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DEGRADATION AND ACTIVATION OF PARATHION ANALOGS BY MICROSOMAL ENZYMES*

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Abstract—Organophosphorus insecticides with substituted or unsubstituted 4-nitrophenyl ester structure were examined for their metabolism *in vitro* by microsomes of rat and rabbit livers and of housefly abdomens. Compounds studied included methyl parathion, parathion, *n*-propyl parathion, *iso*-propyl parathion, dicapthon, Sumithion, Chlorthion and *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate and their P=O analogs. All P=S compounds were metabolized via two oxidative pathways, activation to P=O analogs and cleavage at the aryl phosphate bond. These reactions occurred only in the presence of $NADPH_2$ and O_2. The oxidative cleavage also occurred with one P=O compound, *n*-propyl paraoxon.

OUR PREVIOUS study with ^{35}S-labeled parathion (*O*,*O*-diethyl *O*-4-nitrophenyl phosphorothioate) showed that microsomal enzymes metabolize parathion in two ways *in vitro*.[1] One is activation to paraoxon (*O*,*O*-diethyl *O*-4-nitrophenyl phosphate) with the detached sulfur being bound to the microsomes, and the other is degradation to diethyl phosphorothioic acid and 4-nitrophenol. Both reactions require $NADPH_2$ and O_2, indicating that they are oxidations. Neal[2] has also reached a similar conclusion by using ^{32}P-labeled parathion. Paraoxon is not metabolized by the same system *in vitro*. Therefore, microsomal degradation seemed to be limited to the phosphorothioate structure.[1]

Since aryl phosphate cleavage is a major route *in vivo* of detoxication for phosphorothioate insecticides,[3] compounds related to parathion were examined to see if microsomal degradation also occurred with these compounds. The following compounds (hereafter referred to as P=S compounds) were studied for phenol production and activation: methyl parathion (*O*,*O*-dimethyl *O*-4-nitrophenyl phosphorothioate), parathion (*O*,*O*-diethyl *O*-4-nitrophenyl phosphorothioate), *n*-propyl parathion (*O*,*O*-di-*n*-propyl *O*-4-nitrophenyl phosphorothioate), *iso*-propyl parathion (*O*,*O*-di-*iso*-propyl *O*-4-nitrophenyl phosphorothioate), dicapthon (*O*,*O*-dimethyl *O*-2-chloro-4-nitrophenyl phosphorothioate), Sumithion (*O*,*O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphorothioate), Chlorthion (*O*,*O*-dimethyl *O*-3-chloro-4-nitrophenyl phosphorothioate) and EPN (*O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate). Phenol production from corresponding P=O analogs and possible degradation of phenols were also examined. Most of the work was done with microsomes made from rabbit livers. Microsomes made from rat livers and housefly (*Musca domestica* L.) abdomens were used for comparison.

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MATERIALS AND METHODS

Chemicals. The following insecticides were supplied by the manufacturers: methyl parathion, methyl paraoxon (Shell Chemical Co., New York, N.Y.); parathion, paraoxon, *n*-propyl parathion, *iso*-propyl parathion, *iso*-propyl paraoxon, dicapthon (American Cyanamid Co., Princeton, N.J.); Sumithion, sumioxon (Sumitomo Chemical Co., Ltd., Osaka, Japan); Chlorthion, the P=O analog of Chlorthion (Chemagro Corp., Kansas City, Mo.); and EPN (E. I. DuPont de Nemours and Co., Inc., Wilmington, Del.). *n*-Propyl paraoxon was a gift of Dr. T. R. Fukuto, University of California, Riverside, Cal. The P=O analogs of dicapthon and EPN were prepared by oxidizing dicapthon or EPN with bromine water. All organophosphates were purified by chromatography on silica gel columns. Chloroform was the solvent for the P=S compounds, and *n*-hexane:methanol (10:1) or *n*-hexane:*iso*-propanol (10:1) was used for the P=O compounds. The P=O analog of dicapthon was further purified to remove 2-chloro-4-nitrophenol by shaking a chloroform solution of the compound with a 10% aqueous solution of sodium carbonate. After evaporation of the solvents, all the purified insecticides were stored at − 15°. After purification by chromatography on silica gel columns with chloroform as the solvent, crystals of the following phenols were stored at − 15°: 4-nitrophenol, 2-chloro-4-nitrophenol (Eastman Kodak Co., Rochester, N.Y.); 3-methyl-4-nitrophenol (Sumitomo Chemical Co.); and 3-chloro-4-nitrophenol (obtained by alkaline hydrolysis of the P=O analog of Chlorthion. Reagent grade acetone containing 1% Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) was used to prepare 10^{-1} M (10^{-2} M for some P=O compounds) stock solutions which were stored at − 15°. $NADPH_2$ was purchased from P-L Biochemicals, Inc., Milwaukee, Wis.

Enzyme sources. Liver microsomal pellets were prepared from adult male albino rabbits (obtained locally) and rats (Dan Rolfsmeyer Co., Madison, Wis.) by using hypertonic sucrose according to the method of Palade and Siekevitz.[4] Houseflies of the parathion-resistant Cradson P colony were obtained from Dr. C. N. Smith, U.S. D.A., Gainesville, Fla., and our colony was maintained without insecticidal selection. Fly abdomens were used because Tsukamoto and Casida[5] found that abdomen microsomal preparations were more active with carbamate oxidation than whole fly preparations. They suggested that this might be true with other microsomal oxidations. Preliminary experiments confirmed that such was the case with parathion metabolism. Four-day-old flies were frozen at − 15° or below, and shaken vigorously in a steel can to break the bodies into parts. The material was sifted with a U.S. Standard No. 10 sieve (2 mm opening) to obtain a mixture of abdomens, predominantly from female flies, and thoraces. The mixture was placed on a tilted board, and thoraces were rolled away by using a vibrator. A homogeneous sample of abdomens was obtained by removing the small number of remaining thoraces by hand. The preparation of abdomens was carried out inside the freezer to prevent thawing. A 10% homogenate of these abdomens in 0·25 M sucrose was fractionated by differential centrifugation (1400 *g*, 10 min; 10,000 *g*, 10 min; and 105,000 *g*, 60 min) and microsomes were obtained as the pellets from the last centrifugation.

Twenty per cent suspensions of all microsomes in 0·25 M sucrose were treated with 1% by volume of 10^{-3} M paraoxon, as described previously,[1] to minimize the loss of P=O compounds produced by activation. The pellets obtained after paraoxon treatment were stored at − 15°. Fly microsomes were used within 2 days because

oxidative activity was difficult to maintain. Mammalian microsomes could be stored for a month or more.

Reaction systems. Microsomes were suspended in 0·25 M sucrose just before being added to the incubation mixture. The standard reaction system for mammalian microsomes contained 1 ml of 20% liver microsomes (microsomal material equivalent to 20 g tissue/100 ml) and the following compounds in a total volume of 4 ml: a particular P=S compound (5×10^{-5} M), $NADPH_2$ (1×10^{-3} M), nicotinamide (0·01 M), KCl (0·15 M), Na_2HPO_4 (8×10^{-3} M) and KH_2PO_4 (2×10^{-3} M). An appropriate concentration of P=O compound or phenol was substituted for the P=S compound to investigate the stability of metabolites. Incubations were carried out in 35-ml round-bottomed centrifuge tubes at approximately 25° for 60 min without shaking. The reaction was stopped with 1 ml of 5% trichloroacetic acid (TCA). For experiments with fly microsomes, the volumes were reduced by one-half and the concentration of microsomes in the incubation mixture was increased to 15 per cent instead of 5 per cent as with liver microsomes. All experiments were done at least twice, unless otherwise mentioned, with duplicate determinations for each.

Gas chromatographic analyses. The P=S and P=O compounds were extracted with 10 ml (liver microsomal samples) or 5 ml (fly microsomal samples) of *n*-hexane (Nanograde, Mallinckrodt Chemical Works, St. Louis, Mo.) from the acidified incubation mixture and determined by gas chromatography. Retention times for the compounds are listed in Table 1. Packard dual-column gas chromatographs, equipped with electron capture detectors, were used.

TABLE 1. RETENTION TIMES (t_R) OF STANDARD COMPOUNDS FOR GAS CHROMATOGRAPHY*

Parent compound	t_R (min)	
	P=S	P=O
Methyl parathion	5·31	7·78
Parathion	7·87	11·52
n-Propyl parathion†	5·51	7·78
iso-Propyl parathion	8·56	12·80
Dicapthon	6·89	10·53
Sumithion	5·22	7·48
Chlorthion	7·68	9·94
EPN†	17·13	26·67

* Three μl of 10^{-6} M solutions of the compounds were injected into 120 cm × 2 mm (i.d.) glass columns packed with 60–80 mesh silylated Chromosorb G, coated with approximately 3% Apiezon N. Standard operating conditions were 50 ml/min N_2; column temperature, 185°; detector temperature, 200°.

† Column temperature, 210°; detector temperature, 215°.

The *n*-hexane extract was dried over anhydrous Na_2SO_4, and at least two injections of 3-μl aliquots were made for each sample. A 3-μl injection of an appropriate organophosphate (10^{-6} M in *n*-hexane) served as the standard. Peak height ratios (sample/standard) were used for computation of compound concentrations. Samples containing high concentrations of the organophosphates were diluted so that the sample peak height was less than twice that of the standard.

Operational conditions were: carrier gas, 50 ml/min of N_2; injector temperature, 200°; column temperature, 185°; and detector temperature, 200°. Temperatures were

increased (column, 210°; detector, 215°) to decrease retention times of EPN, *n*-propyl parathion and their P=O analogs. Glass columns, 120 cm × 2 mm (i.d.) were packed with 60–80 mesh, silylated Chromosorb G (Johns-Manville Products Corp., New York, N.Y.) coated with approximately 3% Apiezon N. Prior to coating, the silylated solid support was prepared as follows. Chromosorb G, 60–80 mesh, was washed with concentrated HCl.[6] The dry solid support was then refluxed for more than 6 hr with a toluene solution containing 5% hexamethyldisilazane, 5% trimethylchlorosilane and 20% pyridine. The silylated Chromosorb G was washed with toluene, methanol, toluene and *n*-hexane in the order mentioned.

Glassware used to measure or to store *n*-hexane solutions of the P=O compounds or metabolites of the P=S compounds was silylated by treating for about 10 min with a toluene solution containing 5% dimethyldichlorosilane and 20% pyridine. This treatment prevented the loss of P=O compounds due to adsorption onto glass surface.

Determination of phenols. The phenol in the acidified incubation mixture was determined after extracting the P=S and P=O compounds for gas chromatographic analyses. The sample was shaken again with 10 ml *n*-hexane to remove any remaining P=S and P=O compounds. The *n*-hexane was discarded; final traces were removed by blowing with N_2. The phenol was extracted with 5 ml (liver microsomal samples) or 2·5 ml (fly microsomal samples) of a chloroform:ether (5:1, v/v) mixture. Four ml (liver microsomal samples) or 2 ml (fly microsomal samples) of the chloroform:ether layer was then shaken with 3 ml of 0·05 M Na_2CO_3 in approximately 24% ethanol (liver microsomal samples) or 0·5 ml of 0·1 M Na_2CO_3 in approximately 24% ethanol (fly microsomal samples). Absorbance of the aqueous layer was determined in a Beckman model DB (liver microsomal samples) or DU (fly microsomal samples) spectrophotometer at 400 mμ. Microcells were used with the latter instrument. A value for a blank was obtained with a similarly treated sample without an organophosphate or a phenol. A calibration curve was prepared for each phenol by processing samples containing known amounts of an authentic phenol in the incubation mixture without an organophosphate or $NADPH_2$.

The procedure was modified for dicapthon, methyl parathion and their P=O analogs. TCA was not added to stop the reaction with dicapthon or its P=O analog because 2-chloro-4-nitrophenol was extracted by *n*-hexane from acidic media. Instead, 1 ml of 0·1 M, pH 7 phosphate buffer was added and the mixture was immediately shaken with *n*-hexane. Prior to phenol extraction, 1 ml of 10% TCA was added. Also, 0·1 N NaOH in approximately 24% ethanol was substituted for the Na_2CO_3 : ethanol solution.

n-Hexane did not extract methyl paraoxon efficiently. Therefore, a *n*-hexane:benzene (1:1) mixture was substituted. Since the mixture extracted a large portion of 4-nitrophenol from acidic media, the procedure for dicapthon analogs was followed with the substitution of a 0·1 M, pH 8 phosphate buffer for the pH 7 buffer.

Identification of metabolites. The following procedures and those already described were employed for identification of metabolites. Thin-layer chromatography was carried out by using precoated silica gel F-254 plates (Brinkmann Instruments, Inc., Westbury, N.Y.). The solvent systems were *n*-hexane:dioxane (1:1) and *n*-hexane:ethyl acetate (1:1). *n*-Hexane used in both systems had been shaken with 12 N HCl and dried over anhydrous Na_2SO_4. *n*-Hexane extracts or chloroform:ether extracts, prepared as described in the preceding sections, were evaporated in Teflon beakers and the

residues were dissolved in acetone for spotting. R_f values varied somewhat from experiment to experiment. Tailing of phenols made R_f values of these compounds less certain. Therefore, authentic compounds were always run on the same plate as the metabolite. Table 2 lists typical R_f values for authentic compounds.

TABLE 2. R_f VALUES OF STANDARD COMPOUNDS FOR TWO SOLVENT SYSTEMS OF TLC

Parent compound*	*n*-Hexane:ethyl acetate (1:1)			*n*-Hexane:dioxane (1:1)		
	P=S	P=O	Phenol	P=S	P=O	Phenol
Methyl parathion	0·37	0·10	0·27	0·46	0·30	0·30
Parathion	0·43	0·18	0·27	0·46	0·35	0·30
n-Propyl parathion	0·47	0·28	0·27	0·48	0·39	0·30
iso-Propyl parathion	0·47	0·24	0·27	0·48	0·39	0·30
Dicapthon	0·41		0·10	0·43		0·29
Sumithion	0·38	0·10	0·30	0·46	0·32	0·32
Chlorthion	0·37	0·11	0·27	0·46	0·31	0·28
EPN	0·44	0·23	0·27	0·43	0·35	0·30

* Approximately 10^{-7} mole of each compound was spotted on precoated silica gel F-254 plates (Brinkmann Instruments, Inc., Westbury, N.Y.). The chromatograms were developed after equilibrium with the solvent vapor for about 1 hr. Distance from the origin to the solvent front was 11 cm.

The pK values of phenols were determined as follows. Each phenolic metabolite was extracted with chloroform:ether as described in the preceding section from 16 ml of a reaction mixture that had been incubated for 2 hr. After evaporation of the solvent, the metabolite was dissolved in 2 ml water and the turbid solution was clarified by centrifugation. Absorbance and pH values were determined for each sample containing 0·2 ml of the metabolite solution and 0·5 ml of a wide-range buffer composed of phosphoric, acetic and boric acids and NaOH.[7] The buffer solutions were prepared to cover the pH range of 3 to 10 at intervals of approximately 0·5 pH unit. An appropriate pH range was selected for each metabolite. A Beckman model DU spectrophotometer with microcells and a Corning model 12 pH-meter with a combination electrode were used for the measurements. The pK values for metabolites and authentic compounds were estimated graphically.[1]

RESULTS

Metabolism of the P=S compounds. Microsomes from the three species effected both degradation and activation of all the P=S compounds only in the presence of $NADPH_2$ (Table 3). Requirement for oxygen was checked for the metabolism of *n*-propyl parathion and Sumithion with rabbit liver microsomes by using Thunberg tubes. Formation of both phenols and P=O analogs was suppressed by more than 75 per cent under anaerobic conditions. The remaining activity was probably due to traces of oxygen remaining. The order of microsomal activities was generally rabbit > rat >> fly. Identity of yellow phenolic metabolites formed by rabbit liver microsomes was confirmed by spectrophotometry, TLC and pK determinations. Each metabolite was extracted as described in Materials and Methods and visible spectra were taken in a Beckman model DB spectrophotometer. Positions of absorption peaks of the metabolites were within 3 mμ of those of authentic samples of the expected phenols,

TABLE 3. METABOLISM OF P=S COMPOUNDS*

Substrate	Metabolite produced (10^{-6} M)											
	Rabbit				Rat				Fly			
	Phenol		P=O analog		Phenol		P=O analog		Phenol		P=O analog	
	$NADPH_2$	No $NADPH_2$	$NADPH_2$	No $NADPH_2$	$NADPH_2$	No $NADPH_2$	$NADPH_2$	No $NADPH_2$	$NADPH_2$	No $NADPH_2$	$NADPH_2$	No $NADPH_2$
Methyl parathion	3·8	0·0	3·7	0·0	2·5	0·0	5·4	0·0	0·9	0·0	1·6	0·0
Parathion	10·7	0·5	10·7	0·3	4·9	0·3	3·7	0·0	1·7	0·0	1·5	0·0
n-Propyl parathion	9·4	0·1	6·0	0·0	3·8	0·0	2·8	0·0	1·3	0·0	0·7	0·0
iso-Propyl parathion	13·8	0·1	3·7	0·1	3·2	0·0	3·0	0·0	1·2	0·0	0·7	0·0
Dicapthon	6·1	1·2	6·0	0·0	2·2	0·0	4·5	0 0	0·9	0·0		
Sumithion	4·5	0·7	7·0	0·0	1·6	0·0	5·1	0·0	1·3	0·0	1·9	0·0
Chlorthion	5·0	0·9	6·1	0·0	2·2	0·0	4·8	0·0	1·0	0·1		
EPN	7·6	0·0	7·2	0·0	6·5	0 0	4·9	0·0	1·3	0·0	1·1	0·0

* Phenols and P=O analogs were determined after incubating 5% mammalian liver or 15% fly abdomen microsomes with approximately 5×10^{-5} M P=S compounds for 1 hr. Concentration of $NADPH_2$, when used, was 10^{-3} M. Small zero-time values were subtracted from the 1-hr values to correct for possible impurities.

although broadness of the peaks and presence of minute air bubbles in some samples resulted in fluctuation of peak positions (Table 4). Co-chromatography of each yellow metabolite (extracted with chloroform:ether) with the authentic phenol, gave a single spot on thin-layer plates. The pK values were within 0·2 of the expected values (Table 4).

TABLE 4. ABSORPTION MAXIMUM AND pK VALUES FOR YELLOW METABOLITES AND AUTHENTIC PHENOLS*

Parent compound	Absorption maximum (mμ)		pK	
	Metabolite	Standard	Metabolite	Standard
Methyl parathion	407	406	7·1	7·1
Parathion	405	406	7·1	7·1
n-Propyl parathion	405	406	7·1	7·1
iso-Propyl parathion	404	406	7·1	7·1
Dicapthon	406	405	5·6	5·5
Sumithion	399	402	7·2	7·4
Chlorthion	396	397	6·6	6·4
EPN	404	406	7·1	7·1
n-Propyl paraoxon	405	406	7·1	7·1
P=O analog of EPN	404	406	7·1	7·1

* Yellow metabolites were prepared by incubating 5% (absorption maximum) or 10% (pK) rabbit liver microsomes with approximately 5×10^{-5} M P=S or $2{\cdot}5 \times 10^{-5}$ M P=O compounds in the presence of 10^{-3} M $NADPH_2$ for about 2 hr. Extraction of metabolites and measurements were done as described in text.

Gas chromatograms of *n*-hexane extracts from the three microsomal systems were then examined. Retention times of the metabolites were identical to those of respective P=O analogs. Metabolites in *n*-hexane extracts were also examined by TLC. Except for the dicapthon metabolite, R_f values of the metabolites were the same as those of respective P=O analogs. Co-chromatography of each metabolite with the authentic P=O analog gave a single spot. The metabolite spots were detected under u.v. light and turned yellow on exposure to NH_3 vapor. Both the metabolite and P=O analog of dicapthon were hydrolyzed on the silica gel plates and the R_f values were not determined.

Metabolism of the P=O compounds. Phenols could have been produced from the P=O compounds formed by the activation reaction rather than directly from P=S compounds. Therefore, phenol production from 10^{-5} M P=O compounds was examined both in the presence and absence of $NADPH_2$ (Table 5).

With rabbit microsomes, the rates of phenol production from methyl paraoxon, *iso*-propyl paraoxon, sumioxon and the P=O analog of dicapthon were clearly too low to account for the phenol formed when P=S compounds were incubated in the presence of $NADPH_2$. Although degradation of paraoxon was greater than in our previous study, direct degradation of parathion has already been shown by using ^{35}S-labeled parathion.[1] Metabolism of P=O analogs of Chlorthion and EPN was relatively high. However, concentrations of the P=O compounds remaining after incubation were $2{\cdot}4 \times 10^{-5}$ M and $1{\cdot}6 \times 10^{-5}$ M respectively, much higher than concentrations of the P=O analogs found in the activation mixtures of the corresponding P=S compounds. Therefore, degradation of the P=O analogs did not account

for all the phenols found in the activation mixtures of Chlorthion and EPN. Since phenol formation from the P=O analogs just mentioned was not enhanced by $NADPH_2$, the phenols were probably formed by hydrolytic reactions rather than by oxidative ones.

TABLE 5. PHENOL PRODUCTION FROM P=O COMPOUNDS*

Substrate	Phenol produced (10^{-6} M)					
	Rabbit		Rat		Fly	
	$NADPH_2$	No $NADPH_2$	$NADPH_2$	No $NADPH_2$†	$NADPH_2$	No $NADPH_2$
Methyl paraoxon	0·5	0·5	1·5	0·0	0·2	0·0
Paraoxon	3·6	4·4	5·4	7·6	0·0	0·0
n-Propyl paraoxon	8·5	0·5	17·9	0·4	0·7	0·0
iso-Propyl paraoxon	0·5	0·5	1·2	0·0	0·0	0·0
P=O analog of dicapthon	1·3	1·4	1·3	1·3	1·4	1·3
Sumioxon	0·6	1·1	0·2	0·0	0·0	0·1
P=O analog of Chlorthion	2·0	2·5	2·8	4·1	0·4	0·4
P=O analog of EPN	5·0	5·9	7·2	8·6	1·1	0·9

* Phenols were determined after incubating 5% mammalian liver or 15% fly abdomen microsomes with approximately $2·5 \times 10^{-5}$ M P=O compounds for 1 hr. Concentration of $NADPH_2$, when used, was 10^{-3} M. Figures have been corrected for zero-time values as in Table 3.

† Data from a single experiment run with duplicate samples.

The metabolism of *n*-propyl paraoxon was unique among the P=O analogs studied. About one-third of the substrate was degraded to form 4-nitrophenol, only if $NADPH_2$ was present, indicating an oxidative reaction. Identity of the phenolic metabolite was confirmed by spectrophotometry and p*K* determination (Table 4) and by TLC. Requirement for oxygen was also confirmed by using Thunberg tubes. The concentration of *n*-propyl paraoxon remaining after incubation in the presence of $NADPH_2$ ($1·4 \times 10^{-5}$ M; Table 5) was much higher than that of the P=O compound formed after incubation of *n*-propyl parathion ($6·0 \times 10^{-6}$ M; Table 3). However, concentrations of 4-nitrophenol produced from the P=O and P=S compounds were similar ($8·5 \times 10^{-6}$ M and $9·4 \times 10^{-6}$ M respectively). Therefore, it was likely that only a part of the 4-nitrophenol produced in the P=S incubation mixture was derived from *n*-propyl paraoxon.

With rat and fly microsomes, the over-all picture was qualitatively similar to that for rabbit microsomal metabolism, although enzyme activities of fly microsomes were much lower than those of mammalian microsomes. The sensitivity of phenol determination for fly microsomes was about 6 times as high as that for mammalian microsomes. The oxidative nature of the metabolism of *n*-propyl paraoxon was also clear. The oxidative degradation of *n*-propyl paraoxon by the rat microsomes was extremely high in contrast to similar metabolism of P=S compounds by the same enzyme source.

Phenol formation from n-propyl parathion and EPN. Due to the high rates of phenol formation from the P=O analogs, direct formation of 4-nitrophenol from *n*-propyl parathion and EPN was obscure. Evidence already mentioned indicated the presence of the direct route. Experiments were performed by using rabbit liver microsomes to clarify the relative significance of direct and indirect routes of phenol formation. The

time courses of metabolism of the P=S compounds were followed over a 2-hr period (Fig. 1). Also, 4-nitrophenol produced in 10 min from three different concentrations of the P=O analogs was determined (Table 6). The concentration of 4-nitrophenol and *n*-propyl paraoxon formed from *n*-propyl parathion at 10 min of incubation was $4{\cdot}8 \times 10^{-6}$ M and $3{\cdot}4 \times 10^{-6}$ M respectively (Fig. 1). Since the concentration of

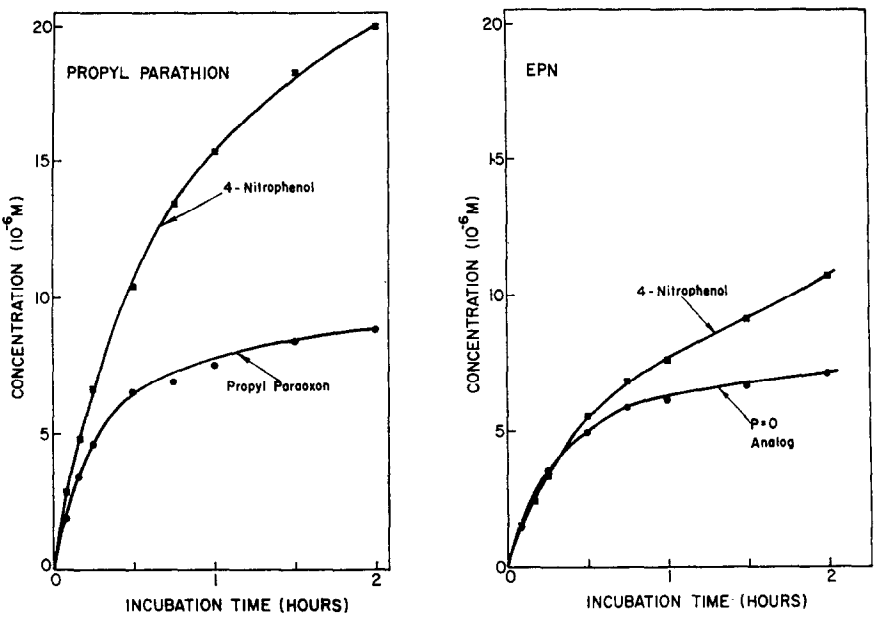


FIG. 1. Time courses of the production of P=O analogs and 4-nitrophenol from *n*-propyl parathion and EPN. The incubation mixtures contained 5×10^{-5} M parent compound, 5% rabbit liver microsomes and 10^{-3} M $NADPH_2$. Metabolites were assayed as described in Materials and Methods.

TABLE 6. EFFECT OF SUBSTRATE CONCENTRATION ON THE METABOLISM OF *n*-PROPYL PARAOXON AND THE P=O ANALOG OF EPN*

	n-Propyl paraoxon		P=O analog of EPN	
Initial substrate concentration (M)	4-Nitrophenol produced (10^{-6} M)	Substrate remaining (10^{-6} M)	4-Nitrophenol produced (10^{-6} M)	Substrate remaining (10^{-6} M)
1×10^{-5}	2·3	7·2	0·8	8·8
5×10^{-6}	1·6	3·7	0·7	4·4
$2{\cdot}5 \times 10^{-6}$	1·0	1·4	0·3	1·9

* 4-Nitrophenol was determined after 10 min of incubation of 5% rabbit liver microsomes with the indicated concentration of the P=O compound in the presence of 10^{-3} M $NADPH_2$. Data are from a single experiment with duplicate samples.

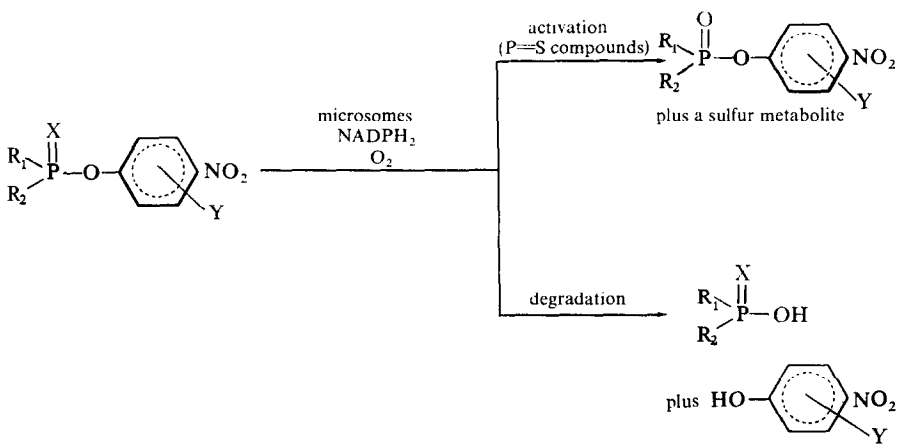
n-propyl paraoxon was lower than $3{\cdot}4 \times 10^{-6}$ M during the first 10 min of incubation, the maximum amount of the phenol expected from *n*-propyl paraoxon is $1{\cdot}3 \times 10^{-6}$ M (by interpolation from Table 6). Therefore, at least $3{\cdot}5 \times 10^{-6}$ M out of $4{\cdot}8 \times 10^{-6}$ M of 4-nitrophenol is derived directly from *n*-propyl parathion. Since a small amount of

n-propyl paraoxon was degraded, activation of *n*-propyl parathion in 10 min should have been slightly higher than $3{\cdot}4 \times 10^{-6}$ M. Similarly, at least $2{\cdot}0 \times 10^{-6}$ M out of $2{\cdot}3 \times 10^{-6}$ M of 4-nitrophenol was derived directly from EPN, and slightly greater than $2{\cdot}5 \times 10^{-6}$ M of EPN was activated in the first 10 min of incubation.

Metabolic stability of phenols. The four phenols obtainable from the eight organophosphates were incubated with rabbit, rat or fly microsomes for 1 hr in the presence or absence of $NADPH_2$. Phenol concentrations were 10^{-5} M for mammalian microsomes or 10^{-6} M for fly microsomes. No metabolism was indicated by the spectrophotometric procedure. Therefore, phenols found in the incubation mixtures containing P=S or P=O compounds represent the actual enzymatic activities for phenol production.

DISCUSSION

The eight P=S compounds having a substituted or unsubstituted 4-nitrophenyl ester structure were oxidatively cleaved to give a 4-nitrophenol. Since EPN was metabolized oxidatively, oxidative cleavage of the aryl phosphate bond is not confined to triesters of phosphorothioic acid. Contrary to expectations, a P=O compound, *n*-propyl paraoxon, was also degraded oxidatively to give 4-nitrophenol. Therefore, the P=S structure is not a necessary condition for the oxidative degradation being discussed. While all P=S compounds examined were degraded, it cannot be concluded that the P=S structure is a sufficient condition for oxidative cleavage. Although high lipid solubility was generally considered a prerequisite for a compound to be metabolized by microsomal oxidations, Mazel and Henderson[8] reported that no correlation was obvious between the rate of oxidative demethylation of substrates by microsomal enzymes and their chloroform:water partition coefficient. However, the reaction scheme *in vitro* previously suggested for parathion[1] may be extended to a more general form:



For the compounds examined in this study, R_1 is an alkoxy group and R_2 is identical to R_1, except for EPN where R_2 is a phenyl group. X is sulfur, except for *n*-propyl paraoxon where it is oxygen. Y is hydrogen, except for dicapthon, Chlorthion and Sumithion where it is Cl or CH_3 substitution on the ring.

Neal and DuBois[9] suggested that the sex and age difference in toxicity of EPN, parathion, methyl parathion and Chlorthion among animals might be explained by the enzymes that produce phenols from these compounds in the presence of $NADPH_2$ or an $NADPH_2$-generating system *in vitro*. Unfortunately, for the major part of their work, they chose EPN whose P═O analog is hydrolyzed to a considerable extent. They concluded that the phenols were produced by hydrolysis after desulfuration of P═S compounds. Although this does occur to some extent, our results show that the direct degradation of P═S compounds is a major route of phenol production in the system *in vitro* in the presence of $NADPH_2$ and O_2. The more recent report by Neal[2] on the microsomal metabolism of ^{32}P-parathion is in agreement with our conclusion.

With few exceptions, similar amounts of a phenol and a P═O analog were produced from a given P═S substrate by a particular microsomal system (Table 3). More activation than degradation often occurred with the four dimethyl phosphorothioates (methyl parathion, dicapthon, Sumithion and Chlorthion), whereas the reverse was true with the higher homologs. Variation of the ratio of activation/degradation among enzyme sources, especially with *iso*-propyl parathion, indicates that activation and oxidative degradation are catalyzed by separate enzyme systems. The order of microsomal activities was generally rabbit > rat >> fly. Part of the differences in metabolite production may be due to microsomal preparation procedures. The rabbit and rat microsomes were prepared by the same procedure and may not represent the same portion of the activity in the original liver homogenates. The fly microsomes were prepared from entire abdomens rather than from a specialized tissue. In the case of mammalian microsomes, variation among the small number of animals used may also have contributed to the activity differences.

Except for *n*-propyl paraoxon, phenol production from P═O compounds did not contribute much to the phenol found in the P═S incubation mixtures. However, the metabolic picture of P═O compounds is more complex than that of P═S compounds. In some cases, especially with paraoxon and the oxygen analog of EPN, phenol production was considerable and was probably due to phosphatases. $NADPH_2$ seemed to have an inhibitory effect. This might be partly due to lowering of the substrate concentration by another $NADPH_2$-requiring reaction. The inhibitory effect might have masked any small amount of oxidative degradation present. A low degree of oxidative degradation might be present in the case of *iso*-propyl paraoxon. Similar amounts of phenol were produced from the P═O analog of dicapthon, regardless of the enzyme source or the presence of $NADPH_2$, suggesting nonenzymatic hydrolysis. Some spontaneous hydrolysis might also have occurred with P═O analogs of Chlorthion and EPN.

Sun *et al.*[10] discussed synergism of sesamex with various phosphorothioates, phosphorodithioates and their P═O analogs in the housefly. Although the mechanism for the synergism might be complex, their data might be an indication of oxidative degradation *in vivo* similar to that described in this report. Dialkyl phosphorothioic acids and dialkyl phosphorodithioic acids have been reported as major metabolites *in vivo* of P═S compounds, including those without the 4-nitrophenyl ester structure.[3, 11–15] Also, hydrolytic enzymes have been assumed to be responsible for the degradation of P═O compounds.[16] Further studies are in progress to examine whether the microsomal oxidation also effects the seemingly hydrolytic metabolism of such compounds.

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