Thiocarbamate Herbicide Metabolism: Microsomal Oxygenase Metabolism of EPTC Involving Mono- and Dioxygenation at the Sulfur and Hydroxylation at Each Alkyl Carbon

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Metabolism of *S*-ethyl dipropylthiocarbamate (EPTC) by the mouse liver microsome–NADPH system involves oxidative attack at the following sites in decreasing relative importance: sulfur, α carbon of ethyl group, α carbon of propyl group, β carbon of propyl group, γ carbon of propyl group, and β carbon of ethyl group. The metabolites hydroxylated at the carbons α to the nitrogen and sulfur decompose at physiological pH, yielding *S*-ethyl *N*-propylthiocarbamate (*N*-depropyl-EPTC) in the former case and carbonyl sulfide and acetaldehyde from the latter compound. [^{14}C]EPTC sulfoxide is further oxidized to [^{14}C]EPTC sulfone which is most easily detected with microsomal preparations pretreated with unlabeled EPTC sulfone to block protein sites undergoing carbamoylation. These findings are consistent with previous studies on in vivo metabolism of EPTC in mammals which indicate major involvement of the sulfoxide intermediate and further suggest that hydroxylation of the carbon α to the sulfur is an important mechanism for thiocarbamate cleavage.

S-Ethyl dipropylthiocarbamate (EPTC) is metabolized in mammals and a liver microsomal oxygenase (MO) system by both sulfoxidation and N-depropylation (Casida et al., 1974, 1975a,b; Hubbell and Casida, 1977; Lay et al., 1975). EPTC sulfoxide readily carbamoylates glutathione (GSH) to yield its *S*-(dipropylcarbamoyl) derivative in a reaction facilitated by GSH *S*-transferases of the liver cytosol (Casida et al., 1974, 1975a,b; Hubbell and Casida, 1977; Lay et al., 1975). Further MO oxidation of EPTC sulfoxide would yield EPTC sulfone, which has not been detected as a metabolite of EPTC either because it does not form or it does not persist (Casida et al., 1975b). On oral administration of EPTC to rats, the sulfoxide serves as an intermediate in metabolism of about half of the dose, while much of the remaining portion undergoes ester cleavage by an unknown mechanism(s) (Hubbell and Casida, 1977; Lay et al., 1975).

The following areas of EPTC metabolism require clarification: (1) possible sulfone formation; (2) the mechanism of ester cleavage not involving the sulfoxide intermediate; (3) identification of metabolites from oxidation at the *N*-propyl and *S*-ethyl substituents. These aspects were examined using the mouse liver MO system.

MATERIALS AND METHODS

Spectroscopy. Nuclear magnetic resonance (NMR) spectra were obtained with a Perkin-Elmer R32B 90-MHz spectrometer. Samples were dissolved in deuteriochloroform using tetramethylsilane as the internal standard. Infrared (IR) spectra were recorded on a Perkin-Elmer 457 IR spectrophotometer using chloroform solutions. Chemical ionization–mass spectra (CI–MS) were obtained with a Finnigan Model 1015D mass spectrometer in combination with a System Industries Model 150 control system using methane as the reagent gas with a CI source pressure of 0.5–1.0 Torr.

Chemicals. The EPTC derivatives are designated by abbreviations as shown in Figure 1. EPTC, *N*-dePr-EPTC, and [*ethyl*-^{14}C]EPTC were supplied by Stauffer Chemical Co. (Richmond, Calif.). Previously reported procedures were used to prepare EPTC sulfoxide and sulfone (Casida et al., 1975a,b), [^{14}C=O]EPTC and its sulfoxide and sulfone derivatives (Hubbell and Casida, 1977), and [*ethyl*-^{14}C]EPTC sulfoxide (Casida et al., 1975b).

Three monohydroxy derivatives of EPTC (β-HOEt-EPTC, β-HOPr-EPTC, and γ-HOPr-EPTC) were synthesized for this study. They were purified by thin-layer chromatography (TLC) (solvent system B, Table I), their purities were established by finding only single TLC spots in six different solvent systems (Table I), and they were identified by IR, NMR, and CI–MS (Table II). To prepare β-HOEt-EPTC, β-mercaptoethanol (106 mg, 1.36 mmol) and triethylamine (1 mL) were added to EPTC sulfone (140 mg, 0.63 mmol) in methanol (2 mL). This mixture was held for 15 h at 25 °C prior to solvent evaporation and product isolation in 45% yield. β- and γ-HOPr-EPTC were synthesized in 20–30% yields from ethyl chlorothioformate (prepared by the general procedure of Hubbell and Casida, 1977) and the appropriate amines. The intermediate amines (2- and 3-hydroxypropylpropylamines) were prepared by refluxing a mixture of 2- or 3-hydroxypropylamine (7.5 g, 0.1 mol) and 1-bromopropane (12.3 g, 0.1 mol) in ethanol (35 mL) for 20 h. The following purification procedure gave ~20% recovery of the desired hydroxypropylpropylamines (NMR and TLC criteria): evaporation to dryness under reduced pressure; extracting the resulting oil first with 30% aqueous sodium hydroxide and then with water; addition of sodium hydroxide to the water extract to give 10% (w/v) concentration, followed by back-extraction of the desired products into ether.

The metabolism studies described below used derivatization procedures to analyze acetaldehyde after conversion to the *N*-acetyl derivative (*N*-acetyl-TTCA) of 2,5,5-trimethylthiazolidine-4-carboxylic acid (TTCA) (Nagasawa et al., 1975) and carbonyl sulfide (COS) after conversion to diisobutylurea (DIBU). These ^{14}C products would form on decomposition of [^{14}C]α-HOEt-EPTC as shown in Scheme I. The ^{14}C label of [^{14}C=O]α-HOEt-EPTC would appear in DIBU and of [*ethyl*-^{14}C]α-HOEt-EPTC would appear in TTCA and *N*-acetyl-TTCA. Standard TTCA [pure by TLC in 1-butanol–water–glacial acetic acid (50:25:11); see Nagasawa et al., 1975] was prepared by reacting DL-penicillamine (PA) (150 mg) with 1.2 molar equiv of acetaldehyde in methanol (10 mL) for

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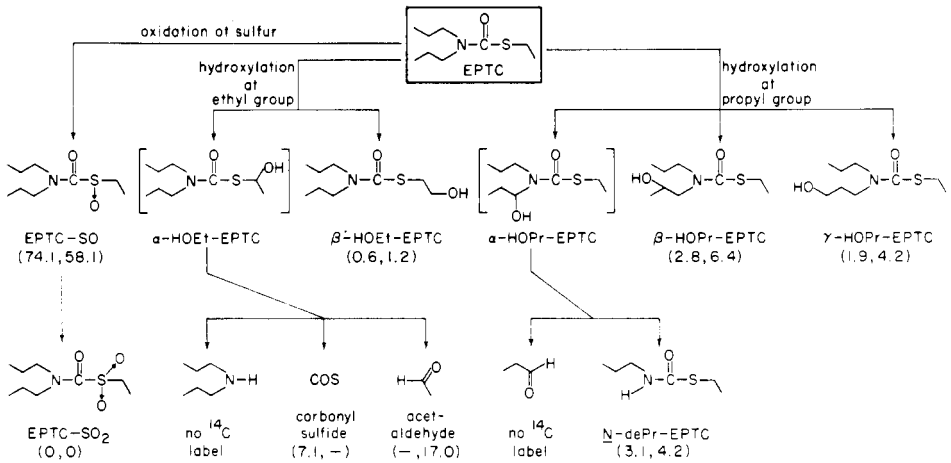


Figure 1. Metabolic pathways for EPTC in mouse liver microsome–NADPH system. Numbers in parenthesis are normalized yields for each metabolite from [^{14}C=O]EPTC and [*ethyl*-^{14}C]EPTC, respectively, calculated as metabolite amount relative to total metabolized EPTC. The yield for [*ethyl*-^{14}C]EPTC sulfoxide includes compound recovered without degradation and that portion that reacted with PA. Unstable intermediates are shown in brackets.

Table I. Chromatographic Characteristics of EPTC and Its Metabolites or Their Derivatives on Silica Gel Thin-Layer Chromatoplates

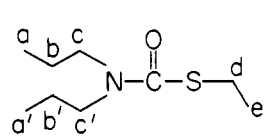
Compound[a]	TLC R_f[b] values in indicated solvent systems[c]							
	A	B	C	D	E	F	G	H
EPTC	0.52	0.55	0.64	0.67	0.34	0.59		
EPTC-SO	0.14	0.07	0.22	0.13	0.02	0.10		
EPTC-SO_2	0.44	0.46	0.62	0.63	0.20	0.56		
N-dePr-EPTC	0.38	0.44	0.61	0.61	0.18	0.51		
β-HOPr-EPTC	0.31	0.21	0.50	0.40	0.03	0.28		
γ-HOPr-EPTC	0.24	0.14	0.41	0.27	0.21	0.21		
β-HOEt-EPTC	0.27	0.20	0.47	0.36	0.04	0.29		
DIBU (COS)			0.38	0.23				
N-Acetyl-TTCA (CH_3CHO)							0.48	0.30

[a] See Figure 1 and intext figure in Materials and Methods section for structures. In the case of derivatives, the compounds derivatized are given in parentheses. [b] Silica gel 60 F-254 chromatoplates (20 × 20 cm, 0.25 mm gel thickness; EM Laboratories, Elmsford, N.Y.). [c] TLC solvent systems are as follows: A = *n*-hexane-acetone (6:1); B = *n*-hexane-ethyl acetate (3:2); C = ethyl acetate; D = diethyl ether; E = *n*-hexane-diethyl ether (4:1); F = toluene-ethyl acetate (3:2); G = chloroform-glacial acetic acid-*n*-hexane (7:2:1); H = diethyl ether-*n*-hexane-glacial acetic acid (15:9:4).

Table II. Spectroscopic Data for EPTC and Three of Its Hydroxylated Metabolites

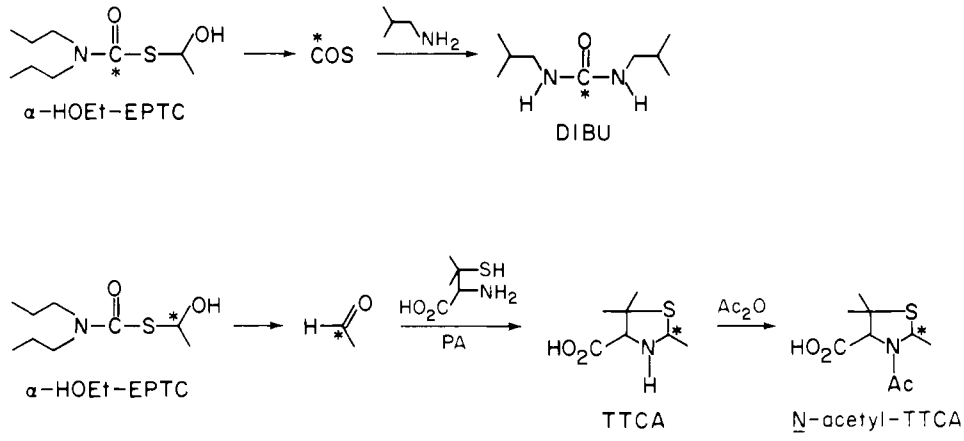
Parameter	Compound			
	EPTC	β-HOEt-EPTC	β-HOPr-EPTC	γ-HOPr-EPTC
Group		IR frequency (cm^{-1})		
C=O	1630	1630	1630	1630
O-H		3415, 1050	3415, 1110	3415, 1065
Proton[a]		NMR chemical shift, δ (multiplicity)[b]		
a	0.90 (t)	0.90 (t)	0.90 (t)	0.90 (t)
a′	0.90 (t)	0.90 (t)	1.25 (m)	3.60 (m)
b	1.60 (m)	1.65 (m)	1.60 (m)	1.65 (m)
b′	1.60 (m)	1.65 (m)	4.05 (m)	1.75 (m)
c	3.25 (t)	3.30 (t)	3.30 (m)	3.25 (t)
c′	3.25 (t)	3.30 (t)	3.40 (m)	3.50 (m)
d	2.90 (q)	3.15 (t)	2.90 (q)	2.90 (q)
e	1.25 (t)	3.85 (t)	1.25 (m)	1.25 (t)
C-O*H*		2.80 (s)	2.50 (s)	2.75 (s)
Fragment		CI-MS, *m/e* (rel intensity)		
$[M + 1]^+$	190 (100)	206 (10)	206 (100)	206 (25)
$[Pr_2NCO]^+$	128 (42)	128 (100)		
$[Pr(HOPr)NCO]^+$			144 (48)	144 (100)

[a] Protons designated as shown in insert figure. The hydroxyl substituents appear at e (β-HOEt-EPTC), b′ (β-HOPr-EPTC), and a′ (γ-HOPr-EPTC).



[b] Integrations are consistent with the formulations in all cases.

Scheme I



16 h at 25 °C and evaporation to dryness. It was acetylated on treatment with 2 molar equiv of acetic anhydride (Ac_2O) in methanol solution for 1 h at 25 °C. The acetylated derivative was isolated by TLC (solvent system G). The methyl ester of TTCA was obtained in methanol solution at 0 °C on addition of diazomethane (ether solution); solvent evaporation gave the pure material. The *N*-acetyl derivative and methyl ester of TTCA were characterized by NMR and CI–MS. DIBU was obtained by reacting phosgene with 4 molar equiv of isobutylamine in benzene (>95% yield) or by addition of COS (Matheson Gas Products, East Rutherford, N.J.) to excess isobutylamine in methanol, followed by holding for 1 h at 25 °C, evaporation of the solvent and excess amine, and addition of ether. DIBU was isolated pure (appropriate NMR and CI–MS) in each case on washing the benzene or ether solution with acid and evaporation of the solvent.

Chromatography. Table I gives the TLC conditions and R_f values for analysis of the EPTC metabolites or their derivatives. Identification of ^{14}C metabolites involved cochromatography in each of the indicated solvent systems using two-dimensional development with selected combinations of these systems. Unlabeled thiocarbamates and DIBU were detected with ninhydrin as previously reported (Casida et al., 1975b; Hubbell and Casida, 1977) whereas TTCA and its derivatives were visualized under ultraviolet (UV) light or with iodine vapor.

Qualitative analysis of COS in the gas phase above MO reaction mixtures described below involved gas chromatography (GC) using a silicic acid column and a thermoconductivity detector (Thornsberry, 1971) with metabolite identification by comparison of retention time with authentic standard.

MO Reactions. Liver microsomes from male albino mice were prepared by centrifugation of a 20% (w/v) liver homogenate in phosphate buffer (0.1 M, pH 7.4) at 14 500*g* for 15 min, recovery of the supernatant fraction, and subjecting it to centrifugation at 105 000*g* for 1 h. The microsomal pellet was washed once with buffer by resuspension and recentrifugation and finally was made up in buffer to the original 20% homogenate equivalent. In special studies, the microsomal preparations as above were treated with 2×10^{-3} M EPTC sulfone for 20 min at 25 °C, with subsequent washing by recentrifugation and resuspension at 0 °C; these preparations are referred to as carbamoylated microsomes.

^{14}C=O substrates (0.68 μmol of EPTC, 0.36 μmol of EPTC sulfoxide, or 0.45 μmol of EPTC sulfone) were incubated with reduced nicotinamide adenine dinucleotide phosphate (NADPH) (0 or 3.6 μmol) and microsomal preparation (1.5 mL, 20% liver fresh weight equivalent) for 50 min at 37 °C. In order to trap ^{14}COS and $^{14}CO_2$, the incubations were carried out in flasks with an inlet permitting continuous O_2 flow and an outlet through two tubes, each containing folded filter paper (to increase the surface area) saturated with 80% (v/v) isobutylamine in methanol. The metabolite reactions were terminated by adding 0.2 mL concentrated hydrochloric acid following which the O_2 flow through the gas phase of the system was continued for an additional 10 min. This trapping system is relatively efficient for $^{14}CO_2$ and ^{14}COS since the first trap contains >30 times more ^{14}C than the second trap. The filter papers were washed with diethyl ether, the ether was extracted twice with 1 N hydrochloric acid to remove excess isobutylamine, and the ether-soluble material was then analyzed by TLC (solvent systems C and D). ^{14}C material lost during the acid extractions was assumed to be $^{14}CO_2$.

Each acidified ^{14}C=O substrate reaction mixture on removal from the gassing system was cooled to 0 °C and extracted with chloroform (3 × 1.5 mL) to obtain essentially complete recovery of unmetabolized substrate and relatively stable metabolites retaining the ester linkage. Precipitated protein was collected by centrifugation and washed with absolute ethanol (2 mL) followed by acetone (2 mL) to separate protein-bound ^{14}C (analyzed by combustion with a Packard Sample Oxidizer) from the portion that was not protein bound; no significant amount of ^{14}C was recovered in these washes. Following determination of the ^{14}C content of the aqueous and chloroform fractions by liquid scintillation counting (LSC), the chloroform-soluble products after drying (anhydrous sodium sulfate) were separated by TLC, quantitated by LSC, and identified by TLC cochromatography. To minimize EPTC loss by volatilization, 2 mg of unlabeled EPTC were added to the chloroform extract before TLC development and the gel region containing EPTC (visualized under UV) was recovered immediately after the solvent developments for LSC. Quantitative data are corrected for the losses of EPTC (~20%) on volatilization and of EPTC sulfoxide (30–35%) and sulfone (45%) on TLC decomposition using the appropriate ^{14}C compounds as controls within each experiment.

The MO reactions with [*ethyl*-^{14}C]EPTC and [*ethyl*-^{14}C]EPTC sulfoxide were carried out as above except that they lacked the isobutylamine trap, contained 13.5 mmol of PA in the incubation mixtures, and were incubated in air. This procedural modification minimized further metabolism of [^{14}C]acetaldehyde because of its rapid conversion to [^{14}C]TTCA. The derivatization seems to be nearly quantitative since no loss of ^{14}C materials was evident in the form of acetaldehyde or other derivatives.

Table III. Metabolism of EPTC, EPTC Sulfoxide, and EPTC Sulfone by Mouse Liver Microsome-NADPH Systems

Compound or fraction	% of initial ^{14}C following incubation with microsomes plus indicated components[a]				
	^{14}C=O label without PA			Ethyl-^{14}C label with PA	
	EPTC	EPTC-SO	EPTC-SO_2	EPTC	EPTC-SO
Products Retaining Ester Linkage					
EPTC	31.3 (99.8)	0.0	0.0	49.9 (95.5)	0.0
EPTC-SO	50.9	87.7 (98.7)	0.0	25.3	89.6 (94.1)
EPTC-SO_2	0.0	1.6	38.1 (54.6)	0.0	0.0
N-dePr-EPTC	2.1	0.0	0.0	2.1	0.0
β-HOPr-EPTC	1.9	0.0	0.0	3.2	0.0
γ-HOPr-EPTC	1.3	0.0	0.0	2.1	0.0
β-HOEt-EPTC	0.4	0.0	0.0	0.6	0.0
Unknowns[b]	0.0	0.0	0.4 (0.4)	0.0 (1.0)	0.3 (0.3)
Cleavage Products					
COS	4.9	0.0	0.0	[c]	[c]
CO_2	1.3	0.0	0.0	[c]	[c]
CH_3CHO	[c]	[c]	[c]	8.5 (0.8)	0.0
Unknowns					
Organosoluble metabolites	1.4[d]	6.1[e]	20.9[f]	1.0[g]	0.0
Product of EPTC-SO reaction with PA[h]	[c]	[c]	[c]	3.8 (1.2)	4.3 (3.5)
Aqueous fraction[i]	1.5 (0.2)	3.4 (0.7)	5.9 (3.7)	3.3 (1.4)	5.3 (1.6)
Protein bound	0.6	1.2 (0.6)	4.4 (15.1)	0.2 (0.1)	0.5 (0.5)
Loss	2.4	0.0	30.3 (26.2)	0.0	0.0

[a] The data are given for systems containing NADPH followed in parenthesis by the appropriate control without NADPH. Where data are not given in the absence of NADPH the values are 0.0. Average of three experiments in each case. [b] Origin on TLC. [c] Not detectable with indicated labeling position. [d] Includes ^{14}C products at origin plus two ^{14}C products with R_f values in the indicated solvent systems as follows: (1) 0.45 (A), 0.45 (B); (2) 0.36 (A), 0.32 (B). [e] Includes two ^{14}C products with R_f values in the indicated solvent systems as follows: (1) 0.15 (A), 0.11 (B); (2) 0.07 (A), 0.04 (B). [f] Includes two ^{14}C products with R_f values in the indicated solvent systems as follows: (1) 0.12 (A), 0.11 (B); (2) 0.06 (A), 0.03 (B). These are products from cleavage of the ester linkage since they are not detected with [*ethyl*-^{14}C]EPTC-SO_2 in comparable experiments without PA. [g] One or more products at R_f 0.01–0.05 with both solvent systems A and B. [h] R_f values of 0.41 (G) and 0.25 (H). [i] May include metabolites other than cleavage products.

TTCA and *N*-acetyl-TTCA were characterized by TLC cochromatography, using a methanol extract of the acidified and lyophilized reaction mixture for TLC of [^{14}C]TTCA as above and a chloroform extract of the acidified reaction mixture for cochromatography of *N*-acetyl-[^{14}C]TTCA (prepared as below) in solvent systems G and H. In the quantitative studies, unlabeled acetaldehyde (50 μL) was added to each incubated reaction mixture which was then held 1 h at 25 °C. The chloroform-soluble materials recovered by extraction were analyzed as with the ^{14}C=O substrates. The aqueous phase of the ethyl-^{14}C reactions, which contains all the [^{14}C]-TTCA, was then treated with Ac_2O (50 μL) and pyridine (30 μL) and held 8 h at 25 °C. This solution was acidified with 0.2 mL of concentrated hydrochloric acid and extracted with chloroform (2 × 1.5 mL) for TLC of *N*-acetyl-[^{14}C]TTCA using solvent system G and quantitation by LSC. Further analysis of the aqueous and protein fractions proceeded as with the ^{14}C=O substrates.

RESULTS

Products Retaining Ester Linkage. The products of MO metabolism of [^{14}C=O]- and [*ethyl*-^{14}C]EPTC, -EPTC sulfoxide, and -EPTC sulfone are quantitated in Table III. The rate of metabolism is faster for [^{14}C=O]EPTC and its sulfoxide without PA than for the corresponding ethyl-^{14}C substrates with PA. This results from MO inhibition by PA rather than differences in oxygen concentration based on direct comparisons with and without PA in air and oxygen atmospheres.

EPTC sulfoxide is the major MO metabolite of EPTC. EPTC sulfone is not detected as an EPTC metabolite using normal microsomes but it is evident in trace amount (0.3%) in the presence but not in the absence of NADPH fortification of carbamoylated microsomes. The carbamoylated microsomes are slightly less active than normal microsomes in EPTC metabolism by sulfoxidation and carbon hydroxylation. Other ester metabolites are, in decreasing amounts, *N*-dePr-EPTC from degradation of α-HOPr-EPTC, β-HOPr-EPTC, γ-HOPr-EPTC, and β-HOEt-EPTC.

EPTC sulfoxide is relatively resistant to MO attack, the major identified ester product being EPTC sulfone (Table III); higher yields of EPTC sulfone are recovered with carbamoylated microsomes. No MO metabolites are identified with EPTC sulfone as the substrate.

Cleavage Products. α-HOEt-EPTC decomposes to give COS and acetaldehyde so the yield for this unstable thiocarbamate can be estimated from two types of analyses. Both procedures establish that hydroxylation of EPTC to give α-HOEt-EPTC is second in importance to sulfoxidation in the metabolism of EPTC by the MO system. Higher values are obtained by the acetaldehyde method than by the COS derivatization procedure. The presence of COS in the gas phase above the reaction mixture was confirmed by GC.

EPTC sulfoxide and EPTC sulfone do not undergo hydroxylation at the α carbon of the ethyl group since COS (and CO_2) are not detected as metabolites of these substrates, nor is acetaldehyde liberated on metabolism of EPTC sulfoxide.

EPTC sulfoxide reacts with PA to give an unidentified product, possibly the PA–ethyl disulfide or other product retaining the *S*-ethyl label. The amount of this product appears to be NADPH dependent with EPTC but not with EPTC sulfoxide as the substrate.

Products in the aqueous fraction appear in increasing amounts in the sequence EPTC, EPTC sulfoxide, and EPTC sulfone. Although these water-soluble metabolites are not identified because of their relatively small amounts,

their quantity is increased by NADPH fortification. The protein-bound fraction is relatively small except with [^{14}C=O]EPTC sulfone as the substrate. This fraction from EPTC sulfone is decreased on adding NADPH. The protein-bound ^{14}C from EPTC sulfoxide metabolism is lower with carbamoylated microsomes than with normal microsomes.

The only major losses occur with EPTC sulfone incubated in the presence or absence of NADPH. These losses are possibly attributable to $^{14}CO_2$ liberation on acidification prior to chloroform extraction. While it is possible that free [^{14}C]carbonate is present in the reaction mixtures, it appears more likely that EPTC sulfone reacts with a microsomal component(s) to form an acid-labile derivative. No attempt was made to define the nature of this reaction.

DISCUSSION

Figure 1 summarizes the findings on mouse liver MO metabolism of EPTC. The amount of each metabolite is expressed as a percentage of total metabolite formation to facilitate comparison of the products with the two [^{14}C]EPTC preparations.

Sulfoxidation is the major MO reaction of EPTC. This MO system lacks the GSH and GSH *S*-transferase components necessary for detoxification of EPTC sulfoxide. In rats in vivo about half of the EPTC dose is metabolized via the sulfoxide and the *S*-(dipropylcarbamoyl) derivative of GSH (Hubbell and Casida, 1977). Although the sulfoxide undergoes MO metabolism to form the sulfone, this is not likely to occur in vivo because of the ease of sulfoxide detoxification on reaction with GSH; the sulfone is sufficiently reactive that it would probably not be detectable in any case.

A major portion of a [^{14}C=O]EPTC dose but not of a [^{14}C=O]EPTC sulfoxide dose is metabolized in rats by a pathway leading to $^{14}CO_2$ liberation (Hubbell and Casida, 1977). Findings from the MO studies strongly suggest that this pathway involves formation of [^{14}C=O]α-HOEt-EPTC followed by decomposition to ^{14}COS which undergoes further metabolism to $^{14}CO_2$. Thus, COS is metabolized in MO systems and in vivo to CO_2 (Dalvi et al., 1974, 1975; De Matteis and Seawright, 1973). Acetaldehyde is also released on decomposition of α-HOEt-EPTC. The lower recoveries of COS than of acetaldehyde are attributable to the efficiency of PA as a trapping system for acetaldehyde, preventing its further metabolism, and the incomplete recoveries of COS either because of less efficient trapping or further metabolism. The isobutylamine trap is appropriate to detect CO_2 formation but not for quantitative studies on this product. The inclusion of PA in the reaction mixture decreases the proportion of sulfoxidation relative to alkyl carbon hydroxylation either by modifying the conformation of the cytochrome P-450 oxygenation site or by altering metabolic site specificity through other mechanisms.

EPTC not only undergoes major reactions of sulfoxidation and hydroxylation at the α carbon of the ethyl group but it is also hydroxylated at each of the other alkyl carbon atoms, the preference for hydroxylation at these sites decreasing in the sequence: α-HOPr, β-HOPr, γ-HOPr, and β-HOEt. Previous studies with mammals establish a large variety of products formed on EPTC metabolism (Lay et al., 1975; Hubbell and Casida, 1977; Ong and Fang, 1970). Most of these can be accounted for via the sulfoxide and GSH conjugate pathway. The present findings that EPTC undergoes MO carbon hydroxylation at each position of the *N*-propyl and *S*-ethyl groups establish possible pathways leading to many of the unidentified in vivo metabolites by combinations of various sites of hydroxylation, further oxidation of the alcohol metabolites, and formation of various conjugates.

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