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SULFOXIDATION OF THIOETHER-CONTAINING PESTICIDES BY THE FLAVIN-ADENINE DINUCLEOTIDE-DEPENDENT MONOOXYGENASE OF PIG LIVER MICROSOMES*

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Abstract—In the presence of NADPH and under aerobic conditions, thioether-containing organophosphorus and carbamate pesticides were oxidized by the FAD-dependent monooxygenase (EC 1.14.13.8) purified from pig liver microsomes. The stoichiometric relationship between NADPH and substrate during the course of the reaction was 1:1, and the product, in the case of disulfoton and phorate, was the sulfoxide. The product was optically active and further oxidation to the sulfone was not apparent. Furthermore, the sulfoxides of disulfoton, phorate and croneton were not substrates for this enzyme. *n*-Octylamine, a known cytochrome P-450 inhibitor, increased the rate of sulfoxidation reactions catalyzed by the FAD-dependent monooxygenase. Structure–activity relationships were investigated using thirty-nine possible substrates. Structural changes around the thioether sulfur that affect nucleophilicity or that cause steric hindrance tended to decrease the sulfoxidation rate. With phosphorodithioates, changes around the phosphorus atom also affected oxidation of the thioether sulfur. Although neither the thiono nor the thiol sulfur atoms were attacked, substitution of either sulfur by oxygen decreased sulfoxidation. Thioether-containing O,O-dimethyl phosphorodithioates were not oxidized as readily as their O,O-diethyl analogs. Tetram and its analogs, which contain a tertiary amine group, were also substrates for this enzyme, presumably forming the *N*-oxide.

Organophosphorus and carbamate pesticides are metabolized extensively by mammals, insects and plants [1–5], microsomal mixed function oxidases being primarily responsible for the oxidative reactions involved [6–9]. Such biotransformations increase the polarity and/or the ability to inhibit cholinesterase [1, 3, 4, 6, 7]. Incubation of thioether-containing pesticides with microsomal enzyme preparations under aerobic conditions and in the presence of NADPH results in the formation of sulfoxides and to a lesser extent sulfones [1, 2, 4, 6, 7]. With the majority of organophosphorus insecticides, other than phosphates and phosphorothiolates, sulfoxidation is followed by oxidative desulfuration of the thiono sulfur to yield the corresponding oxygen analog (oxon) [1, 4, 6, 7].

The nature and substrate specificity of the enzymes involved in sulfur oxidation have not been well characterized but have generally been considered to be the cytochrome P-450-dependent monooxygenase system [6–10]. However, there are other distinctly different enzymes present in hepatic microsomes. For example, the FAD-dependent monooxygenase (EC 1.14.13.8) is present in substantial amounts in hepatic microsomes and is a different entity from either of the two other microsomal flavoproteins, NADPH–cytochrome P-450 reductase and NADH–cytochrome b_5 reductase [8, 9, 11]. This flavoprotein was originally characterized as a relatively non-specific amine oxidase. Subsequent studies by Ziegler and co-workers [12, 13] demonstrated that it also catalyzed the oxidation of sulfur-containing compounds such as cysteamine and thioureas and is better described as a sulfur oxidase.

In a preliminary report [14] we described experiments which demonstrated the role of the FAD-dependent monooxygenase in the oxidation of sulfur-containing pesticides. This paper is a detailed account of these and subsequent experiments.

MATERIALS AND METHODS

Chemicals. The microsomal FAD-dependent monooxygenase that was purified to homogeneity from pig liver [15, 16] was provided by Professor D. M. Ziegler of the University of Texas at Austin. NADPH was purchased from the Sigma Chemical Co. (St. Louis, MO). Disulfoton and phorate sulfoxides were synthesized as described by Metcalf *et al.* [17]. They were purified by preparative thin-layer chromatography (TLC) on silica gel 60 F254 plates (2.0 mm thickness; E. Merck) chloroform–acetone (9:1) solvent system 1, and identified by NMR (60 MHz, $CDCl_3$, TMS internal standard) and mass spectroscopy. The pesticides presented in Tables 3 and 4 were obtained as indicated in the footnotes.

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O,O-Diethyl *S*-phenylphosphorodithioate was synthesized in a reaction similar to that reported for fonofos [18]. The product was purified by preparative TLC using hexane–acetone (5:1) as the developing solvent (R_f 0.65). NMR data: δ, 1.39 (H_6, m, CH_3); δ, 4.22 (H_4, m, CH_2); δ, 7.46 (H_5, m, aromatic).

Enzyme assay. The sulfoxidation of the pesticides by the FAD-dependent monooxygenase was measured by following the substrate-dependent oxidation of NADPH spectrophotometrically at 340 nm using an Aminco DW-2 UV-Vis spectrophotometer. Unless indicated otherwise, the reaction medium contained 0.05 M potassium phosphate buffer (pH 7.4) and 0.1 mM NADPH in a total volume of 3.0 ml and was incubated at 37° with continuous stirring. After an initial 3-min incubation for temperature equilibration, the reaction was started by the addition of the enzyme solution to a final concentration of approximately 1 nmole/ml, and the endogenous NADPH oxidation rate was recorded for 30 sec. The substrate (25 and/or 50 μM) was then added in 5 μl of acetone, and the oxidation of NADPH was recorded for an additional 30–60 sec. Reaction velocities were calculated from the substrate-dependent NADPH oxidation during the first 10–30 sec.

Metabolism of [methylene-^{14}C]phorate. [Methylene-^{14}C]phorate (sp. act. 9.7 μCi/μmole; Amersham Corp., Arlington Heights, IL) was incubated with the monooxygenase reaction medium at a final concentration of 50 μM (1.98×10^5 dpm). The substrate-dependent NADPH oxidation was monitored spectrophotometrically, as indicated above, until the rate returned to the initial endogenous rate (2.5 min). The reaction was stopped, and the product was recovered by extracting the incubation mixtures four times with an equal volume of chloroform. The chloroform solution was dried over $MgSO_4$, filtered, and evaporated under a stream of nitrogen. The residue was dissolved in 167 μl chloroform, and 10 μl aliquots were applied, together with authentic phorate and phorate sulfoxide, to 20×20 silica gel G F254 chromatoplates (0.1 mm thickness; Brinkmann Instruments, Westbury, NY). Over 93% recovery of the radioactivity was obtained. Similar recoveries were obtained with the controls in which no NADPH was added to the reaction media. The chromatoplates were developed in two dimensions in the following solvent systems described in Table 1: 1 and 4; 2 and 1; 3 and 4. Compounds were located by means of u.v. fluorescence and autoradiography on X-ray films.

Similar experiments were performed using unlabeled disulfoton and phorate at a final concentration of 100 μM (five replicates each) and analyzing the products by TLC (one dimensional, see Table 1). Compounds were visualized by u.v. fluorescence and by spraying with 2,4-dibromoquinone-4-chloromide. Products were identified by cochromatography and comparison of R_f values with those of authentic standards.

Optical activity of enzymatically produced disulfoton sulfoxide. The optical activity of the sulfoxide was measured by optical rotary dispersion spectroscopy in a model ORD/UV5 (Jasco Manufacturing Co., Easton, MD). The sulfoxide was enzymatically synthesized by incubating 0.5 mM disulfoton in a 3 ml reaction medium containing 1 mM NADPH and 2 nmoles/ml of the monooxygenase maintained at 37° in a metabolic shaker (both for 1.5 hr). Ten replicas were used and the incubation period was determined by means of a preliminary experiment in which the substrate-dependent NADPH oxidation was monitored spectrophotometrically. Following incubation, the replicas were combined and extracted three times with equal volumes of chloroform. The chloroform extracts were combined, dried over $MgSO_4$, and filtered, and the solution was evaporated to a small volume. An aliquot was analyzed by TLC using solvent system 1 (Table 1) which indicated the presence of the sulfoxide and some unreacted substrate. To avoid possible racemization, no attempt was made to purify the sulfoxide or estimate its concentration. Optical activity was measured in 1.2 ml (total volume) quartz cuvettes (1 cm light path). The sulfoxide was dissolved in 0.6 ml and scanned between 550 and 300 nm to detect optical activity. The solution was essentially particle free.

RESULTS

The chromatographic and spectroscopic data for disulfoton, phorate, and their sulfoxides are summarized in Tables 1 and 2.

Disulfoton and phorate were both metabolized rapidly by the monooxygenase. Under the conditions described, the reaction was essentially complete in 2–3 min (Fig. 1). From double-reciprocal plots of substrate/velocity data the apparent K_m and V_{max} values were 21.3 and 28.0 μM and 394 and 334 nmoles NADPH·min^{-1}·(mg protein)$^{-1}$ respectively. The reaction stoichiometries indicated that

Table 1. Thin-layer chromatographic R_f values of disulfoton, phorate and their sulfoxides

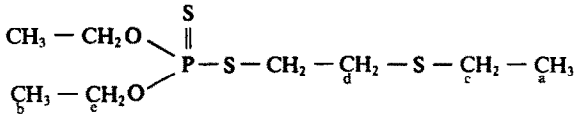
Solvent system*	R_f values			
	Disulfoton	Disulfoton sulfoxide	Phorate	Phorate sulfoxide
(1) Chloroform–acetone (9:1)	0.94	0.41	0.90	0.58
(2) Hexane saturated with methanol	—	—	0.73	0.04
(3) Chloroform–ether (9:1)	0.94	0.20	0.88	0.38
(4) Hexane–ethyl acetate–benzene (2:4:1)	0.90	0.07	0.88	0.28
(5) Benzene–acetone (9:1)	0.72	0.19	—	—

* Developed to 18 cm on silica gel G chromatograms, as described in Materials and Methods.

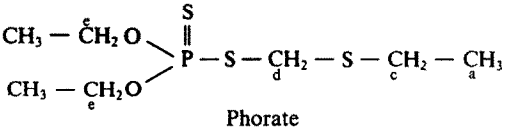
Table 2. Spectroscopic data for disulfoton, phorate and their sulfoxides

Parameter	Disulfoton	Disulfoton sulfoxide	Disulfoton sulfone	Phorate	Phorate sulfoxide
Proton*			NMR chemical shift (δ)		
a	1.3 (t)	1.43 (t)	1.43 (t)	1.34 (t)	1.36 (t)
b	1.4 (t)	1.43 (t)	1.43 (t)	1.42 (t)	1.36 (t)
c	2.63 (q)	2.82 (q)	3.08 (q)	2.73 (q)	2.82 (m)
d	3.03 (m)	3.3 (m)	3.52 (m)	4.0 (d)	3.97 (d)
e	4.2 (m)	4.22 (m)	4.2 (m)	4.22 (m)	4.25 (m)
			MS, *m/e*		
	274	290		260	276

* Protons are designated as shown below:



Disulfoton



Phorate

1 mole of NADPH had been oxidized per mole of substrate added (Fig. 1). This ratio remained constant over a 10-fold (5–50 μM) range of substrate concentration.

Thin-layer chromatographic analyses of the chloroform extract from the reaction mixture containing [methylene-^{14}C]phorate indicated that only one oxygenated metabolite was formed. The substrate was oxidized completely to its corresponding sulfoxide, no other metabolites or unmetabolized substrate having been detected. Similar results were obtained for unlabeled disulfoton. Sulfoxidation was essentially NADPH dependent since no product was formed in its absence and, in the absence of NADPH, [^{14}C]phorate was quantitatively recovered.

Optical rotary dispersion studies indicated that the disulfoton sulfoxide formed enzymatically was optically active (Fig. 2) and, thus, that oxygen addition was, at least in part, stereospecific. As expected, chloroform, disulfoton and chemically synthesized disulfoton sulfoxide did not exhibit optical activity. No attempt was made to elucidate the absolute configuration of the sulfoxide.

Structure–activity relationships. Thioether-containing organophosphates and carbamates were generally good substrates for the monooxygenase (Table 3), and the rates of metabolism of different substrates varied from 26.4 to 0.3 nmoles NADPH·min^{-1}·(nmole enzyme)$^{-1}$. Of the phosphorodithioates, disulfoton and phorate exhibited the highest rate of sulfoxidation [V_{max} of 15.8 and 12.7 nmoles NADPH·min^{-1}·(nmole enzyme)$^{-1}$.

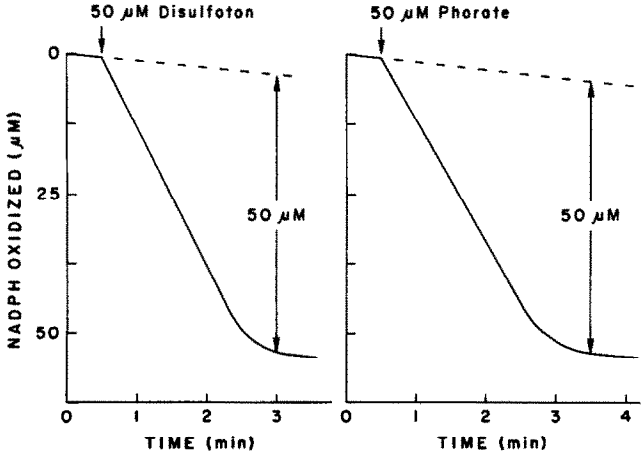


Fig. 1. Oxidation of disulfoton and phorate by the FAD-dependent monooxygenase from pig liver microsomes.

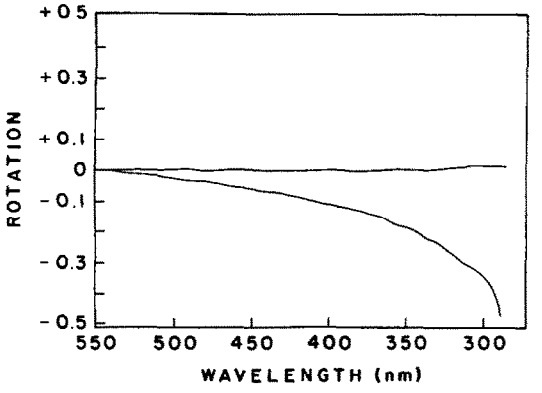


Fig. 2. Optical rotation of disulfoton sulfoxide formed by the FAD-dependent monooxygenase from pig liver microsomes.

Substitution of either the thiono or thiol sulfur by an oxygen atom (compounds 2, 3 and 5) reduced the rate of sulfoxidation. This effect was further enhanced by substitution of the *O,O*-diethyl groups by *O,O*-dimethyl groups (compounds 6–9). Substitution of the ethylene group of disulfoton by a methylene or aryl group (compounds 4, 5 and 8) reduced activity, and this effect was further enhanced by substituting the alkyl group of the ethylthio with an aryl or tertiary butyl group (compounds 9–11). Compounds lacking a thioether sulfur (compounds 12–16), as well as disulfoton and phorate sulfoxides, are not substrates. However, NADPH oxidation profiles for compounds 12–14 showed a brief initial oxidation for about 10 sec, followed by a return to the endogenous rate. This may have been due to initial binding of the substrate, followed by inhibition. Sulprofos (compound 20) was an excellent substrate for the monooxygenase [26.4 nmoles·min^{-1}·(nmole enzyme)$^{-1}$ and was oxidized at a much higher rate than the other pesticides tested. The phosphorothiolate, profenophos (compound 24), appeared to be a marginal substrate. However, although it is possible that the thiol sulfur could potentially be metabolized, the stoichiometries and product identification for this compound and sulprofos have not been determined. The phosphoramidates, methamidophos and acephate, as well as MOCAP (compounds 21–23) were not substrates for the monooxygenase.

The carbamate pesticides tested were generally not as good substrates as the phosphorodithioates. Thiofanox was metabolized rapidly at a rate of 9.5 nmoles NADPH·min^{-1}·(nmole enzyme)$^{-1}$. The rate was much less for its analog, aldicarb, while methomyl was not oxidized to any detectable extent. Methiocarb and croneton were metabolized at a lower rate, 2.8 and 3.2 nmoles NADPH·min^{-1}·(nmole enzyme)$^{-1}$, respectively. The triazine herbicides, metribuzin and terbutryn, the thiocarbamates, EPTC and cycloate, as well as croneton sulfoxide were not substrates. The oxidation of compounds 1–4, 7–11, 13, 15, 18, 21, 26 and 29 was measured following the addition of *n*-octylamine (3 mM) and the rate of oxidation increased by 1.8- to 3.0-fold for oxidizable substrates. *n*-Octylamine did not initiate metablism of non-substrates.

Tetram, a phosphorothiolate with a tertiary amine group replacing the thioether moiety, was also a substrate for the enzyme (Table 4) although the rate of oxidation was low. However, replacing the *N,N*-diethyl- by *N,N*-dimethyl groups and/or the oxyphosphoryl with the thiophosphoryl analog enhanced the rate of oxidation. Reaction stoichiometries determined for *O,O*-diethyl-*S*-(*N,N*-dimethylaminoethyl) phosphorodithioate (compound 3) indicated that 1 mole of NADPH are oxidized per mole of substrate added. Attempts to identify the product of this reaction were unsuccessful. Since tertiary amines are known to be oxidized to their corresponding *N*-oxides [12, 13, 19] by the FAD-dependent monooxygenase, the method of Craig and Purushothaman [20] was used in an attempt to synthesize the *N*-oxide of compound 3 (Table 4) which proved unsuccessful due to breakdown of the product. Thin-layer chromatography of methanol–water (1:1) extracts of the enzymatic reaction mixture similarly indicated considerable breakdown. Aminocarb and chlordimeform (compounds 4 and 5; Table 4) were not oxidized to any appreciable extent.

DISCUSSION

The FAD-dependent monooxygenase catalyzed the sulfoxidation of thioether-containing pesticides. Reaction stoichiometries indicated the formation of a monooxygenated product, and TLC analysis clearly demonstrated that the thioether sulfur of these compounds was oxidized to the corresponding sulfoxide. Disulfoton sulfoxide formed enzymatically was optically active, indicating that the reaction was stereospecific. Neither the thiophosphoryl nor the thiol sulfur atoms were oxidized by this monooxygenase. This reaction was established in detail for disulfoton and phorate, and by analogy it appears probable that the other substrates tested were oxidized in a similar manner. Elimination of NADPH from the reaction medium led to quantitative recovery of the substrate. Similar studies on non-pesticidal substrates by Poulsen *et al.* [13] showed an identical effect on elimination of oxygen. All substrates studied were metabolized primarily by oxidative attacks on the sulfur (Table 3) or nitrogen (Table 4) atoms.

Thirty-nine compounds were tested as possible substrates. Although the experiments were not structured in such a way as to evaluate binding and catalytic activity separately, they did demonstrate the effect of structure on overall activity and hence the potential role of this monooxygenase *in vivo*.

The structure–activity relationships indicate that changes around two centers present in phosphorodithioates affected the rate of thioether sulfoxidation. One of these is the phosphorus atom. Substitution by oxygen of either the thiono or the thiol sulfur atoms, which are not oxidized themselves, reduced activity. This was possibly due to the difference in electronegativity between the sulfur and oxygen atoms. Reduced activity was also observed in substrates with *O,O*-dimethyl substitutions (Table 3). The second center is the thioether present in both phosphorodithioate and carbamate pesticides. Thus,

Table 3. Structure–activity relationships in the sulfoxidation of thioether-containing pesticides by the FAD-dependent monooxygenase

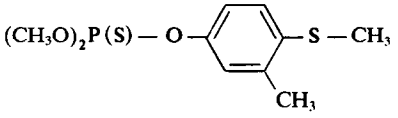
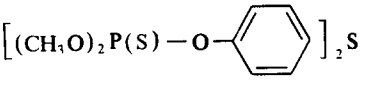
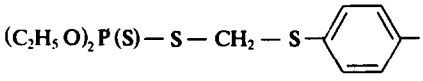
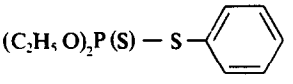
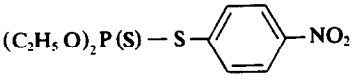
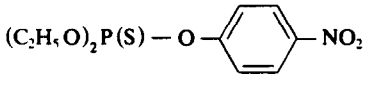
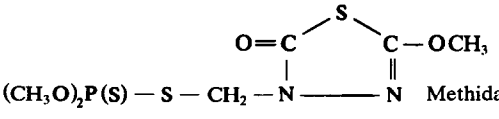
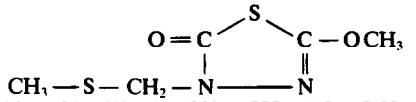
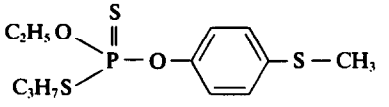
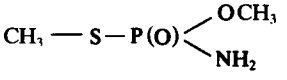
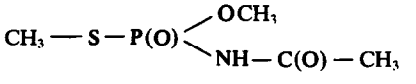
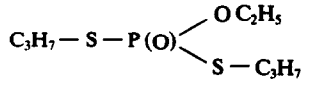
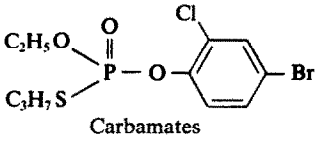
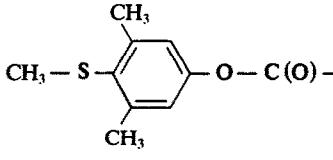
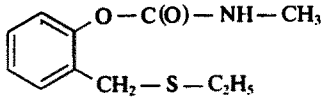
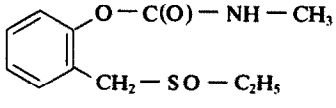
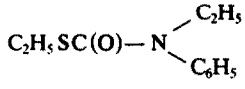
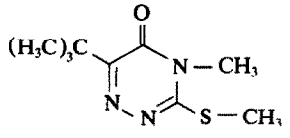
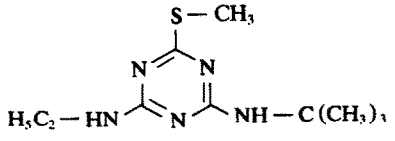
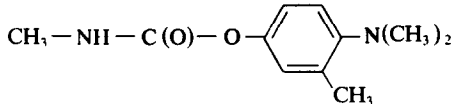
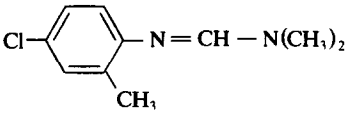
	Substrate*	Name	Activity ± S.D. [nmoles NADPH·min^{-1}·(nmole enzyme)$^{-1}$]
	Organophosphates		
1.	$(C_2H_5O)_2P(S)—S—CH_2—CH_2—S—C_2H_5$	Disulfoton	11.51 ± 0.42
2.	$(C_2H_5O)_2P(S)—O—CH_2—CH_2—S—C_2H_5$	Demeton O (thiono)	6.98 ± 0.59
3.	$(C_2H_5O)_2P(O)—S—CH_2—CH_2—S—C_2H_5$	Demeton S (thiolo)	4.24 ± 0.84
4.	$(C_2H_5O)_2P(S)—S—CH_2—S—C_2H_5$	Phorate	9.58 ± 0.90
5.	$(C_2H_5O)_2P(O)—S—CH_2—S—C_2H_5$	Phorate oxon	1.52 ± 0.27
6.	$(CH_3O)_2P(S)—S—CH_2—CH_2—S—C_2H_5$	Thiometon	5.27 ± 0.31
7.	$(CH_3O)_2P(O)—S—CH_2—CH_2—S—C_2H_5$	Demeton S (methyl)	2.47 ± 0.28
8.		Fenthion	7.54 ± 0.32
9.		Abate	0.0000
10.		Cl Carbophenothion	1.45
11.	$(C_2H_5O)_2P(S)—S—CH_2—S—C(CH_3)_3$	Counter	3.77 ± 0.41
12.	$(C_2H_5O)_2P(S)—S—CH_2—CH_2—C(CH_3)_3$		0.00
13.			0.00
14.			0.00
15.		Parathion	0.00
16.		Methidathion	0.00
17.			1.37 ± 0.14
18.	$(C_2H_5O)_2P(S)—S—CH_2—CH_2—SO—C_2H_5$	Disulfoton sulfoxide	0.00
19.	$(C_2H_5O)_2P(S)—S—CH_2—SO—C_2H_5$	Phorate sulfoxide	0.00
20.		Sulprofos	26.43 ± 0.66
21.		Methamidophos	0.00
22.		Acephate	0.00
23.		MOCAP	0.00

Table 3 (*continued*)

Substrate*	Name	Activity ± S.D. [nmoles NADPH·min^{-1}·(nmole enzyme)$^{-1}$]
24. 	Profenophos	0.30 ± 0.03
Carbamates		
25. $CH_3-S-CH_2-C(=N-O-C(O)-NH-CH_3)-C(CH_3)$	Thiofanox	9.53 ± 0.88
26. $CH_3-S-C(CH_3)_2-CH=N-O-C(O)-NH-CH_3$	Aldicarb	1.15 ± 0.12
27. $CH_3-S-C(CH_3)=N-O-C(O)-NH-CH_3$	Methomyl	0.00
28. 	Methiocarb	2.82 ± 0.03
29. 	Croneton	3.16 ± 0.20
30. 	Croneton sulfoxide	0.00
31. $C_2H_5SC(O)-N(C_3H_7)_2$	EPTC	0.00
32. 	Cycloate	0.00
Triazine herbicides		
33. 	Metribuzin	0.00
34. 	Terbutryn	0.00

* Compounds 1, 4, 8–11, 16, 21–23, 26, 27, 33 and 34 were purchased from Chem Service, West Chester, PA; 1 and 4 from the City Chemical Co., New York, NY; 2, 3, 7, 29, 30 were provided by the Mobay Chemical Corp., Kansas City, MO; 5, 6, 20, 24, 25, 28, 31 and 32 by the Environmental Protection Agency, Research Triangle Park, NC; 12 and 17 by Professor W. C. Dauterman, NCSU, Raleigh, NC; 14 and 15 by Dr. A. A. Nomeir, NIEHS, Research Triangle Park, NC; 13 was synthesized [17]; and 18 and 19 were synthesized [16]. All substrates were tested at 25 and 50 μM with the exception of some of the disulfoton series which were tested at 25 μM only. The results indicated that 25 μM was essentially saturating for all substrates tested at both concentrations.

Table 4. Oxidative metabolism of tertiary amine-containing pesticides by the FAD-dependent monooxygenase

	Substrate*	Name	Activity ± S.D. [nmoles NADPH·min^{-1}·(nmole enzyme)$^{-1}$]
1.	$(C_2H_5O)_2P(O)—S—CH_2—CH_2—N(C_2H_5)_2$	Tetram†	0.59 ± 0.14
2.	$(C_2H_5O)_2P(O)—S—CH_2—CH_2—N(CH_3)_2$	†	10.73 ± 0.11
3.	$(C_2H_5O)_2P(S)—S—CH_2—CH_2—N(CH_3)_2$	†	16.18 ± 0.38
4.		Aminocarb‡	0.00
5.		Chlordimeform‡	0.00

* Compounds 1–3 were provided by Professor W. C. Dauterman, NCSU, Raleigh, NC; 4 and 5 came from Chem Service, West Chester, PA.

† Activity was measured for their water soluble oxalate salts at a final concentration of 100 μM, 3 ml reaction volume.

‡ Activity was measured at a final concentration of 50 μM, 3 ml reaction medium.

structural changes on the thioether moiety that affect the oxidation potential and/or increase steric hindrance of the sulfur atom (i.e. reactivity) apparently affect enzyme–substrate binding and decrease the rate of sulfoxidation. This is best illustrated by the sulfoxides of disulfoton and phorate and by abate which are not substrates. Methidathion (compound 16) was not a substrate for the monooxygenase; however, its *in vivo* metabolite, 4-methylthiomethyl, 2-methoxy, 1,3,4-thiodiazol-5-one [21, 22] (compound 17) was oxidised to the corresponding sulfoxide. There was no apparent correlation between water solubility (of those compounds for which this value is known) and substrate activity.

Pesticides containing thioether sulfur are known to be metabolized to their corresponding sulfoxides and, to a lesser extent, to their sulfones [1–6]. Similarly, certain thiourea compounds have been shown to be oxidized by the FAD-dependent monooxygenase, first to their corresponding sulfenic and then to their sulfinic acids [13, 23]. However, the K_m values for the second oxidation were much higher than those for the first. In this study, we found that the pesticide sulfoxides were not oxidized at concentrations up to 100 μm. Thus, further oxidation may be due to another oxidase or in some cases may be non-enzymatic.

In general, carbamates were not as good substrates as the organophosphorus pesticides, and the thiocarbamate and triazine herbicides were not oxidized to any measurable extent. It is known, however, that their sulfoxides are the primary metabolites formed, both *in vivo* and *in vitro* [24–29]. It is interesting to compare the thiocarbamates (EPTC and cycloate) with the phosphoroamidates (methamidophos and acephate) and the phosphorodithiolates (MOCAP) with which the monooxygenase is also inactive. As with the thiocarbamates, there is biochemical evidence that the sulfoxide may be formed following the incubation of methamidophos with microsomal preparations [30].

Cyanatryn, a triazine herbicide and a structural analog of terbutryn (compound 34), is not oxygenated by the rat liver NADPH-dependent monooxygenase [29]. However, upon incubation with liver microsomes from rats, rabbits or humans, the *S*-oxide was rapidly formed. Microsomal oxidation was also inhibited by metyrapone and carbon monoxide, suggesting that the *S*-oxygenation is catalyzed by cytochrome P-450 [29].

Tetram and its analogues (Table 4) were also good substrates for the monooxygenase. Although the product from the oxidation of these tertiary amine-containing pesticides has not been isolated, it appears that the corresponding *N*-oxide forms. This is based on the following evidence: the thiono and thiolo sulfur atoms of phosphorodithioates are not oxidized; on incubation with the monooxygenase tertiary amines, such as *N*,*N*-dimethylaniline, *N*,*N*-dimethyl-*n*-octylamine and trimethylamine, they have been shown to be oxidized to their corresponding *N*-oxides [15, 19]. From the limited number of tetram analogs, it appears that substitution around the phosphorus atom affects the rate of oxidation in a manner similar to the thioether oxidation of other phosphorodithioates. The *N*,*N*-dimethyl analogs were better substrates than the corresponding *N*,*N*-diethyl analog of tetram, in agreement with other tertiary and secondary amines studied [15]. It should be noted that the monooxygenase did not catalyze the oxidative *N*-demethylation of tertiary amines or the oxidation of the carbamoyl moiety of carbamates and thiocarbamates (Table 3).

Our findings demonstrate the role of the FAD-dependent monooxygenase from pig hepatic microsomes in the oxidative metabolism of pesticides, and it is apparent that it may be a mechanism of considerable importance. In agreement with the above, we found that sulfoxidation was not inhibited by *n*-octylamine, a known inhibitor of cytochrome P-450-dependent oxygenation, and that organophosphates lacking a thioether sulfur (compounds 12–16) were not substrates for the FAD-dependent monooxygenase.

Parathion and other phosphorothioates are known to be metabolized to their oxygen analogs by cyto-

chrome P-450 [11, 31, 32]. Thus, it is apparent that these two monooxygenases oxidize different sites of the same or different compounds, although it is entirely possible that overlap in substrate specificity occurs with other substrates.

In this regard, we recently reported that the phosphonodithioate pesticide, fonofos (*O*-ethyl, *S*-phenylethylphosphonodithioate), and its phenylphosphonodithioate analog are also metabolized by the FAD-dependent monooxygenase [33] even though these compounds lack a thioether sulfur [33]. Our findings demonstrate the necessity and importance of defining the functions and relative importance of different microsomal enzymes both *in vitro* and *in vivo*.

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