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Rat Liver Microsomal Metabolism of Propyl Halides

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SUMMARY

The *in vitro* metabolism of 1-propyl halides (chloride, bromide, and iodide) by hepatic microsomes from phenobarbital-induced rats was examined. The following metabolites were detected: propene, 1,2-epoxypropane, 1,2-propanediol, propionic acid, and undefined species bound to protein (for propyl chloride). The addition of exogenous glutathione to the incubation mixture led to the production of *S*-(1′-propyl)glutathione and *S*-(2′-hydroxy-1′-propyl)glutathione. The ratio of the metabolites resulting from C_1-C_2 functionalization [propene, 1,2-propanediol, and *S*-(2′-hydroxy-1′-propyl)glutathione] to that resulting from C_1 functionalization (propionic acid) increased as the halide progressed down the halide order chloride bromide, and iodide. Mechanisms which rationalize the distribution of propyl halide metabolites as a function of the halide are discussed. The preferred mechanism interprets that the results obtained are a consequence of the partitioning of the initial metabolic transformation between α-hydroxylation and halogen oxygenation pathways.

INTRODUCTION

The widespread use of organohalides in commerce, industry, and medicine and their persistence has resulted in these materials' being major environmental pollutants. Many of the compounds in this class are carcinogenic. Some organohalides are potent direct alkylating agents, and their carcinogenic or other toxic effects may be attributed to this property. However, a number of these compounds require bioactivation to exhibit toxicity. This activation process in many cases requires metabolism by the cytochrome P-450-containing monooxygenases (1–4).

Extensive studies of *in vivo* and *in vitro* metabolism have led to several postulated mechanisms for the cytochrome P-450-mediated metabolism of organohalides (R_1R_2CHX), including oxidation of the halogen-bound carbon via carbon-hydrogen bond oxygen insertion (R_1R_2COHX) (3–5), reduction of the carbon-halogen bond generating a halide ion (X^-) and a carbon-centered free radical ($R_1R_2\dot{C}H$) (6–8), and the direct halogen oxidation to a hypervalent organohalogen species ($R_1R_2CHX{=}O$) (4, 9).

Research in this laboratory has focused on the direct halogen oxidation process. These studies have consisted of a comparison of the chemistry of hypervalent organohalide species and the products of rat hepatic microsomal and purified cytochrome P-450 monooxygenase-catalyzed oxidation of halogen-containing compounds. These studies have demonstrated that iodosobenzene, a stable hypervalent organohalide, is a product of cytochrome P-450 monooxygenase-catalyzed oxidation of iodobenzene (10). In order to develop an understanding of the chemical mechanisms of the generation of such hypervalent organohalide species, we have examined the chemistry of the related (but unstable) alkyliodoso species, which are generated *in situ* by peracid oxidation of alkyliodides (9). These transient hypervalent organoiodide species are potent electrophiles whose reaction processes appear to proceed through incipient or demonstrable carbocations.

The principal focus of the studies reported here was to determine the metabolic products resulting from the cytochrome P-450-catalyzed metabolism of the propyl halide series (chloride, bromide, iodide). Of particular importance was the influence of the halogen substituent in the halide series on the distribution and types of metabolic products seen. 1-Propyl halides were chosen for these studies since the propyl moiety offers relatively few options to the cytochrome P-450 monooxygenase system that are not associated with RCH_2X metabolism.

EXPERIMENTAL PROCEDURES

Materials

n-[1-^{14}C]Propyl bromide was purchased from Amersham Corporation, The Radiochemical Centre (Amersham, England). The compound had a specific activity of 6.9 mCi/mmole and was diluted with carrier to 1.62 mCi/mmole. An aliquot was determined to be ≥97.2% radiochemically pure by gas-liquid chromatographic iso-

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lation and radioassay. *n*-[1-^{14}C]Propyl chloride and propyl iodide were synthesized by nucleophilic displacement of the corresponding bromide either by lithium chloride in dimethyl sufoxide or by sodium iodide in acetone. The synthesized *n*-[1-^{14}C]propyl chloride and propyl iodide had radiochemical purities, as determined by gas-liquid chromatographic analysis, of ≥98.1% and ≥95.7%, respectively. 1-Chloropropane, 1-bromopropane, 1-iodopropane, 1,2-epoxypropane, 1-propanol, and 1,2-dibromopropane were purchased from Aldrich Chemical Company (Milwaukee, Wisc.). Propionic acid and 1,2-propanediol were purchased from Eastman Chemical Company (Rochester, N. Y.). Propene and propane were purchased from Matheson Speciality Gases (East Rutherford, N. J.). Glucose-6-phosphate, NADP, glutathione, and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Company (St. Louis, Mo.).

Incubation Conditions

Hepatic microsomes were prepared from phenobarbital-treated male Sprague-Dawley rats as described elsewhere (11) and were stored at −70° in potassium chloride (150 mM)/Tris hydrochloride (10 mM) buffer (pH 7.4) containing 20% (v/v) glycerol.

Unless otherwise noted, incubations were carried out at 37° for 60 min in Teflon-sealed vials (10 ml) in a final volume of 4.0 ml. The incubation mixtures contained rat hepatic microsomal protein (28.5 mg; 44.7 nmoles of cytochrome P-450), potassium chloride (150 mM)/Tris hydrochloride (10 mM) buffer (pH 7.4), [1-^{14}C]propyl halide (1.12 mmoles added in acetone/methanol or dimethyl sulfoxide/methanol) and an NADPH-generating system employing NADP (0.5 mM), glucose-6-phosphate dehydrogenase (4.0 units), glucose-6-phosphate (5.0 mM), and magnesium chloride (5.0 mM). The controls did not contain the NADPH-generating system.

Analytical Methods

Proton magnetic resonance spectra were recorded at 100 MHz with a JEOL MH-100 spectrometer with tetramethylsilane or sodium 3-(trimethylsilyl)-1-propanesulfonate as internal standards. ^{13}C-NMR spectra were recorded at 22.50 MHz by employing a JEOL FX-90Q Fourier transform spectrometer with deuteriochloroform (77.0 ppm) as internal standard. Low-resolution mass spectra were obtained with either a Finnigan Series 3200F or a Ribermag 10-10b quadrapole gas chromatograph/mass spectrometer. Elemental analyses were performed by Galbraith Laboratories (Knoxville, Tenn.).

Microsomal cytochrome P-450 concentrations were determined by the method of Omura and Sato (12), and microsomal protein contents were determined by the method of Lowry *et al.* (13).

A Packard 7400 Series gas chromatograph equipped with flame ionization detection and a 2-m 5% SP-2340 on 100/120 Chromosorb WAW glass column was employed for detection and for isolation for radioassay of metabolites. For detection and quantification of volatile metabolites the head space (0.5 ml) of the [1-^{14}C]propyl halide incubations was analyzed by gas chromatography, and the column effluent was collected at various retention times in scintillation fluid and assayed for radioactivity. The water-soluble metabolites were isolated by removal of the microsomes by Ca^{2+} precipitation (14) and filtration of the resultant supernatant with an Amicon DIAFLO apparatus and a PM-10 membrane. The filtrate was extracted continuously with diethyl ether for 24 hr, the ethereal layer was separated, and the ether was evaporated at 30° under a stream of nitrogen. A nearly quantitative (≥95%) extraction of the propyl halide metabolites was achieved by using this procedure. The residue from the ether extracts was dissolved in methanol (200 μl) and analyzed for specific metabolites by gas chromatography as described above.

The amount of radioactivity from the [^{14}C]propyl halides bound to microsomal protein was determined by homogenizing the isolated microsomes (14) in methanol (10 ml) followed by centrifugation (5000 × *g*) to remove any noncovalently bound radioactivity. This process was repeated until the amount of radioactivity in the methanol washes was equal to the background. The microsomes washed in this way were solubilized using methanolic potassium hydroxide (20%, 2 ml) at room temperature overnight, and aliquots (0.4 ml) were assayed by liquid scintillation spectrometry.

The formation of glutathione adducts was determined by incubation of the propyl halides with hepatic microsomes as noted above in the presence of 1 mM glutathione. The incubation medium was then filtered as described above, and the filtrate was lyophilized to dryness. The residue was dissolved in methanol (0.5 ml), and aliquots (50 μl) were applied to preparative DEAE-cellulose thin-layer chromatographic plates along with authentic *S*-propyl glutathione. The plates were developed with the solvent system 1-propanol/0.1 M ammonium carbonate (4:1). *S*-Propyl glutathione was visualized with ninhydrin reagent. Cellulose in the area of the plate corresponding in R_F to that of *S*-propyl glutathione was removed, placed in scintillation vials, and assayed for radioactivity by liquid scintillation spectrometry.

Synthesis of Glutathione Adducts

S-(1′-Propyl)glutathione. A solution of 1-iodopropane (850 mg, 5.0 mmoles) in ethanol (5 ml) was added to an aqueous solution of sodium hydroxide solution (1.0 N, 10 ml) containing glutathione (1.540 g, 5.0 mmoles) in the dark. The solution was rendered homogeneous by the addition of ethanol (~5 ml), and the mixture was warmed to 55° for 72 hr. The reaction mixture was then lyophilized to dryness, dissolved in a 4:1 solution of 1-propanol/0.1 M ammonium carbonate, and chromatographed on a column (2 × 30 cm) of DEAE-cellulose (Whatman, DE-32). The column was eluted with the 1-propanol/0.1 M ammonium carbonate solvent system. The *S*-propylglutathione derivative was further purified by preparative thin-layer chromatography on DEAE-cellulose and the solvent system 1-propanol/0.1 M ammonium carbonate (4:1). The amorphous white powder (0.120 g, 0.342 mmole, 6.8%) prepared in this way had an NMR spectrum consistent with that of *S*-(1′-propyl)glutathione. ^{1}H-NMR (in ^2H_2O) δ: 1.21 (3 *h*, *t* J = 6.4 Hz), 1.79 (2 *H*, sext J = 6.4 Hz), 2.33 (2 *H*, *t* J = 7.2 Hz), 2.55 (2 *H*, *d* J = 7.4 Hz), 2.63 (2 *H*, *t* J = 6.4 Hz), 3.03 (2 *H*, *m*), 3.77 (3 *H*, *m*), 4.63 (1 *H*, *dd* J = 6.4, 7.4 Hz); ^{13}C-NMR (in ^2H_2O)

parts per million: 175.5, 174.9, 173.9, 172.2, 54.2, 53.3, 43.0, 33.8, 32.9, 31.5, 26.3, 22.3, 12.7. Elemental analysis of the product:

$C_{13}H_{23}N_3O_6S$

Calculated:	C 44.65,	H 6.64
Found:	C 44.29,	H 6.72

These results are also consistent with the structure *S*-(1′-propyl)glutathione.

S-(2′-Hydroxyl-1′-propyl)glutathione. Glutathione (4.61 g, 15.0 mmoles) and 1,2-epoxypropane (4.20 g, 75.0 mmoles) were dissolved in aqueous sulfuric acid (1.0 N, 30 ml) and allowed to stand under nitrogen at room temperature for 24 hr. The mixture was extracted four times with chloroform (10 ml each time), the aqueous phase was evaporated to dryness, and the residue was chromatographed on a column (2 × 30 cm) of DEAE-cellulose (Whatman, DE-32). The solvent system was 1-propanol/0.1 M ammonium carbonate (4:1). This procedure yielded an oily, amorphous solid (172 mg, 0.470 mmole, 3.1%), which was a mixture of the two diastereomers of *S*-(2′-hydroxy-1′-propyl)glutathione. This product had an NMR spectrum consistent with that of *S*-(2′-hydroxy-1′-propyl)glutathione. ^{1}H-NMR (in ^2H_2O) δ: 1.38 (3 *H*, *d* J = 7.0 Hz), 2.34 (2 *H*, *d* J = 7.2 Hz), 2.60 (2 *H*, *t* J = 6.4 Hz), 2.72 (2 *H*, *m*), 3.03 (2 *H*, *m*), 3.80 (4 *H*, *m*), 4.65 (1 *H*, *dd* J = 6.4, 7.4); ^{13}C-NMR (in ^2H_2O) parts per million: 174.8, 173.8, 172.9, 171.4, 68.0, 54.2, 53.4, 41.6, 40.2, 33.7, 31.4, 26.2, 21.4. Elemental analysis of the product:

$C_{13}H_{23}N_3O_7S$

Calculated:	C 42.73,	H 6.35
Found:	C 42.10,	H 6.68

These results are also consistent with the structure of the product's being *S*-(2′-hydroxy-1′-propyl)glutathione.

RESULTS

The studies reported here were carried out with rat hepatic microsomes from phenobarbital-induced Sprague-Dawley rats. However, the involvement of the cytochrome P-450 monooxygenase system in the formation of the observed microsomal metabolites was established by examining the metabolism of propyl iodide by a reconstituted cytochrome P-450 monooxygenase system purified from the livers of rats pretreated with phenobarbital (15) and noting the similarity in the metabolites with those obtained using hepatic microsomes. Because of the large amount of the cytochrome P-450 monooxygenase system needed for these studies, hepatic microsomes rather than a reconstituted system were used.

The incubation period of hepatic microsomes with the propyl halides was standardized at 60 min to ensure adequate metabolite production. However, the metabolism of propyl iodide was not linear over the 1-hr incubation time.

A volatile metabolite was detected in the head space of the incubation containing propyl bromide and propyl iodide. This compound was tentatively identified as propene by gas chromatographic/mass spectroscopic analysis. The mass spectrum of this compound was invariably contaminated by higher molecular weight impurities, precluding precise structure determination and differentiation from propane, a mechanistically feasible metabolite resulting from one- or two-electron reduction of the propyl halide. To differentiate between these possible enzyme-catalyzed products, the unambiguous structure determination of a higher molecular weight derivative of propene was pursued. Thus, bromination of the head space derived from the microsomal incubation with propyl iodide was carried out. Analysis of the head space revealed that propene had been converted to 1,2-dibromopropane. The structure of 1,2-dibromopropane was unambigously confirmed by gas chromatography/mass spectroscopy. The data for formation of propene from the various propyl halides are shown in Table 1.

The formation of a trace amount (<40 pmoles/nmole of cytochrome P-450/60 min) of an additional volatile metabolite, identified as 1,2-epoxypropane through comparison with an authentic sample by gas chromatography/mass spectrometry, was detected in the incubation medium containing propyl iodide but not in the incubation media containing propyl chloride or propyl bromide. The presence of additional volatile metabolites (e.g., propane, propanal, and 1-propanol), which was anticipated on the basis of mechanistic considerations, could not be confirmed despite a sensitive assay procedure (~10 pmoles/incubation).

Analysis of the ether extract of the aqueous phase obtained from the microsomal incubation with each propyl halide produced evidence for two additional metabolites. The structure of these metabolites were shown to be the propionic acid and 1,2-propanediol by comparison of their mass spectra with that of authentic compounds. The data for formation of propionic acid and 1,2-propa-

TABLE 1

Formation of metabolites of [1-^{14}C]halopropanes

Halide	Metabolite[a]		
	Propene	1,2-Propanediol	Propionic acid
	pmoles metabolite/nmole cytochrome P_{450}/hr		
[1-^{14}C]Chloropropane			
Complete incubation system	<10[b]	1250 ± 94	263 ± 18
Minus NADPH	<10	<10	<10[b]
[1-^{14}C]Bromopropane			
Complete incubation system	151.2 ± 30.0	3290 ± 1450	637 ± 280
Minus NADPH	22.3 ± 5.5	<10	<10
[1-^{14}C]Iodopropane			
Complete incubation system	984.6 ± 24.0	2180 ± 510	112 ± 28
Minus NADPH	18.4 ± 6.1	<10	<10

[a] These values represent means of (at least) duplicate determinations ± standard deviation. All values were determined by gas chromatographic isolation of the metabolite by effluent gas trapping and radioassay.

[b] On the basis of the specific activity of the propyl halides used in these experiments, the limit of detection was approximately 10 pmoles of metabolite per incubation.

nediol are shown in Table 1. No evidence for the existence of additional water-soluble metabolites was obtained.

The addition of glutathione (1.0 M) to the incubation mixtures containing the propyl halides produced a substantial increase in the amount of water-soluble, non-ether-extractable radioactivity. Evidence for the formation of *S*-(1′-propyl)glutathione and *S*-(2′-hydroxyl-1′-propyl)glutathione was provided by chromatography on DEAE-cellulose against authentic standards (Table 2). The structure of the *S*-(1′-propyl)glutathione adduct formed in the incubation medium containing propyl iodide was verified by obtaining the chemical ionization mass spectrum of the isolated metabolite and comparing it with that of authentic synthetic material. The amount of *S*-(2′-hydroxy-1′-propyl)glutathione formed in the incubation with propyl iodide was insufficient to obtain a mass spectrum. However, the behavior on DEAE-cellulose thin-layer chromatography was identical with that of authentic *S*-(2′-hydroxy-1′-propyl)glutathione. In the case of propyl chloride and propyl iodide, the formation of *S*-propyl glutathione was NADPH-dependent, suggesting the involvement of cytochrome P-450-containing monooxygenase systems. In the case of propyl bromide the data are anomalous. No dependence on NADPH could be demonstrated consistently for formation of *S*-propyl glutathione from propyl bromide. In fact, the presence of NADPH consistently decreased the amount of *S*-propyl glutathione formed in comparison with the incubation carried out in the absence of NADPH. The formation of *S*-(2′-hydroxy-1′-propyl)glutathione was shown to be NADPH-dependent for all three propyl halides. There was a suggestion (<20 nmoles/nmole of cytochrome P-450/60 min) of NADPH-stimulated binding of radioactivity to microsomal macromolecules in the incubation medium containing propyl chloride but not in the incubations containing propyl bromide or propyl iodide.

DISCUSSION

Cytochrome P-450-containing monooxygenases catalyze several diverse oxidation reactions which have as a common unifying feature the formation of new covalent bonds in the substrate molecule with the incorporation of one atom of oxygen (5). In addition, these enzymes are capable of the reductive metabolism of several classes of compounds with a high oxidation status, including tetrahalomethanes, arene oxides, and tertiary amine oxides (6–8).

The present study has attempted to define the initial chemical transformations involved in the metabolism by the cytochrome P-450-containing monooxygenase systems of propyl halides (chloride, bromide, and iodide). In this study both the structure of the metabolic products and the relative distribution of these metabolites as a function of the carbon-bound halide have been examined.

A comparison of the formation of the various metabolites as a function of the halogen substituent on the propyl group is complicated to some extent by the apparently anomalous behavior of propyl bromide in these incubations. For example, NADPH-stimulated *S*-propyl glutathione was absent, in contrast to propyl chloride and propyl iodide. In addition, large standard deviations were experienced in the formation of propionic acid and 1,2-propanediol in the incubations containing propyl bromide. Nevertheless, if the rates of formation of propene are examined (Table 1), one sees an increase as one progresses down the halide order: chloride < bromide < iodide. There is the same tendency in the rates of formation of *S*-(2′-hydroxy-1′-propyl)glutathione. In the case of 1,2-propanediol (Table 1) there is also a general trend for an increase in rate of formation as one progresses down the halide order: chloride < iodide ≤ bromide. When the data with propyl bromide are ignored, the formation of *S*-propyl glutathione (Table 2) is greater with propyl iodide than with propyl chloride. In the case of propionic acid (Table 1), the amounts formed appear, in general, to be the reverse of those of the other metabolites, namely iodide < chloride ≤ bromide.

Additional insight into the chemical mechanism of organohalide metabolism by cytochrome P-450 could have been obtained by a knowledge of the rates (V_{max}) for the disappearance of starting propyl halide. However, the differential volatility and water solubility of the propyl halides precluded the generation of meaningful data from simple halide extraction (e.g., hexane) from the incubation mixture and radioassay. We have obtained qualitative data for the disappearance of starting halide by observing the absolute quantities in the head space after an incubation period versus a control (minus NADPH) incubation. These data suggest that the cytochrome P-450-catalyzed disappearance of propyl halides follows the "halide order" and are consonant with the investigations of others on the metabolism of analogous alkyl halides (6–8): propyl iodide > propyl bromide > propyl chloride.

Metabolites which are difunctionalized (at C_1 and C_2) comprised the major class of metabolic products derived from the propyl halides investigated. We postulate that the formation of propene by dehydrohalogenation is the initial and predominant enzyme-mediated transformation of these propyl halides. Related alkyl halide β-elimination processes are well-established metabolic reactions *in vivo*: the principal mode of mammalian metabolism of 2,2-(4′-chlorophenyl)-1,1,1-trichlorethane

TABLE 2

Formation of glutathione derivatives of [1-^{14}C]halopropane metabolites

Halide	Metabolite[a]	
	S-Propyl glutathione	*S*-(2′-Hydroxy-1′-propyl) glutathione
	pmoles metabolites/nmole cytochrome P_{450} /hr	
[1-^{14}C]Chloropropane		
Complete incubation system	220 ± 10	31 ± 3
Minus NADPH	60 ± 36	20 ± 4
[1-^{14}C]Bromopropane		
Complete incubation system	408 ± 27	147 ± 8
Minus NADPH	696 ± 58	24 ± 4
[1-^{14}C]Iodopropane		
Complete incubation system	1070 ± 20	190 ± 148
Minus NADPH	30 ± 1	9 ± 1

[a] These values represent means of (at least) duplicate determinations ± standard deviation. These values were determined by radioassay of the solubilized microsomal protein for protein-bound adducts and of glutathione adducts isolated by thin-layer chromatography.

(DDT) is by dehydrohalogenation to form 2,2-(4′-chlorophenyl)-1,1-dichloroethylene (DDE) (16, 17) and the *in vivo* metabolism of alkyl bromides has been suggested to proceed by dehydrohalogenation and subsequent olefin epoxidation (18). Our studies implicate the cytochrome P-450 system as being responsible for the dehydrohalogenation of the propyl halides.

The dependence on the carbon-bound halide of the mode of cytochrome P-450-catalyzed metabolism of the propyl halides into either C_1,C_2-functionalized or C_1-functionalized species provides important information relative to the chemical mechanism of metabolism of the propyl halides and, perhaps, other haloalkanes. Atom-selective differences in the metabolism of isoelectronically substituted series of compounds are well documented. Thus, alkyl amines produce predominantly intermediate α-hydroxy amines, whereas the isoelectronic alkyl phosphines generate phosphine oxides; alkyl ethers produce intermediate α-hydroxy ethers, whereas the isoelectronic alkyl sulfides and selenides generate sulfoxides and selenoxides (5, 19–21). We postuate that the metabolic products of alkyl halides reflect a related continuum of the α-hydroxylation and heteroatomic oxygenation processes exhibited by the Group V and Group VI elements. Our proposed mechanism for the cytochrome P-450 monooxygenase-catalyzed metabolism of propyl halides is shown in Fig. 1. The steps thought to be catalyzed by the cytochrome P-450 monooxygenases are indicated by the notation *P-450*. Non-cytochrome P-450-catalyzed reactions (perhaps enzyme-catalyzed) of the proposed intermediates are labeled with letters (*A–I*). Products which are judged too unstable to be isolated are *bracketed*.

We propose that the metabolites formed with each of the propyl halides examined are a consequence of the partitioning of the initial metabolic transformation between α-hydroxylation and heteroatomic oxygenation. α-Hydroxylation of the propyl halide *1* would provide the geminal hydroxy halide *2*, which would subsequently collapse (*A*) to propionaldehyde *3*. This proposed α-hydroxylation pathway leading from an alkyl halide to a *gem*-halohydrin is well documented and underlies several

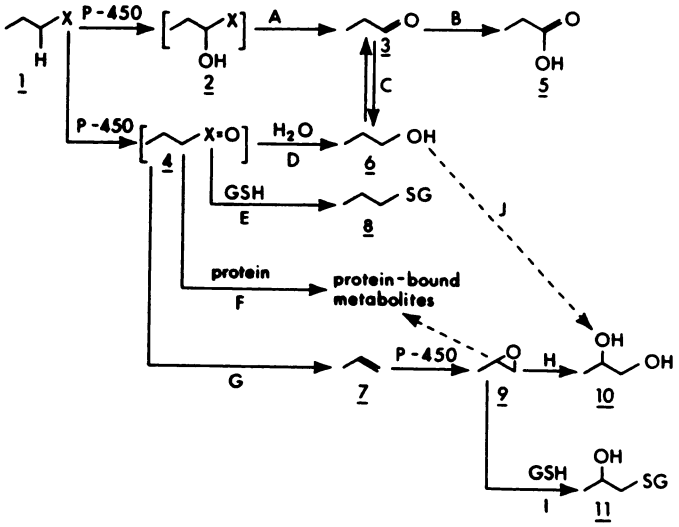

FIG. 1. *Proposed mechanism for metabolism of propyl halides by cytochrome P-450-containing monooxygenases*

critical processes mediated by cytochrome P-450 enzymes, including *N*- and *O*-dealkylation and halocarbon bioactivation (4, 5, 19–21). Oxygenation of the heteroatom of the propyl halide would provide a transient intermediate organohalide oxide *4*, which would rapidly collapse (*G*) via β-elimination or nucleophilic substitution processes (*D, E, F*) as illustrated by model system studies on alkyl iodosyl species (9). The relative contribution of heteroatomic oxygenation apparently increases as the halide progresses down the periodic table from chloride to bromide to iodine. Although heteroatomic oxygenation is well established for the Group V and Group VI elements (5, 19–21), this pathway has only recently received support as a major metabolic reaction for iodide in the Group VII elements (10). Thus, we believe the current data suggest that the propyl halide metabolites and the metabolite distribution dependence on halide are consistent with the intervention, as major initial metabolic products, of organohalogen oxides of the chloride and bromide as well as of the iodide. Experimental support for the postulation of organobromide oxide species has recently appeared with the finding of Nguyen and Martin (22) of the first stable bromosyl derivative. The rationale for the increased predominance of heteroatomic oxygenation over α-hydroxylation as the nonmetallic elements progress down a periodic group column is not clear at present but possibly is related to the difference in the energies between the heteroatom-oxygen bond and the stability of a cation or radical (or an incipient species or a transition state resonance contributor) adjacent to the heteroatom (*vide infra*). Thus, subtle adjustments in the stabilities of the (incipient or resonance form) radicaloid or cationic species or of the heteroatomic oxygen bond (e.g., by 1,1-dihaloalkane substitution) may dramatically alter the course of the metabolic process.

The only metabolic product of the propyl halides detected in this study which we believe was derived from the α-hydroxylation pathway, and most likely generated by oxidation of the intermediate aldehyde *3*, was propionic acid *5*. The reduction of *3* to 1-propanol *6* is also a possible route of metabolism. However, 1-propanol was not detected in these studies. Propionaldehyde *3* also was not observed as a metabolite in these studies, which is consistent with the ease of metabolism of aldehydes in biological systems to carboxylic acids. The lack of observation of propionaldehyde may also have been the consequence of binding of the compound to microsomal protein.

As noted above, the proposed halogen oxide species *4* may undergo two major non-enzymatic reactions: β-elimination and nucleophilic substitution. The relative contributions of these reaction pathways is likely a function of the lifetime and stability of the intermediate halosyl compound, which, in turn, is dependent upon the halide. We suggest that the stability order for these halosyl species is chlorosyl > bromosyl > iodosyl. In order to rationalize the product distribution, β-elimination-generating propene *7* must be the predominant pathway for these postulated halosyl intermediates. The suggestion of NADPH-stimulated protein binding of propyl chloride may be a consequence of a chlorosyl intermediate *4* which is sufficiently stable to escape immediate β-elimination and undergo displacement by a protein-bound

nucleophile (pathway *F*). In an attempt to trap these transient halosyl species, exogenous glutathione was added to the incubation mixture. The formation of the alkylated product, *S*-propyl glutathione *8*, was dependent upon cytochrome P-450 in the case of propyl chloride and propyl iodide. We postulate that the formation of propyl glutathione results from glutathione displacement on the intermediate organohalogen oxide *4* (pathway *E*).

The additional difunctionalized metabolites are most likely derived from further metabolism of propene *7*. Thus, cytochrome P-450-mediated epoxidation of propene *7* would afford 1,2-epoxypropane *9*, which could become hydrolyzed either directly or enzymatically to form 1,2-propanediol *10* (pathway *H*). 1,2-Epoxypropane has been shown to be a substrate for the microsomal epoxide hydratase enzyme (23). If exogenous glutathione is present, epoxide *9* could undergo nucleophilic displacement by glutathione, perhaps catalyzed by a glutathione transferase enzyme (24), to afford *S*-(2′-hydroxy-1′-propyl)glutathione *11* (pathway *I*). It is possible that a portion of 1,2-propanediol *10* is derived from the cytochrome P-450-mediate β-hydroxylation of 1-propanol (pathway *J*). However, incubation of propanol with hepatic microsomes followed by gas chromatographic/mass spectroscopic analysis of the incubation media did not show the presence of *10*. It is additionally possible that a small amount of the hydroxypropyl glutathione adduct *11* is derived from enzyme-catalyzed β-hydroxylation of *S*-propyl glutathione *8*.

Detailed mechanistic interpretations of haloalkane metabolism by cytochrome P-450 enzymes are complicated, since neither the mode of substrate binding (steric factors) nor the intrinsic nature of the oxidation reactions (e.g., free radical, concerted, or other) has been resolved (25, 26). At present, the favored model for the active oxygen species in cytochrome P-450-catalyzed reactions is an oxygen atom transiently bound to the heme iron (27). This ferryl oxygen model is considered to possess a highly electrophilic species of oxygen (28). The mechanism by which oxygen is transferred from the ferryl oxygen species to the substrate has centered on an "oxenoid" transfer scheme in which the ferryl oxygen reacts with substrates in a concerted fashion as something equivalent to a neutral oxygen atom, or "oxene" (27, 28). Recently, experimental evidence has accumulated which suggests that this concerted, oxenoid mechanism for oxygen transfer may not be entirely consistent and that an oxidation scheme involving radicaloid processes of sequential single-electron or hydrogen transfer may be operative (26, 29–34).

If such radicaloid processes for oxygen transfer to halogen-substituted alkanes are considered, conceptually different mechanistic rationales for halocarbon metabolism arise. Although we postulate hypervalent organohalide oxides (iodosyl, bromosyl, and chlorosyl species) as reactive products in the enzymatic activation of some organohalides, other hypervalent halide species may be involved as either intermediates leading to these halogen oxides or as reactive intermediates themselves (Fig. 2). For example, propylhalide cation radicals (e.g., *12*) may be involved as the first discrete intermediate leading to either halogen oxidation (*4*) or α-carbon oxidation via geminal hydrogen removal (e.g., *12* → *13* → *2*). Such sequential one-electron oxidation of haloalkanes occurs electrochemically (35–37) and has been suggested as the mechanism of oxidation with chemical agents, including peracid oxidation of alkyl iodides (38). Experimental evidence for the formation of nitrogen-centered cation radicals as intermediates in the *N*-dealkylation of amines by cytochrome P-450 and related enzymes has been presented through the observation of EPR signals attributable to such species (33, 34). In addition, the rate (V_{max}) of formation of aryl sulfoxides from the corresponding sulfide has been logarithmically correlated with the one-electron oxidation potential of the sulfide, suggesting, circumstantially, that oxygenation proceeds via one-electron oxidation by transfer from sulfide to the active species of the enzyme and subsequent recombination (30). These distinct chemical mechanisms for cytochrome P-450-catalyzed metabolism could have significant consequences with regard to the toxicological implications of metabolism of xenobiotic substances, since the two mechanisms do not necessarily generate identical spectra of metabolic products. For example, the radicaloid proc-

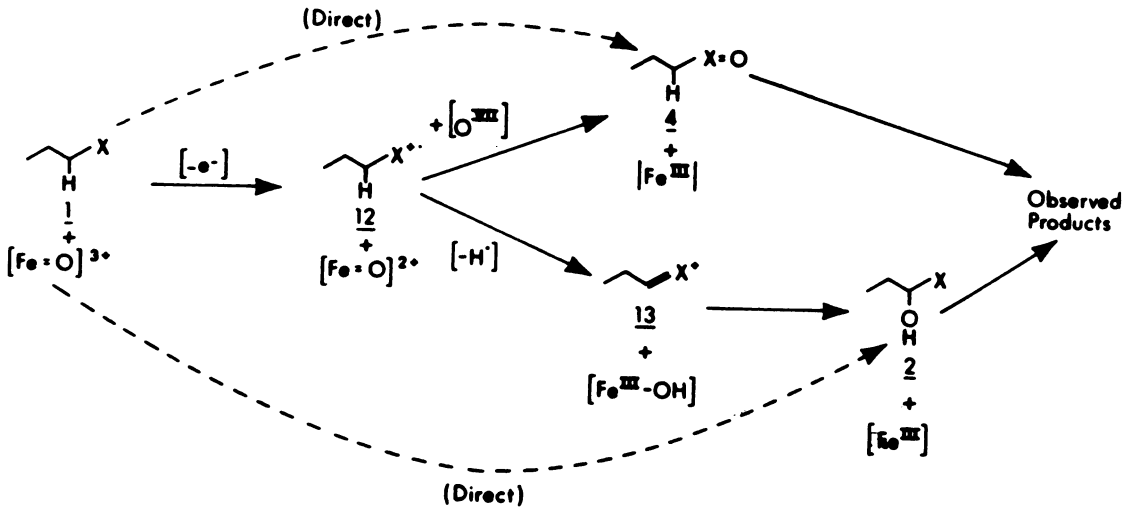

FIG. 2. *Enzymatic oxidation of propyl halide by direct (dashed line) or sequential one-electron transfer (solid lines) mechanisms*

ess must produce discrete, transient radical intermediates which may themselves possess toxic properties.

We suggest that mixed-function, oxidase-catalyzed, halogen oxidation of organohalides is a major metabolic process. These postulated hypervalent organohalide intermediates may constitute previously unrecognized metabolic products of critical relevance to the toxicology of this chemical class.

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