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HEPATIC MICROSOMAL METABOLISM OF THE DICHLOROETHANES*

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Abstract—The binding of 1,1-dichloroethane (1,1-DCE) to the substrate binding site of hepatic microsomal cytochrome P-450, and the stimulation of hepatic microsomal CO-inhibitable NADPH oxidation by 1,1-DCE and 1,2-dichloroethane (1,2-DCE) were enhanced by induction with phenobarbital but not with β-naphthoflavone. Incubation of the dichloroethanes with hepatic microsomes from phenobarbital-treated rats, NADPH-generating system and EDTA resulted in the conversion of 1,1-DCE to acetic acid and to a lesser extent to 2,2-dichloroethanol and probably also mono- and dichloroacetic acid and the conversion of 1,2-DCE to chloroacetaldehyde and to a lesser extent to chloroacetic acid and probably 2-chloroethanol. In addition, reaction mixtures constituted as described above resulted in slight but significant losses (*ca.* 13%) of hepatic microsomal cytochrome P-450. The omission of dichloroethane or the NADPH-generating system from incubation mixtures eliminated the above effects, and SKF-525A or CO diminished or eliminated the effects. Pathways for the metabolism of 1,1-DCE and 1,2-DCE are proposed.

Chlorinated hydrocarbons are widely used in many spheres, including agriculture and industry. 1,1-Dichloroethane (Cl_2HCCH_3) (1,1-DCE) is utilized in relatively small quantities, primarily in the chemical, agricultural and petroleum industries. 1,2-Dichloroethane (ClH_2CCH_2Cl) (1,2-DCE) is utilized as a solvent, pesticide, fumigant and gasoline additive, but its primary industrial application is in the synthesis of vinyl chloride and other chlorinated hydrocarbons [1–3]. 1,2-DCE is one of the most widely produced halocarbons, several million tons being synthesized annually on a global basis [2, 3]. The estimated numbers of workers exposed to 1,1-DCE and 1,2-DCE are approximately five thousand and two million respectively [3]. It has been proposed that seventy-four kg of 1,2-DCE was released into ambient air in one year in the United States alone [1].

Both 1,1-DCE and 1,2-DCE have been shown to be toxic to laboratory animals and man, eliciting primarily hepatoxic and neurologic effects [3]. By most criteria, 1,1-DCE is less toxic than is 1,2-DCE [4–6]. Studies of the possible mutagenicity and carcinogenicity of 1,1-DCE have been negative or inconclusive [7, 8], but it has been recommended by the National Institutes of Health (U.S.A.) that 1,1-DCE be treated with caution in the workplace [3]. In contrast, 1,2-DCE is carcinogenic in rats and mice and mutagenic in several test systems, particularly in the presence of activating enzymes, such as the hepatic GSH transferases and to a lesser extent the hepatic microsomal cytochrome P-450 enzyme system [1, 3, 6, 9–14].

The metabolism of 1,1-DCE has not been characterized extensively. This compound, however, has been shown to bind to the substrate binding site of hepatic cytochrome P-450 [15] and to be dechlorinated by this enzyme system, although the products of this interaction have not been identified [16].

1,2-DCE has been shown to be metabolized *in vitro* and *in vivo* by two distinct pathways. The conjugation of 1,2-DCE with glutathione leads to the formation of ethylene [17] and glutathione conjugates, including the mutagenic species 2-chloroethyl glutathione [18]. The interaction of 1,2-DCE with hepatic microsomal cytochrome P-450 *in vitro* leads to the production of 2-chloroethanol, non-volatile metabolites, and reactive species (proposed at least in part to be 2-chloroacetaldehyde) which bind to cellular protein or added DNA [18]. The urinary metabolites of 1,2-DCE *in vivo* have been reported to be 2-chloroethanol and chloroacetic acid [19, 20].

An investigation of the interaction of 1,1-DCE and 1,2-DCE with multiple forms of rat liver cytochrome P-450 with special reference to the identification of metabolites is reported herein. The relevance of the hepatic microsomal cytochrome P-450 dependent metabolism of the dichlorinated ethanes to their potential carcinogenicity and mutagenicity is assessed.

EXPERIMENTAL

Materials. 1,1-DCE, 1,2-DCE, acetic acid and methyl acetate were reagent grade from Merck Chemicals, Darmstadt, West Germany. β-Naphthoflavone and sodium phenobarbital were obtained from the Aldrich Chemical Co., Milwaukee, WI, U.S.A., and Maybaker, Port Elizabeth, R.S.A., respectively. SKF-525A (β-diethylaminoethyl-2,2-

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diphenylvalerate) was a gift from Smith, Kline & French Ltd., Isando, Transvaal, R.S.A. Cylinders of compressed gases were obtained from Afrox Ltd., Cape Town, R.S.A. NADPH and NADH were purchased from Miles Laboratories, Cape Town, R.S.A. Other reagents were analytical grade and were obtained as described previously [21, 22]. Water was distilled and deionized.

Treatment of animals. Male Long–Evans rats (200 ± 10 g) 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, Cape Town, R.S.A., and water. β-Naphthoflavone and phenobarbital were administered by intraperitoneal injection as described by Nebert *et al.* [23] and Ivanetich *et al.* [24]. Animals were starved overnight (16 hr) prior to being killed.

Preparation of hepatic microsomes. Hepatic microsomes were isolated by differential ultracentrifugation [25] and were suspended in 0.02 M Tris–HCl, pH 7.4, at a concentration of 2 mg protein/ml for spectral assays and at 4 mg protein/ml for assays of metabolite production. The microsomal protein concentration was determined by the method of Lowry *et al.* [26] as modified by Chaykin [27].

Addition of compounds. 1,1-DCE and 1,2-DCE were added below the surface of the hepatic microsomal suspension with a Hamilton μl syringe, and the suspension was vortex mixed for 30 sec.

Spectral assays. Difference spectra of hepatic microsomes (3 ml) plus dichlorinated ethane versus hepatic microsomes were recorded at 25°. The difference in absorbance between the absorbance peak at 385 nm and the trough at 415 nm was taken as the magnitude of the difference spectrum (ΔA).

Rates of NADPH oxidation of reaction mixtures containing hepatic microsomes, NADPH (0.12 mM), and dichlorinated ethane (25 mM) were monitored spectrally at 340 nm at 25°. The reported rates are corrected for non-cytochrome P-450 dependent NADPH oxidation, measured as above but in samples saturated with $CO:O_2$ (80:20, v/v) [28].

The effect of the dichloroethanes on the levels of hepatic microsomal cytochrome P-450 was assayed in incubation mixtures containing hepatic microsomes (2 mg protein/ml), NADPH-generating system [29], EDTA (0.2 mM), and dichloroethane (25 mM). Reaction mixtures were incubated at 30° for 15 min with shaking at 60 cycles/min. Cytochrome P-450 concentrations were determined from measurements of the difference spectrum of CO-ferrocytochrome P-450 versus ferrocytochrome P-450 according to the method of Omura and Sato ($\varepsilon_{450nm-490nm}$ 91 cm^{-1} mM^{-1}) [30]. A Beckman 5230 recording spectrophotometer was used for all spectral studies.

Identification of metabolites of the dichloroethanes. Reaction mixtures containing the dichloroethane (40 mM)*, NADPH-generating system [29], EDTA (0.2 mM) and hepatic microsomes (3–18 ml at 4 mg protein/ml) in 0.02 M Tris–HCl, pH 7.4, were incubated at 30° with shaking at 60 cycles/min for 20 min. Protein was precipitated by the addition of H_2SO_4 plus Na_2SO_4, and the precipitate was removed by centrifugation at 1000 *g* for 10 min [31]. The resulting supernatant fraction was extracted with 4 or 5 ml of diethyl ether, for determination of acetic acid and the mono- and dichloroacetaldehydes and acetic acids; or with 4 or 5 ml of ethyl acetate, for the determination of chlorinated ethanols. The organic phase was removed, dried over anhydrous Na_2SO_4, and analyzed by gas–liquid chromatography. Standards were prepared by adding authentic samples of acetic acid or chlorinated ethanols, aldehydes and acetic acids to hepatic microsomes, precipitating the protein, and extracting as described above.

Carboxylic acids were methylated with diazomethane under nitrogen. Chlorinated aldehydes and methyl esters of chlorinated carboxylic acids were routinely assayed using column I, a 1 m × 6 mm glass column of Chromosorb 101 [32]. Column, detector, and injector temperatures were 200°, 250°, and 200° respectively. Mono- and dichlorinated ethanols were routinely measured on column II, a 3 m × 6 mm glass column of 10% Carbowax 20 M on Chromosorb W (AW). Column, detector, and injector temperatures were 170°, 230°, and 200° respectively [33]. Methyl acetate was assayed using the latter column with injector, oven, and detector temperatures of 120°, 70°, and 140° respectively.

A Packard 428 gas–liquid chromatograph was utilized for the identification of volatile metabolites. Chlorinated metabolites were detected with an electron capture detector, while non-halogenated metabolites were detected with a flame ionization detector. Peak areas were calculated by a Pye-Unicam DP88 computing mini-integrator.

Calculations and statistical analysis. Binding constants (K_s) and maximum extents of binding (ΔA_{max}) were calculated from Hanes plots. Student's *t*-test for unpaired data was used to calculate significant differences between means. A significant difference was taken as $P < 0.01$, with $P < 0.05$ being probably significant. Reported values are means ± standard deviations for, at least, duplicate assays on two or more separate microsomal preparations, unless otherwise specified.

RESULTS

Binding to hepatic microsomal cytochrome P-450. Both 1,1-DCE and 1,2-DCE [27] bound to cytochrome P-450 in hepatic microsomes from induced and uninduced rats, resulting in the production of a type 1 difference spectrum (λ_{max} = 385 nm, λ_{min} = 415 nm). The effect of induction of different forms of cytochrome P-450 on the spectral binding constants (K_s) and the maximum extents of binding (ΔA_{max}) of 1,1-DCE are presented in Table 1. The parameters for the binding of 1,1-DCE were not affected by induction with β-naphthoflavone. However, induction with phenobarbital significantly decreased K_s and increased ΔA_{max} and ΔA_{max} per nmole cytochrome P-450. The K_s for the binding of 1,2-DCE to cytochrome P-450 in microsomes from

* These concentrations of the DCEs did not affect the microsomal suspension (assessed visibly) or convert cytochrome P-450 to cytochrome P-420 (assessed spectrally [30]).

Table 1. Binding and metabolism of dichloroethanes by hepatic microsomal cytochrome P-450*

Compound	Induction	Binding			NADPH oxidation	
		K_s (mM)	ΔA_{max}	ΔA_{max} per nmole cytochrome P-450	nmoles/min/mg microsomal protein	nmoles/min/nmole cytochrome P-450
1,1-DCE	None	3.1 ± 0.2	0.042 ± 0.008	0.024 ± 0.004	1.7 ± 0.8	1.3 ± 0.6
	β-Naphthoflavone	3.0 ± 0.1	0.038 ± 0.006	0.030 ± 0.008	1.2 ± 0.5	0.9 ± 0.3
	Phenobarbital	2.3 ± 0.1†	0.148 ± 0.014†	0.054 ± 0.02†	4.5 ± 0.2†	1.9 ± 0.2‡
1,2-DCE	None				1.7 ± 0.4	1.6 ± 0.6
	β-Naphthoflavone				1.1 ± 0.5	0.8 ± 0.3†
	Phenobarbital	2.35§	0.068§	0.034§	3.7 ± 0.7†	1.7 ± 0.6

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

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

‡ Probably differs significantly from corresponding value for microsomes from uninduced rats, $P < 0.05$.

§ Represents results from a single preparation of hepatic microsomes.

phenobarbital-treated rats (Table 1) compares with that reported by Ivanetich *et al.* [34].

Hepatic microsomal NADPH oxidation. The CO-inhibitable oxidation of NADPH by hepatic microsomes was stimulated by both 1,1-DCE (25 mM) and 1,2-DCE (25 mM) (Table 1). The rates of 1,1-DCE and 1,2-DCE stimulated CO-inhibitable NADPH oxidation per mg microsomal protein were unaffected by β-naphthoflavone induction but were generally increased following phenobarbital induction. In contrast, the rates of NADPH oxidation per nmole cytochrome P-450 were not affected significantly by these inducing agents, except that the rate in the presence of 1,2-DCE was decreased significantly following β-naphthoflavone induction.

Identification of metabolites of dichloroethanes. The metabolism of the dichlorinated ethanes was assessed in reaction mixtures containing microsomes from phenobarbital-induced rats, NADPH-generating system, EDTA and 1,1-DCE (40 mM) or 1,2-DCE (40 mM). Metabolites have been identified as described below. The amounts of metabolites produced over 20 min have been corrected for the levels of metabolites found in reaction mixtures comprised as above, but not incubated (Table 2).

Following incubation of 1,1-DCE with the complete reaction mixture, no 2-chloroethanol was detected (limit of detection, 0.1 nmole per mg protein per 20 min), but measurable amounts were detected on column II of a metabolite which chromatographed as did 2,2-dichloroethanol (retention time, 270 sec). Incubation of 1,2-DCE with the complete reaction mixture led to the production of a metabolite which chromatographed identically to 2-chloroethanol on column II (retention time, 137 sec), whereas 2,2-dichloroethanol (limit of detection, 0.05 nmole per mg protein per 20 min) was not detected on any occasion (Table 2). The identity of mono- and dichloroethanol as metabolites of 1,2-DCE and 1,1-DCE, respectively, was confirmed as follows. Both metabolites were detected by the electron capture detector, which indicates that the metabolites are halogenated. The presence of the alcohol functional group in the two metabolites was confirmed by incubation of ethyl acetate extracts (5 ml) of the incubated reaction mixtures with 0.5 ml acetyl chloride for 1 hr at 50°, which resulted in the peaks of authentic standards of mono- and dichloroethanol and the peaks from reaction mixtures being no longer detectable by gas–liquid chromatography. The metabolite proposed to be 2,2-dichloroethanol had an identical retention time to an authentic sample of 2,2-dichloroethanol (420 sec) on a second gas–liquid chromatography column (column I), but it was not possible to confirm the identity of 2-chloroethanol on this column because of the insensitivity of the electron capture detector to this compound and the very small amounts produced.

1,2-DCE was converted by the hepatic microsomal reaction mixtures to a metabolite which chromatographed identically to chloroacetaldehyde on column I (retention time, 145 sec), while 1,1-DCE was not (limit of detection, 0.05 nmole per mg protein per 20 min) (Table 2). The identity of the metabolite proposed to be chloroacetaldehyde was confirmed as follows. The chromatographic peak which was attributed to chloroacetaldehyde was eliminated by the application of the Fehling's test to extracts of incubation mixtures. Furthermore, the oxidation of the metabolite with alkaline potassium permanganate [35], under conditions which oxidize chloroacetaldehyde to chloroacetic acid, resulted in the conversion of the metabolite to a product, which when methylated with diazomethane (see Experimental section) chromatographed identically to methyl chloroacetate (column I, retention time, 360 sec). Dichloroacetaldehyde was not produced in measurable amounts (limit of detection < 0.05 nmole per mg protein per 20 min) from 1,1- or 1,2-DCE.

Incubation of 1,1-DCE in the complete reaction mixture led to the production of metabolites which, following methylation with diazomethane, chromatographed on column I identically to methyl monochloroacetate and methyl dichloroacetate (retention times of 360 and 550 sec respectively). Following the incubation of 1,2-DCE with the complete reaction

Table 2. Production of metabolites from 1,1- and 1,2-DCE with hepatic microsomes from phenobarbital-induced rats*

Additions	Metabolite production (nmoles/mg microsomal protein/20 min)					
	2-Chloroethanol	2,2-Dichloro-ethanol	Chloro-acetaldehyde	Acetic acid	Chloroacetic acid	Dichloroacetic acid
GS	ND	ND	ND	< 6	ND	ND
1,1-DCE	ND	ND	ND	< 6	ND	ND
1,1-DCE + GS	ND	0.12 ± 0.02	< 0.07 ± 0.03	179 ± 15	0.22 ± 0.08	0.048 ± 0.005
1,1-DCE + GS + CO:O_2				102 ± 12	0.03 ± 0.01†	0.017 ± 0.001†
1,1-DCE + GS + SKF-525A					0.09 ± 0.02†	0.024 ± 0.001†
1,2-DCE	ND	ND	ND	ND	ND	ND
1,2-DCE + GS	0.20 + 0.05†	ND	35 ± 1	ND	0.31 ± 0.01	ND
1,2-DCE + GS + CO:O_2			18 ± 2†		0.16 ± 0.01†	
1,2-DCE + GS + SKF-525A			28 ± 4†		0.23 ± 0.01†	

* In incubation mixtures containing hepatic microsomes from phenobarbital-treated rats, EDTA, and additions as shown. Experimental details are given in the Experimental section. Reported values are means ± S.D. for determinations in triplicate on three to five separate preparations of hepatic microsomes. Abbreviations: GS, NADPH-generating system; and ND, not detectable.

† Differs from corresponding value in the absence of inhibitor, $P < 0.01$.

mixture, a product was formed which, following methylation, chromatographed on column I, identically to methyl chloroacetate; however, no peak was detected corresponding to methyl dichloroacetate. The methylated samples were detected by the electron capture detector, which indicates that they are halogenated compounds. Either the omission of the diazomethane treatment or the neutralization of reaction mixtures prior to extraction eliminated the peaks from the chromatograms, confirming that the metabolites contain the carboxylic acid functional group. In view of the low levels of these metabolites that were produced from 1,1-DCE, it was not possible to further confirm their structure. However, the metabolite of 1,2-DCE which was proposed to be methyl chloroacetate was shown to chromatograph identically to an authentic sample of methyl chloroacetate on column II (retention time, 75 sec).

In view of the relatively low levels of the chlorinated metabolites produced per 20 min from 1,1-DCE, the effect of redistillation of commercially available reagent grade 1,1-DCE on the levels of metabolite production was assessed. In no case were the levels of production of the metabolites per 20 min decreased by this treatment, relative to the data given in Table 2. In extracts of complete incubation mixtures, which had been treated with diazomethane, a peak chromatographing identically to methyl acetate was produced from 1,1-DCE but not from 1,2-DCE (retention time, 110 sec on column II with injector, oven, and detector temperatures at 120°, 70°, and 140° respectively). When extracts of reaction mixtures containing the metabolite proposed to be acetic acid were treated with benzyldimethylphenylammonium chloride according to the method of Richards *et al.* [36], a peak appeared at the retention time of benzyl acetate (225 sec on column II) corresponding to 174 ± 15 nmoles benzyl acetate produced per mg microsomal protein per 20 min.

Following incubation of hepatic microsomes from phenobarbital-induced rats, NADPH-generating system and EDTA, or of hepatic microsomes from phenobarbital-induced rats, EDTA and either 1,1-DCE or 1,2-DCE, no chlorinated metabolites or acetic acid were produced in measurable amounts (Table 2). The inhibitors of cytochrome P-450—CO and SKF-525A [37]—significantly decreased the metabolism of 1,1-DCE to mono- and dichloroacetate and the conversion of 1,2-DCE to chloroacetaldehyde and chloroacetic acid (Table 2). CO and metyrapone decreased the conversion of 1,1-DCE to acetic acid, with negligible levels of acetic acid (< 6 nmoles per mg protein per 20 min) being produced in the presence of the latter inhibitor (Table 2).

Effect of dichloroethanes on the levels of hepatic microsomal cytochrome P-450. The effects of the dichloroethanes on the levels of hepatic microsomal cytochrome P-450 are presented in Table 3. Incubation of 1,1-DCE or 1,2-DCE with hepatic microsomes from untreated or β-naphthoflavone-induced rats in the presence or absence of NADPH-generating system resulted in no detectable change in cytochrome P-450 levels. However, incubation of 1,1-DCE or 1,2-DCE with hepatic microsomes from

Table 3. Effects of dichloroethanes on the levels of hepatic microsomal cytochrome P-450 *in vitro**

		1,1-DCE		1,2-DCE	
		Loss of cytochrome P-450		Loss of cytochrome P-450	
Pretreatment of rat	Additions	nmoles/mg protein	%	nmoles/mg protein	%
None	GS	< 0.01	< 0.1	< 0.01	< 0.1
	DCE	< 0.01	< 0.1	0.02 ± 0.01	2.6 ± 0.7
	DCE + GS	< 0.01	< 0.1	0.03 ± 0.03	3.4 ± 3.4
β-Naphthoflavone	GS	< 0.01	< 0.1	0.03 ± 0.03	3.1 ± 3.1
	DCE	< 0.01	< 0.1	< 0.01	< 0.1
	DCE + GS	< 0.01	< 0.1	< 0.01	< 0.1
Phenobarbital	GS	0.03 ± 0.03	1.3 ± 0.9	0.03 ± 0.01	0.7 ± 0.2
	DCE	0.01 ± 0.01	1.9 ± 1.9	< 0.01	< 0.1
	DCE + GS	0.32 ± 0.10†	12.2 ± 2.4†	0.31 ± 0.09†	13.3 ± 1.6†
	DCE + GS + CO:O_2	0.11 ± 0.05‡	3.7 ± 1.5‡	0.04 ± 0.04‡	1.7 ± 1.7‡

* Reported values are means ± S.D. for determinations in triplicate on three or more preparations of hepatic microsomes. Incubation mixtures contained hepatic microsomes (2 mg protein/ml), EDTA, and additions as indicated. Experimental details are given in the Experimental section. Abbreviation: GS, NADPH-generating system.

† Differs significantly from corresponding value for GS or DCE, $P < 0.01$.

‡ Differs significantly from corresponding value for GS plus DCE in absence of inhibitor, $P < 0.01$.

phenobarbital-induced rats, in the presence of EDTA and an NADPH-generating system, resulted in significantly decreased levels of cytochrome P-450. The decreases in the levels of cytochrome P-450 did not occur in the absence of the NADPH-generating system or the dichloroethane and were diminished by CO.

DISCUSSION

The results presented herein demonstrate that 1,1-DCE is metabolized by hepatic microsomal cytochrome P-450 *in vitro* and confirm that 1,2-DCE is also metabolized by this enzyme system [18]. First, both 1,1-DCE and 1,2-DCE bound to the active site of cytochrome P-450, as assessed by their abilities to produce a Type 1 difference spectrum (see Results) [15, 34, 38]. Both compounds stimulated hepatic microsomal CO-inhibitable NADPH oxidation (Table 1), which can provide a measure of cytochrome P-450 dependent metabolism [28]. Furthermore, the metabolism of 1,1- and 1,2-DCE and their metabolic activation to species capable of decreasing the levels of hepatic cytochrome P-450 required hepatic microsomes and an NADPH-generating system and was inhibited by CO and/or SKF-525A (Tables 2 and 3), which are effective inhibitors of cytochrome P-450 [37].

The form of cytochrome P-450 elevated by β-naphthoflavone, viz. cytochrome P-448, does not appear to bind or metabolize 1,1-DCE or 1,2-DCE. Elevation of the levels of cytochrome P-448 by prior induction with β-naphthoflavone did not affect the extent of binding of 1,1-DCE to hepatic microsomal cytochrome P-450 or the stimulation of the rate of hepatic microsomal CO-inhibitable NADPH oxidation by 1,1-DCE and 1,2-DCE (Table 1), and it did not result in measurable metabolic activation of either compound to species capable of decreasing the levels of cytochrome P-450 (Table 3).

In contrast, the form of cytochrome P-450 induced by phenobarbital appears to bind and metabolize both 1,1-DCE and 1,2-DCE. Elevation of this form of cytochrome P-450 increased ΔA_{max} and ΔA_{max} per nmole cytochrome P-450 for the binding of 1,1-DCE to cytochrome P-450. Furthermore, elevation of the phenobarbital inducible form of cytochrome P-450 slightly increased the affinity of the enzyme for 1,1-DCE and resulted in increased rates of 1,1- and 1,2-DCE stimulated CO-inhibitable NADPH oxidation per mg microsomal protein (Table 1), and in measurable metabolic activation of 1,1- and 1,2-DCE to reactive species capable of decreasing the levels of hepatic microsomal cytochrome P-450 (Table 3). The report of Guengrich *et al.* that the hepatic microsomal conversion of 1,2-DCE to unidentified nonvolatile metabolites is decreased following induction with polycyclic hydrocarbons and increased following induction with phenobarbital [18] is consistent with the above proposals.

The metabolism of 1,1-DCE by hepatic microsomal cytochrome P-450 resulted in the production of acetic acid as the major metabolite and 2,2-dichloroethanol and probably also mono- and dichloroacetic acids as minor metabolite(s) (see Results) (Table 2). On the basis of these results, pathways for the metabolism of 1,1-DCE are proposed (Fig. 1). The initial steps in the metabolism of 1,1-DCE are proposed to involve cytochrome P-450 dependent hydroxylations (probably via oxygen insertion, see Ref. 39) at either C-1 or C-2. Hydroxylation at C-1 results in the production of an unstable α-haloalcohol which can lose HCl to form acetyl chloride or, in a much less favorable reaction, undergo a chlorine shift to yield chloroacetyl chloride. Both of these acyl chlorides are expected to react with water to yield the free acids or to be able to react with cellular constituents. Hydroxylation at C-2 produces 2,2-dichloroethanol, which can be further oxidized to dichloroacetaldehyde and dichloro-

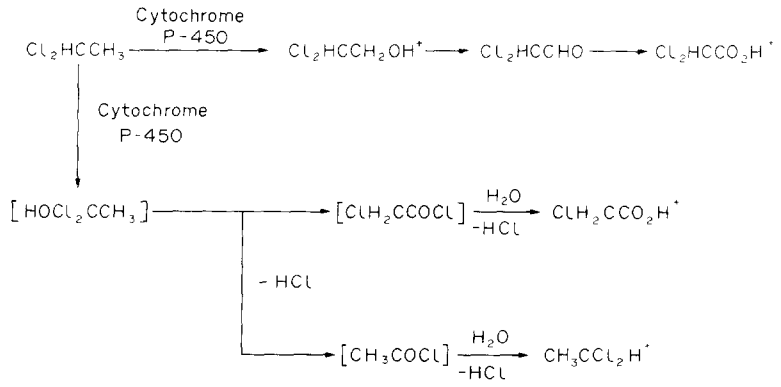

Fig. 1. Proposed pathways for the metabolism of 1,1-DCE. Key: (+) Identified metabolite from the hepatic microsomal cytochrome P-450 enzyme system (see Results). Brackets enclose unstable species.

acetic acid. It is anticipated that the oxidation of 2,2-dichloroethanol may be catalyzed by the cytochrome P-450 enzyme system or by alcohol and aldehyde dehydrogenase contaminants of hepatic microsomal preparations.

The proposed pathways for the metabolism of 1,2-DCE are supported by the identification of 2-chloroethanol and chloroacetic acid as urinary metabolites of 1,2-DCE [19, 20], of glutathione conjugates and ethylene as products of the interaction of 1,2-DCE with GSH and GSH transferases [17, 18], and of 2-chloroethanol [18], chloroacetaldehyde and chloroacetic acid as metabolites of 1,2-DCE from the hepatic microsomal cytochrome P-450 enzyme system *in vitro* (see Results) (Table 2). It is proposed that hepatic cytochrome P-450 may hydroxylate 1,2-DCE with the loss of chlorine to yield 2-chloroethanol or may catalyze an oxygen insertion at either of the two equivalent carbon atoms to yield an unstable α-haloalcohol which rearranges non-enzymically to chloroacetaldehyde. In view of the observation that the rate of production of chloroacetaldehyde greatly exceeds the rates of production of 2-chloroethanol and chloroacetate (Table 2) and the report that NADPH plus hepatic microsomes catalyzes the conversion of chloroacetaldehyde to 2-chloroethanol, but not the reverse reaction [18], it is anticipated that the oxygen insertion reaction proceeds at a far greater rate than the oxidative dehalogenation of 1,2-DCE. It would appear that metabolism of 1,2-DCE by cytochrome P-450 may be the rate-limiting step in the metabolism of this compound *in vivo*, since the inhibition of this enzyme *in vivo* with CCl_4 decreases metabolism of 1,2-DCE, while induction of this enzyme with phenobarbital *in vivo* increases the metabolism of 1,2-DCE [40].

The extent of dechlorination of 1,1-DCE was calculated from the data presented in Table 2 to be 10-fold greater than that of 1,2-DCE. This result compares with the report of Van Dyke and Wineman [16] that ^{36}Cl-1,1-DCE was dechlorinated by hepatic microsomes at an approximately 25-fold greater rate than was 1,2-DCE.

If the metabolism of the DCEs is assumed to be linear for 20 min, the rates of metabolite production would be 8.9 and 1.7 nmoles per mg microsomal protein per min (Table 2) from 1,1- and 1,2-DCE respectively. If the extents of metabolite production in the presence of CO : O_2 are subtracted from these values, the apparent rates of CO-inhibitable metabolite production from 1,1- and 1,2-DCE are calculated to be 3.9 and 0.9 nmoles per mg microsomal protein per min (Table 2). The latter rate for 1,1-DCE compares to the rate of 1,1-DCE stimulated CO-inhibitable NADPH oxidation, while that for 1,2-DCE is approximately one-quarter of the rate

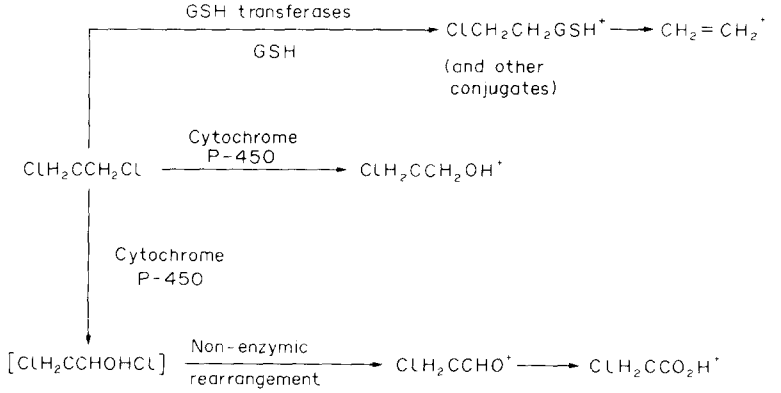

Fig. 2. Proposed pathways for the metabolism of 1,2-DCE. Key: (+) Identified metabolites. Brackets enclose unstable species.

of 1,2-DCE stimulated NADPH oxidation. It is therefore possible that 1,2-DCE, but not 1,1-DCE, is a partial uncoupler of hepatic cytochrome P-450.

It would appear that the metabolism of 1,1-DCE and 1,2-DCE by cytochrome P-450 is important in mediating the toxic effects of these compounds. 1,1-DCE and 1,2-DCE are converted by cytochrome P-450 to a variety of chlorinated metabolites, some of which (e.g. chloroacetaldehyde and acetyl chloride) are reactive species which would be expected to be more toxic than the parent compounds.

In addition, cytochrome P-450 may also play a role in the activation of 1,1- and 1,2-DCE to mutagenic species. The proposed metabolic pathways for both compounds contain several reactive species, including chloroacetaldehyde which is thought in part to mediate the mutagenic and carcinogenic effects of vinyl chloride (see, for example, Ref. 41) and unstable reactive species such as α-haloalcohols and acyl chlorides (Figs. 1 and 2). Furthermore, both 1,1- and 1,2-DCE are converted by cytochrome P-450 to reactive species which can inactivate the enzyme, apparently by modifying its heme group (Table 3).

The relatively greater rate of metabolism of 1,1-DCE relative to 1,2-DCE by the hepatic microsomal cytochrome P-450 system is not consistent with the relatively greater toxicity, mutagenicity and carcinogenicity of 1,2-DCE. Therefore, it would appear that both the hepatic cytochrome P-450 enzyme system and the hepatic GSH transferases play an important role in the activation of 1,2-DCE to mutagenic and carcinogenic species (see, for example, Refs. 11, 12 and 14).

REFERENCES

1. *IARC Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Humans* **20**, 429 (1979).
2. L. Fishbein, *Mutation Res.* **32**, 267 (1976).
3. J. C. Parker, G. E. Casey, L. J. Bahlman, N. A. Leidel, D. Rose, H. P. Stein, A. W. Thomas and J. M. Lane, *NIOSH Current Intelligence Bulletin 27, Am. ind. Hyg. Ass. J.* **40**, A46 (1979).
4. T. H. Hofmann, H. Birnstiel and P. Jobst, *Arch. Tox.* **27**, 248 (1971).
5. G. D. Divincenzo and W. J. Krasavage, *Am. ind. Hyg. Ass. J.* **35**, 21 (1974).
6. L. J. Bahlman, N. A. Leidel, J. C. Parker, H. P. Stein, A. W. Thomas, B. S. Woolf and J. D. Millar, *NIOSH Current Intelligence Bulletin 25, Am. ind. Hyg. Ass. J.* **39**, A35 (1978).
7. V. F. Simmon, K. Kauhanen and R. G. Tardeff, in *Progress in Genetic Toxicology* (Eds. D. Scott, B. A. Bridges and F. H. Sobel), p. 249. Elsevier/North Holland Biomedical Press, New York (1977).
8. National Cancer Institute Report, DHEW/PUB/NIH-78-1316, NCI-CG-TR-66, *Gov. Rep. Announce Index (US)* **78**, 113 (1978).
9. E. K. Weisburger, *Environ. Hlth Perspect.* **21**, 7 (1977).
10. U. Rannug and C. Ramel, *J. Toxic. environ. Hlth* **2**, 1019 (1977).
11. U. Rannug, A. Sundvall and C. Ramel, *Chem. Biol. Interact.* **20**, 1 (1978).
12. S. Banerjee and B. L. van Duuren, *J. natn. Cancer Inst.* **63**, 707 (1979).
13. M-T. King, H. Beikirch, K. Eckhardt, E. Gocke and D. Wild, *Mutation Res.* **66**, 33 (1979).
14. U. Rannug and B. Beije, *Chem. Biol. Interact.* **24**, 265 (1979).
15. O. Pelkonen and H. Vainio, *Fedn Eur. Biochem. Soc. Lett.* **51**, 11 (1975).
16. R. A. Van Dyke and C. G. Wineman, *Biochem. Pharmac.* **20**, 463 (1971).
17. J. C. Livesey and M. W. Anders, *Drug Metab. Dispos.* **7**, 199 (1979).
18. F. P. Guengrich, W. M. Crawford, J. Y. Domoradzki, T. L. Macdonald and P. G. Watanabe, *Toxic. appl. Pharmac.* **55**, 303 (1980).
19. M. G. Kokarovtseva and N. I. Kiseleva, *Farmak. Toks.* **41**, 118 (1978).
20. S. Yllner, *Acta pharmac. tox.* **30**, 257 (1971).
21. A. K. Costa and K. M. Ivanetich, *Biochem. Pharmac.* **29**, 2863 (1980).
22. K. M. Ivanetich and L. H. van den Honert, *Carcinogenesis* **2**, 697 (1981).
23. D. N. Nebert, J. K. Heidema, H. W. Strobel and M. J. Coon, *J. biol. Chem.* **248**, 7631 (1973).
24. K. M. Ivanetich, J. J. Bradshaw, J. A. Marsh, G. G. Harrison and L. S. Kaminsky, *Biochem. Pharmac.* **25**, 773 (1976).
25. J. L. Holtzman and M. L. Carr, *Archs Biochem. Biophys.* **150**, 227 (1972).
26. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
27. S. Chaykin, *Biochemistry Laboratory Techniques*, p. 20. John Wiley, New York (1966).
28. B. Stripp, N. Zampaglione, M. Hamrick and J. R. Gillette, *Molec. Pharmac.* **8**, 189 (1972).
29. J. A. Marsh, J. J. Bradshaw, S. A. Lucas, L. S. Kaminsky and K. M. Ivanetich, *Biochem. Pharmac.* **26**, 1601 (1977).
30. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
31. K. C. Leibman and J. D. Hindman, *Analyt. Chem.* **36**, 348 (1964).
32. L. T. Sennello, *Biochem. Med.* **8**, 345 (1953).
33. E. R. Garrett and L. H. Lambert, *J. pharm. Sci.* **55**, 812 (1966).
34. K. M. Ivanetich, S. A. Lucas, J. A. Marsh, M. R. Ziman, I. D. Katz and J. J. Bradshaw, *Drug Metab. Dispos.* **6**, 218 (1978).
35. R. L. Shriner, R. C. Fuson and D. Y. Curtin, *Systematic Identification of Organic Compounds*, p. 252. John Wiley, New York (1964).
36. R. G. Richards, C. L. Mandenhall and J. MacGee, *J. Lipid Res.* **16**, 395 (1975).
37. M. W. Anders, *A. Rev. Pharmac.* **11**, 37 (1971).
38. P. S. Cox, L. J. King and D. V. Parke, *Xenobiotica* **6**, 363 (1976).
39. G. Loew, J. Trudell and H. Motulsky, *Molec. Pharmac.* **9**, 152 (1973).
40. Yu. Yu. Bonitenko, A. A. Bruk and N. A. Gosteva, *Chem. Abstr.* **92**, 354606 (1979).
41. F. P. Guengrich, W. M. Crawford and P. G. Watanabe, *Biochemistry* **18**, 5177 (1979).