naphthoflavone did not affect the values of K_s, K_M, ΔA_{max} or V_{max} for tetrachloroethylene (Tables 1 and 2). In addition, SKF 525-A, which is an effective inhibitor of cytochrome P-448 [35], was a relatively poor inhibitor of the spectral binding and metabolism of tetrachloroethylene (Table 3).

At least two distinct forms of cytochrome P-450—those induced by pregnenolone-16α-carbonitrile and by phenobarbital—do appear to bind and metabolize tetrachloroethylene. Induction by either of these agents resulted in alterations in K_s and/or K_M and in increased ΔA_{max} and/or V_{max} values for the binding and metabolism of tetrachloroethylene (Tables 1 and 2). Furthermore, metyrapone, which is a relatively specific inhibitor of the phenobarbital-induced form of cytochrome P-450 [35], was an effective inhibitor of the spectral binding and metabolism of tetrachloroethylene by hepatic microsomes from phenobarbital-induced rats (Table 3).

Cytochrome P-450 induced by phenobarbital appears to metabolize tetrachloroethylene more effectively than any other form of the enzyme. Although phenobarbital and pregnenolone-16α-carbonitrile enhanced V_{max} (per nmole cytochrome P-450) for the metabolism of tetrachloroethylene to similar extents, the K_M for tetrachloroethylene was 10-fold lower following phenobarbital induction, than following pregnenolone-16α-carbonitrile induction (Table 2).

The major product of the interaction of tetrachloroethylene with the hepatic microsomal cytochrome P-450 system *in vitro* was trichloroacetic acid, which is also the major metabolite of tetrachloroethylene *in vivo* and with perfused liver *in situ* [3, 7–10]. The interaction of tetrachloroethylene with the hepatic microsomal cytochrome P-450 system also resulted in the production of trichloroacetyl derivative(s) of cellular constituents, as shown earlier for the metabolism of tetrachloroethylene in perfused liver [9]. Inasmuch as 2,2,2-trichloroethanol was not produced in appreciable amounts from tetrachloroethylene in the presence of hepatic microsomes, it appears that the cytochrome P-450 system is not directly involved in the production of this minor metabolite of tetrachloroethylene *in vivo* [3].

In terms of the proposed metabolic pathway for tetrachloroethylene (Scheme 1), it appears that the cytochrome P-450 enzyme system may be the sole enzyme system involved in the conversion of tetrachloroethylene to free trichloroacetate and to the trichloroacetyl moiety covalently bound to cellular constituents. The covalently bound trichloroacetyl group is probably in ester or amide linkage to alcohol or amino functional groups of cellular constituents and presumably arises in a non-enzymic reaction of the epoxide or acyl chloride derivatives of tetrachloroethylene with cellular constituents. Thus, the cytochrome P-450 system appears to catalyze the metabolic activation of tetrachloroethylene to reactive species that may mediate the toxic effects of the parent compound.

There are many similarities between the hepatic microsomal metabolism of tetrachloroethylene and of another chlorinated ethylene, viz. trichloroethylene, which is also thought to be metabolically activated by hepatic cytochrome P-450. The equilibrium constants for the binding and metabolism of tetrachloroethylene by hepatic microsomal cytochromes P-450 (Tables 1 and 2) are similar to those reported for the interaction of trichloroethylene with this enzyme system (*ca.* 0.4 mM) [19]. In addition, tetrachloroethylene and trichloroethylene stimulate hepatic microsomal NADPH oxidation to similar extents (Table 2) [19]. The maximum rates of metabolism (V_{max}) of tetrachloroethylene by the cytochrome P-450 system in hepatic microsomes from differently induced rats are, however, approximately 30-fold lower than the corresponding maximum rates of metabolism of trichloroethylene (Table 2) [19].

The relative rates of metabolism of tetrachloroethylene and trichloroethylene by hepatic microsomes *in vitro* correlate well with the respective extents of metabolism of these two compounds *in vivo*. Tetrachloroethylene, which is metabolized at a much slower rate than is trichloroethylene *in vitro*, is metabolized to a much smaller extent *in vivo* and has a much longer biological half-life than trichloroethylene (Table 2) [3, 7, 8, 19, 36]. Furthermore, the relative extents of metabolism of tetrachloroethylene and trichloroethylene correlate with the extents of their deleterious effects on cellular constituents. For example, tetrachloroethylene does not affect the levels of microsomal cytochromes P-450 and heme *in vitro* (see Results), whereas trichloroethylene (and another asymmetrical haloethylene, vinyl chloride) degrade the heme of hepatic microsomal cytochromes P-450 *in vitro* and *in vivo* [14, 15, 19, 37]. In addition, in the presence of microsomal activating enzymes, tetrachloroethylene is non-mutagenic, whereas trichloroethylene is mutagenic [4, 5].

The lower chemical reactivity of tetrachloroethylene compared to that of trichloroethylene (and vinyl chloride) may not, however, be only a consequence of the relatively low rate of metabolism of tetrachloroethylene. It has been proposed that the reactive metabolite of tetrachloroethylene, viz. tetrachloroethylene oxide, is much less reactive than the epoxides of unsymmetrically substituted chlorinated ethylenes, such as trichloroethylene and vinyl

chloride [9]. Consequently, the less severe deleterious effects of tetrachloroethylene as opposed to those of trichloroethylene and vinyl chloride may reflect both a lower rate of metabolism of tetrachloroethylene and its conversion to a relatively less toxic metabolic intermediate.

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