In Vitro Metabolism of Vamidothion and Its Thio Analogue by Rat and Mouse Liver

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The in vitro metabolism of vamidothion [*O,O*-dimethyl *S*-[2-[[1-(methylcarbamoyl)ethyl]thio]ethyl] phosphorothioate] and its thio analogue thiovamidothion [*O,O*-dimethyl *S*-[2-[[1-(methylcarbamoyl)-ethyl]thio]ethyl] phosphorodithioate] was investigated on rat and mouse liver subcellular fractions. Vamidothion was rapidly oxidized to the sulfoxide, which was the principal metabolite with no further oxidation to the sulfone. A low level of hydrolysis products was detected but only at low substrate concentrations. Thiovamidothion was extensively oxidized to thiovamidothion sulfoxide, vamidothion, and vamidothion sulfoxide in addition to oxidative hydrolytic products. The major route of in vitro metabolism of vamidothion and thiovamidothion in both species appears to involve the cytochrome P-450 dependent monooxygenase system, as demonstrated by use of cytochrome P-450 inhibitors. The FAD-containing monooxygenase (sulfur oxidase) does not appear to be involved in the oxidation of the thioether moiety. Also, the glutathione *S*-transferases showed moderate activity toward thiovamidothion but not toward vamidothion.

Vamidothion, Kilval, Kilvar [*O,O*-dimethyl *S*-[2-[[1-(methylcarbamoyl)ethyl]thio]ethyl] phosphorothioate], is a systematic insecticide and acaricide that is widely used in the control of sap-sucking insects and mites present on fruit trees and field crops. Vamidothion may be considered a combination of demeton-*S*-methyl (Metasystox) and dimethoate (Rogor) since structurally it has similar functional groups, i.e. dimethyl phosphorothioate, thioether, and N-substituted carboxamide groups.

The oxidation of the thioether moiety in organophosphorus insecticides to the sulfoxide has been observed in plants in vivo (Metcalf et al., 1954, 1955, 1957; Fukuto et al., 1956; Fukuto and Metcalf, 1969) and in animals in vivo and in vitro (Rao and McKinley, 1969; Bull et al., 1970; Bull and Stokes, 1970; Bull, 1965, 1972). In the case of the thioate and dithioate triesters containing a thioether moiety, the compound is subject to oxidation at both sites (Bowman and Casida, 1958). Oxidation of the sulfide to the sulfoxide and then to the sulfone and the desulfuration of the phosphorus atom have generally been considered and oxidative activation, leading to an increase in anticholinesterase activity (Eto, 1974; Kulkarni and Hodgson, 1980).

The N-substituted carboxamide group in organophosphorus triester compounds is subject to cleavage of the C–N bond in animals in vivo and in vitro leading to a decrease in anticholinesterase activity (Dauterman and Casida, 1959; Chen and Dauterman, 1971a,b; El-Oshar and Dauterman, 1979). In addition to the hydrolysis of the amide bond by amidase action, oxidative N-dealkylation to form the unsubstituted amide has also been reported in vivo and in vitro (Bull and Linquist, 1964, 1966; Lucier and Menzer, 1970; Tseng and Menzer, 1974).

The enzyme system(s) involved in sulfoxide and sulfone formation has generally been assumed to be the cytochrome P-450 dependent monooxygenase system. This assumption, however, may not be valid in all cases, in view of the existence of another monooxygenase, a flavoprotein (FAD) enzyme (Ziegler, 1980).

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Recent studies have shown the involvement of a FAD-containing monooxygenase (sulfur oxidase) in the sulfoxidation of many organophosphate and carbamate insecticides and other thioether-containing xenobiotics (Hajjar and Hodgson, 1980, 1982; Poulson, 1981).

Metabolism of vamidothion and dimethoate have been studied in mammals, insects, and plants, in vivo and in vitro (Morikawa and Saito, 1966). Both compounds were hydrolyzed to water-soluble metabolites (mono- and dimethyl phosphate and phosphorothioate). The carboxylic acid derivative was detected only with dimethoate, in rats, plants, and insects, whereas desmethylvamidothion was detected only with plants.

The present study was undertaken to investigate the metabolism of vamidothion and thiovamidothion in rat and mouse liver subcellular fractions and to determine the enzyme(s) system(s) involved.

MATERIALS AND METHODS

Chemicals. [^{14}C]Vamidothion [*O,O*-dimethyl *S*-[2-[[1-(methylcarbamoyl)[1,2-^{14}C]ethyl]thio]ethyl] phosphorothioate] (sp act. 27 mCi/mmol), nonradioactive vamidothion, and vamidothion sulfoxide and sulfone were obtained from Rohne-Poulenc, France, via Rohne-Poulenc Japan, Ltd., and Shionogi & Co., Ltd. Desmethylvamidothion was prepared by demethylating vamidothion with sodium iodide. [*methoxy*-^{14}C]Vamidothion (sp act. 0.3 mCi/mmol) and thiovamidothion (sp act. 0.9 mCi/mmol) were prepared by reacting the ammonium salt of *O,O*-[^{14}C]dimethyl phosphorothioate or *O,O*-[^{14}C]dimethyl phosphorodithioate, respectively, with 2-[(2-chloroethyl)-thio]-*N*-methylpropionamide. Thiovamidothion sulfoxide was prepared by oxidation of the sulfide compound with 30% hydrogen peroxide.

All compounds were purified by TLC and characterized by IR and NMR spectroscopies prior to use. The radiochemical purities of all compounds were determined to be >99.0% as demonstrated by TLC.

Nonradioactive potassium *O,O*-dimethyl phosphorothioate was prepared by alkaline hydrolysis of *O,O*-dimethyl phosphorochloridothioate (Fletcher et al., 1950), whereas ammonium *O,O*-dimethyl phosphorodithioate was prepared by the method of Hoegberg and Cassaday (1951).

NADPH, NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and reduced glutathione were pur-

0021-8561/87/1435-0138$01.50/0

chased from Sigma Chemical Co., St. Louis, MO. SKF-525A [2-(diethylamino)ethyl 2,2-diphenylpentanoate] was obtained from Smith, Kline and French Laboratories, Philadelphia, PA. *n*-Octylamine was obtained from Aldrich Chemical Co., Milwaukee, WI. Cytochrome P-450 reductase antibodies were obtained from Dr. P. Levi, NCSU.

Animals. Random-bred white male rats of the Dublin Sprague–Dawley strain weighing 250 g and random-bred white male mice of the Dublin ICR strain weighing 35 g were obtained from Dominion Laboratories, Dublin, VA.

Enzyme Preparation. The rats and mice were decapitated, and their livers were immediately removed and homogenized (10%, w/v) in ice-cold 0.1 M phosphate buffer, pH 7.4, for 30 s, utilizing a Brinkman Polytron homogenizer. Subcellular fractions were separated by differential centrifugation as previously described (Motoyama and Dauterman, 1972). The subcellular fractions obtained were 10000*g* supernatant, 10000*g* precipitate (mitochondria), 105000*g* precipitate (microsomes), and 105000*g* supernatant (soluble fraction). Portions of the 10000*g* supernatant and the soluble fraction were dialyzed for 24 h against 60 vol of the homogenization buffer. The microsomes were resuspended in the same buffer at a volume equal to the 105000*g* supernatant.

Incubation System. The reaction mixture consisted of 1.5 mL of enzyme solution equal to 0.15 g of wet liver, 0.5 μmol of vamidothion (0.08 μCi), or 0.7 μmol of thiovamidothion or 0.7 μmol of thiovamidothion sulfoxide (0.1 μCi), 50 μL of NADPH regenerating system (contained at final concentration 5.0 μmol of NADP, 5.0 μmol of glucose 6-phosphate, and 1.0 unit of glucose 6-phosphate dehydrogenase), or 10, 20, of 40 μmol GSH and was diluted to a final volume of 2.0 mL with 0.1 M phosphate buffer. Buffer or boiled enzyme was used as a control. The reaction mixture was placed in a shaking water bath at 37 °C, and the incubation time was varied from 0 to 2 h. When selective cytochrome P-450 inhibitors were used, the microsomal preparation was incubated with (3.0 mM final concentration) *n*-octylamine hydrochloride (Hanzlik et al., 1978, 1980) or cytochrome P-450 reductase antibodies (5 mg of antireductase protein/mg of microsomal protein (Tynes and Hodgson, 1983) for 5–7 min at 4 °C prior to the addition of substrate and cofactor. Also the microsomal preparation was incubated with either SKF 525-A (4 μmol) or piperonyl butoxide (4 μmol) for 5 min at 37 °C in the presence of NADPH, after which the substrate was added.

In order to deactivate the FAD-containing monooxygenase, microsomes were incubated for 60 min at 37 °C, and then substrate and cofactor were added (Poulsen et al., 1979; Kedderis and Rickert, 1983). At the end of the incubation time the enzyme reaction was stopped by the addition of 1.5 mL of ethanol and the solution was thoroughly mixed and centrifuged at 10000*g* for 7 min. The ethanolic solution was decanted, and the amount of radioactivity in 0.05-mL aliquots was determined in a Packard Model 3330 liquid scintillation counter using Scintiverse II cocktail. The efficiency was corrected using [^{14}C]-*n*-hexadecane as an internal standard.

Preliminary Characterization of Metabolites. Fifty-microliter aliquots of the ethanolic solutions were spotted on 5 × 20 or 20 × 20 cm Polygram Sil G/UV254 (0.25-mm-thick) precoated plates and developed in solvent systems I–VII (Table I). Two-dimensional development using solvent VI followed by solvents II, IV, or VII resulted in the separation of the highest number of thiovamidothion metabolites. Metabolites were detected by scanning the TLC plates on a Berthold LB2832 automatic TLC linear analyzer. Known standards were used for cochromatography and were visualized under UV light at 254 nm and by color development when the plates were sprayed with either 0.5% 2,6-dibromo-*N*-chloro-*p*-benzoquinone imine in cyclohexane (Menn et al., 1957) or 0.25% palladium chloride in 0.2 N HCl. The amount of each individual metabolite formed after 2.0-h incubation was quantitated. The R_f values for reference compounds in the various solvent systems used are presented in Table I.

Table I. R_f Values of Vamidothion, Thivamidothion, and Possible Metabolites

compound	solvent system[a] I	II	III	IV	V	VI	VII
vamidothion	0.6	0.86	0.85	0.82	0.15	0.6	
thiovamidothion		0.93	0.92	0.9	0.5	0.82	
vamidothion sulfoxide	0.3	0.7	0.4		0.0	0.15	
thivamidothion sulfoxide		0.8	0.76	0.75	0.1	0.4	
O-desmethyl thiovamidothion		0.82		0.17		0.0	0.65
dimethyl phosphorothioic acid ester		0.70		0.13		0.0	0.5
dimethyl phosphoric acid ester		0.35		0.0		0.0	0.1
dimethyl phosphorodithioic acid ester		0.90		0.3		0.0	0.7
monomethyl phosphorothioic acid ester		0.1		0.0		0.0	0.02

[a] Key: I, acetonitrile–methanol (90:10, v/v); II, acetonitrile–water (85:15, v/v); III, methylene chloride–ethanol (90:10, v/v); IV, ethyl acetate–ethanol–ammonium hydroxide (70:25:5, v/v/v); V, benzene–ethyl acetate–acetic acid (50:48:2, v/v/v); VI, chloroform–acetone (55:45, v/v); VII, acetonitrile–water–ammonium hydroxide (80:18:2, v/v/v).

Large-Scale Incubation Mixture. In order to generate a sufficient amount of thiovamidothion metabolites for spectral analysis, the in vitro system was enlarged.

One hundred milliliters of the enzyme solution from rat liver microsomes or the soluble fraction (dialyzed for 24 h against phosphate buffer) was used. The substrate was added at a final concentration of 0.7 mM. The NADPH regenerating system and GSH were added as described earlier. The reaction mixture was incubated at 37 °C for 2.5 h after which the reaction was stopped by the addition of an equal volume of dichloromethane. The organic phase and the aqueous phase were separated by centrifugation at 10000*g*. The aqueous phase was reextracted three times, the organic phases were combined and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum followed by a gentle stream of nitrogen. The aqueous phase was concentrated by lyophilization and the residue dissolved in 3–5 mL of distilled water. An excess amount of methanol was added to precipitate the soluble proteins followed by centrifugation at 10000*g* for 5 min. The supernatant was decanted, and the methanol was evaporated under nitrogen at 25 °C. The water-phase concentrate was passed through several PreSep C-18 columns (Fisher Scientific Co.), and the radioactivity was extracted from the columns with methanol, concentrated, and used for TLC.

Separation of Metabolites. Vamidothion and vamidothion sulfoxide were separated on a 20 × 20 cm Polygram Sil G/UV 254 (0.1-mm-thick) precoated plate by solvent system VI. The radioactive compounds were extracted from the silica gel with methanol and evaporated, and the compound was dissolved in dichloromethane.

Column Chromatography. Metabolites of the thiovamidothion were separated as follows: The organic phases from five microsomal runs were combined, concentrated,

Table II. Metabolism of Vamidothion by Subcellular Fractions of Rat and Mouse Liver and the Effect of Cofactors[a]

subcellular fraction	metabolites[b] rat	mouse
10000*g* supernatant + no cofactor	0.0	0.0
10000*g* supernatant + NADPH	53.0 ± 2.5	42.0 ± 1.0
10000*g* supernatant + GSH (10 μol)	0.0	0.0
10000*g* precipitate + no cofactor	0.0	0.0
10000*g* precipitate + NADPH	14.5 ± 2.4	10.0 ± 1.3
microsomes + no cofactor	0.0	0.0
microsomes + NADPH	65.0 ± 3.0	51.0 ± 2.6
soluble fraction + no cofactor	0.0	0.0
soluble fraction + GSH (10 μmol)	0.0	0.0
control[c]	0.0	0.0
control[d]	0.0	0.0

[a] All values expressed as percent of total metabolites and are the average of four experiments (mean ± SE). [b] Incubation time 60 min. [c] Boiled 10000*g* supernatant. [d] 0.1 M potassium phosphate buffer (pH 7.4).

and subjected to Florisil column chromatography as described by Patchett and Batchelder (1961).

The water phase of the soluble fraction was concentrated by lyophilization and applied to an ion-exchange column and eluted as described by Chopade et al. (1981). No attempt was made to separate the water-soluble metabolites formed by the microsomal fraction.

Spectroscopic Analyses. Radioactive metabolites eluted from the Florisil column were further purified by TLC and then passed through a PreSep C-18 column prior to preparation for FT-IR and FT-NMR spectroscopy.

RESULTS AND DISCUSSION

The effect of added cofactors on the metabolism of vamidothion by rat and mouse liver subcellular fractions is presented in Table II. Without addition of any cofactor, no metabolism of vamidothion occurred after 1 h of incubation. However, at a low substrate concentration (0.1 mM), about 12.0% and 9.0% of vamidothion equivalents were metabolized in the rat and mouse 10000*g* supernatants, respectively. The water-soluble metabolites formed in the soluble fractions amounted to less than 10.0%. Upon addition of the NADPH regenerating system to the 10000*g* supernatant, the mitochondrial and the microsomal fraction, a moderate amount of metabolism occurred with the mitochondria (14.0%, 11.0%) in the rat and mouse preparations, respectively, and a substantial increase in the metabolic activity was observed with 10000*g* supernatant and microsomal fractions. The highest activity was associated with the microsomal fraction, and the metabolic products amounted to 65.0% of vamidothion equivalents in the rat and 52.0% in the mouse. On the other hand, addition of GSH at a final concentration of 5.0, 10.0, or 20.0 mM to the dialyzed or nondialyzed 10000*g* supernatant or the soluble fraction did not effect the metabolic activity.

Incubation of thiovamidothion with rat and mouse liver preparations without cofactors added (Table III) resulted in a moderate amount of metabolism by the 10000*g* supernatants and the soluble fractions. After 1 h of incubation, the metabolic products amounted to 22.0% and 16.0% in the rat and mouse liver 10000*g* supernatant and 17.0% and 14.0% in the soluble fractions, respectively. Addition of the NADPH regenerating system to the 10000*g* supernatant, the mitochondrial and microsomal fractions, resulted in a substantial increase in metabolic activity. The amount of metabolites reached maxima of 87.0%, 20.0%, and 83.0% in the various rat preparations and 63.0%, 15.0%, and 59.0% in the various mouse preparations.

Table III. Metabolism of Thiovamidothion by Subcellular Fractions of Rat and Mouse Liver and the Effect of Cofactors[a]

subcellular fraction	metabolites[b] rat	mouse
10000*g* supernatant + no cofactor	22.0 ± 2.0	16.0 ± 1.4
dialyzed 10000*g* supernatant + no cofactor	7.5 ± 1.6	6.0 ± 1.3
10000*g* supernatant + NADPH	87.0 ± 4.4	63.0 ± 3.0
10000*g* supernatant + GSH (10 μmol)	21.0 ± 2.5	17.0 ± 2.0
dialyzed 10000*g* supernatant + GSH (10 μmol)	20.0 ± 1.5	14.0 ± 1.5
10000*g* precipitate + no cofactor	0.0	0.0
10000*g* precipitate + NADPH	20.0 ± 1.0	15.0 ± 1.0
10000*g* precipitate + GSH (10 μmol)	0.0	0.0
microsomes + no cofactor	7.0 ± 1.0	4.0 ± 1.6
microsomes + NADPH	83.0 ± 3.6	59.0 ± 2.2
soluble fraction + no cofactor	17.5 ± 1.8	14.0 ± 1.0
dialyzed soluble fraction + no cofactor	7.0 ± 0.7	5.0 ± 1.3
dialyzed soluble fraction + GSH (10 μmol)	19.0 ± 2.5	13.0 ± 2.5
control[c]	0.0	0.0
control[d]	0.0	0.0

[a] All values expressed as percent of metabolites and the average of four experiments (mean ± SE). [b] Incubation time 60 min. [c] Boiled 10000*g* supernatant. [d] 0.1 M potassium phosphate buffer (pH 7.4).

Addition of (5.0, 10.0, 20.0 mM) GSH to 10000*g* supernatant or the soluble fraction did not result in any significant increase in metabolic activity. Dialysis of the subcellular fractions resulted in a >50.0% decrease in metabolic activity.

From the degradation study presented in Tables II and III, it was observed that a 2.0-h incubation period was adequate to investigate the metabolic behavior of both compounds in rat and mouse liver microsomes and soluble fraction. When vamidothion was incubated for 2.0 h with rat or mouse liver microsomes fortified with NADPH, the parent compound was rapidly metabolized (oxidized) to an organic soluble metabolite. The product(s) formed following 2.0-h incubation amounted to 85.0% and 62.0% of vamidothion equivalents with rat and mouse liver microsomes, respectively. Two-dimensional TLC using solvent system VI followed by solvent systems II, IV, or VII resolved one metabolite, identified as vamidothion sulfoxide on the basis of cochromatography with a standard. No other metabolite was detected.

Incubation of thiovamidothion with rat or mouse liver microsomes and NADPH for 2 h resulted in more than 90.0% and 70.0% of thiovamidothion equivalents being metabolized. Figure 1 shows the metabolism of vamidothion and its thio analogue by rat and mouse liver microsomes. Two-dimensional TLC of thiovamidothion metabolites using solvent system VI followed by solvent II resolved three organic-soluble metabolites and five water-soluble metabolites. Cochromatography of the unknown organic soluble metabolite with known standards revealed that they were thiovamidothion sulfoxide, vamidothion, and vamidothion sulfoxide. The amounts of each organic-soluble metabolite and the water-soluble metabolite are presented in Figure 2. Vamidothion was also incubated for 2.0 h with either dialyzed rat or mouse liver soluble fraction supplemented with (5.0 mM) GSH. No significant increase in metabolic activity was observed when compared with the dialyzed enzyme solution. On the other hand, incubation of thiovamidothion with dialyzed rat or mouse liver soluble fractions supplemented with 5.0 mM GSH resulted in approximately 20.0% and 15.0% of thiovamidothion being metabolized to water-soluble metabolites in rat and mouse preparations, re-

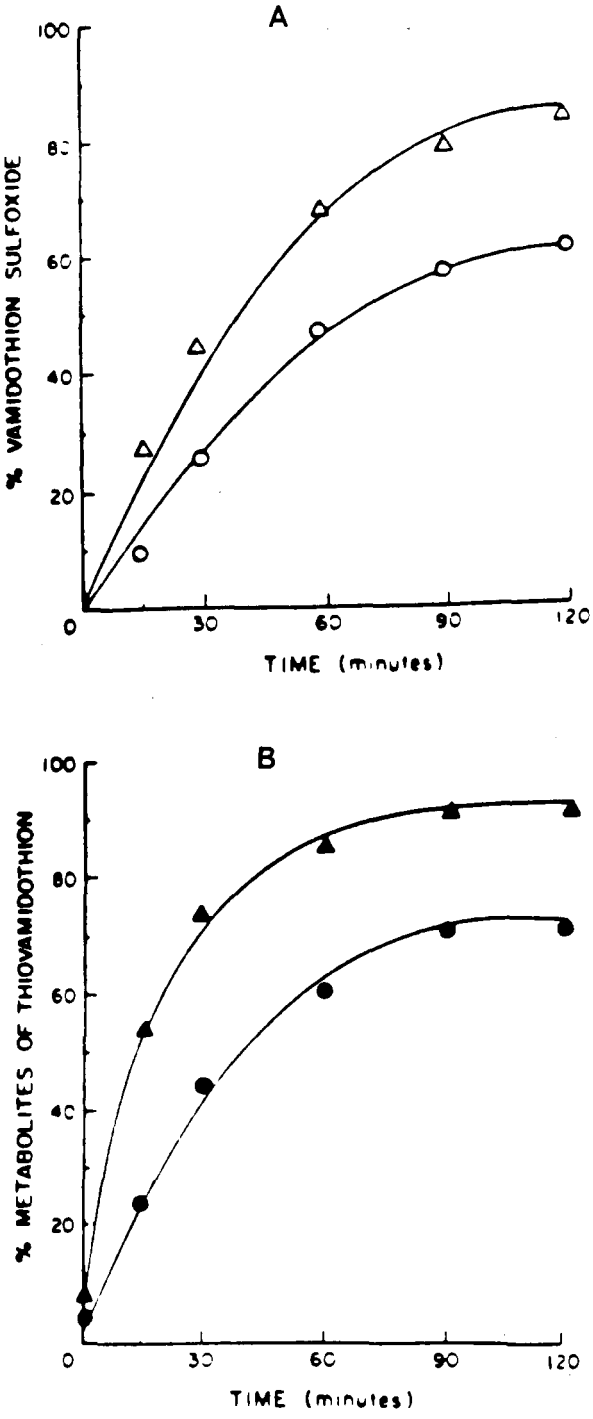

Figure 1. A. Oxidation of vamidothion to the sulfoxide by rat (△) and mouse (○) liver microsomes. B. Oxidative metabolism of thiovamidothion in rat (▲) and mouse (●) liver microsomes.

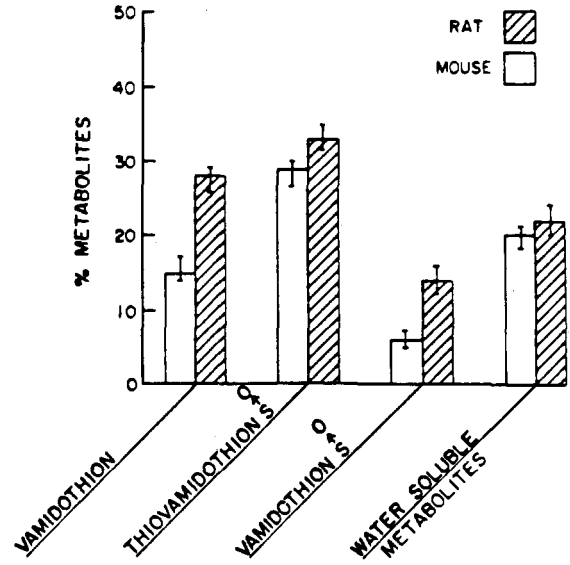

Figure 2. Metabolites of thiovamidothion generated by rat and mouse liver microsomes.

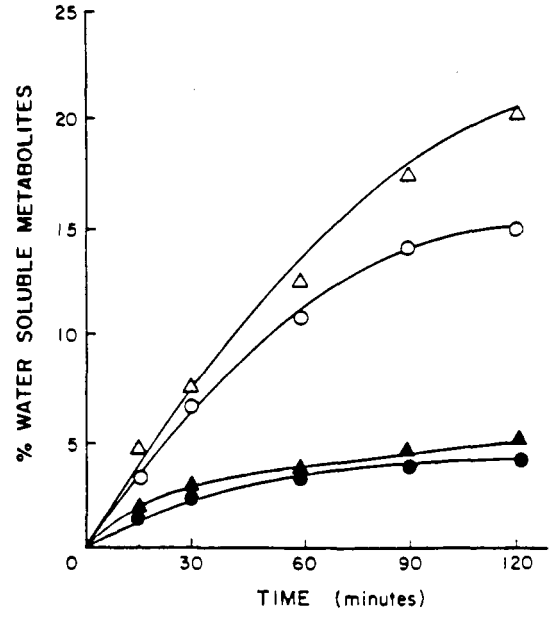

Figure 3. Degradation of thiovamidothion by rat (△) and mouse (○) liver dialyzed soluble fraction + GSH and by rat (▲) and mouse (●) liver dialyzed soluble fraction with no GSH added.

spectively, whereas dialyzed enzyme solutions alone resulted in about 5.0% water-soluble metabolites being formed.

Vamidothion was not metabolized by rat or mouse liver subcellular fractions unless NADPH was added to the incubation mixtures, whereas thiovamidothion was metabolized at a moderate rate by the 10000*g* supernatant and the soluble fractions, and addition of NADPH substantially increased the metabolic activity. This would indicate that monooxygenases are probably involved in the metabolism of the two compounds.

When the results of vamidothion metabolism by rat and mouse liver dialyzed soluble fraction in the presence or absence of GSH were compared with the results of thiovamidothion, it became evident that glutathione *S*-transferase (soluble enzyme) did not play any role in vamidothion metabolism but was responsible for some metabolism of thiovamidothion (Figure 3), and the amount of added GSH was equivalent to the endogenous level.

Effect of Microsomal Oxidase Inhibitors. Table IV shows the effect of selective MFO inhibitors on the metabolism of vamidothion and thiovamidothion by rat and mouse liver microsomes. Incubation of rat or mouse liver microsomes with piperonyl butoxide (2.0 mM) and SK-F525A (2.0 mM) (known cytochrome P-450 inhibitors) at 37 °C for 5 min in the presence of NADPH and oxygen prior to the addition of substrate resulted in more than 90.0% inhibition of vamidothion oxidation to the sulfoxide and more than 75.0% inhibition of thiovamidothion oxidation. When 3.0 mM *n*-octylamine or antireductase (well-known P-450 inhibitors) (Tynes and Hodgson, 1983) were used, the results were similar with more than 80% inhibition of vamidothion sulfoxidation and more than

Table IV. Effect of Inhibitors on the Metabolism of Vamidothion and Thiovamidothion by Rat and Mouse Liver Microsomes[a]

	% inhibition			
	vamidothion		thiovamidothion	
treatment	rat	mouse	rat	mouse
control[b]	0.0	0.0	0.0	0.0
contro6[c]	7.0 ± 1.5	8.3 ± 3.0	11.0 ± 2.0	9.0 ± 4.2
piperonyl butoxide (2.0 mM)	85.4 ± 4.0	83.0 ± 6.0	74.1 ± 5.0	78.0 ± 3.6
SKF 525-A (2.0 mM)	88.1 ± 2.7	90.0 ± 2.4	79.0 ± 4.6	84.0 ± 3.1
n-octylamine (3.0 mM)[d]	86.7 ± 3.0	85.8 ± 3.2	81.7 ± 6.0	86.8 ± 3.0
antireductase[d]	82.0 ± 4.0	87.0 ± 2.6		
heat treatment	4.5 ± 2.0	6.1 ± 2.6	4.7 ± 1.6	7.1 ± 2.0

[a] All values are expressed as percent inhibiton and are the average of three replicates (mean ± SE). [b] Substrate and cofactor were added the same time. [c] Microsomes were incubated with inhibitor cofactor for 5 min prior to addition of substrate. [d] Microsomes were incubated with inhibitor at 4 °C for 5 min prior to addition of substrate and cofactor. [e] Preincubation at 37 °C for 1 h prior to the addition of substrate and cofactor.

70.0% inhibition of thiovamidothion oxidation. Preincubation of the rat and mouse liver microsomes at 37 °C for 1 h prior to addition of substrate and cofactor, to deactivate FAD-containing monooxygenase, resulted in no significant decrease in metabolic activity.

The findings indicate that the enzyme(s) involved in vamidothion sulfoxidation as well as oxidation of thiovamidothion is a cytochrome P-450 dependent monooxygenase system. The FAD-containing monooxygenase [sulfuroxidase, which has been reported to be involved in sulfoxidation of many thioether-containing pesticides including organophosphates (Hajjar and Hodgson, 1980, 1982)] is not involved in sulfoxidation of either vamidothion or thiovamidothion. This conclusion was supported by evidence obtained with mouse liver purified cytochrome P-450 in a reconstituted system and mouse liver purified FAD-containing monooxygenase. Only the cytochrome P-450 showed activity whereas the FAD-containing monooxygenase showed no activity.

Metabolic Behavior of the Sulfoxides. Incubation of either vamidothion sulfoxide or thiovamidothion sulfoxide with rat or mouse liver microsomes fortified with NADPH or with rat or mouse liver soluble fraction and GSH resulted in no further metabolism of vamidothion sulfoxide to sulfone. However, thiovamidothion sulfoxide was metabolized to vamidothion sulfoxide and other oxidative hydrolytic products (Table V). The glutathione *S*-transferase did not show any activity toward vamidothion sulfoxide, but it metabolized thiovamidothion sulfoxide slightly.

Characterization of the Metabolites. *Organic-Soluble Metabolites.* As was mentioned earlier in this study, vamidothion and its metabolite were separated on TLC using solvent system VI. Cochromatography of the resultant metabolite with known standards strongly suggested that the metabolite was the sulfoxide.

GC–MS did not resolve the identity of the metabolite due to thermal degradation of the compound. However, FT-IR and FT-NMR spectroscopies provided information to verify the identity of vamidothion sulfoxide (data available on request).

Florisil column chromatography of the organic-soluble metabolites of thiovamidothion resolved three radioactive peaks. Two-dimensional TLC demonstrated that the first peak was the parent compound, the second peak was vamidothion, and the third peak was resolved into two compounds believed to be thiovamidothion sulfoxide and vamidothion sulfoxide.

Table V. Metabolisum of Vamidothion Sulfoxide and Thiovamidothion Sulfoxide by Subcellular Fractions of Rat and Mouse Liver[a]

	% metabolism			
	thiovamidothion sulfoxide		vamidothion sulfoxide	
subcellular fraction	rat	mouse	rat	mouse
10000*g* supernatant + no cofactor	21.0 ± 2.0	15.0 ± 1.0	0.0	0.0
10000*g* supernatant + NADPH	35.0 ± 3.0	26.0 ± 2.5	0.0	0.0
microsomes + no cofactor	6.4 ± 0.5	5.0 ± 1.0	0.0	0.0
microsomes + NADPH	27.0 ± 1.0	18.0 ± 1.5	0.0	0.0
soluble fraction + no cofactor	10.0 ± 1.0		0.0	0.0
soluble fraction + GSH	12.0 ± 2.0		0.0	0.0
control[b]	0.0		0.0	0.0

[a] All values are expressed as percent of the total substrate and are the average of three experiments (mean ± SE). [b] Phosphate buffer and boiled 10000*g* supernatant.

After further purification of vamidothion and separation of the two sulfoxides on TLC and further cleanup on Prep-Sep C-18 column, FT-IR and FT-NMR spectroscopies provided sufficient information to verify the structure of the metabolites (data available on request).

NADPH-Dependent Water-Soluble Metabolites. Microsomal preparations from neither species metabolized vamidothion to water-soluble metabolite. Attempts to generate sufficient amounts of the water-soluble metabolites at a low substrate concentration for spectral analysis was not successful. However, the small amount of metabolites produced in this case were believed to be hydrolytic products, i.e. cleavage of the P–S bond resulting in the formation of dimethylphosphoric acid and 2-[(2-mercaptoethyl)thio]-*N*-methylpropionamide. This was demonstrated by TLC of the alkaline hydrolysis products of the parent compound.

Water-soluble metabolites of thiovamidothion formed by the microsomal preparation from both species were identified by cochromatography with standards in various solvent systems. They were identified as dimethyl phosphorothioic acid ester (the major water-soluble metabolite), dimethyl phosphoric acid ester, dimethyl phosphorodithioic acid ester, monomethyl phosphorothioic acid ester, and a sulfur-containing unknown. The unknown gave a positive reaction when sprayed with palladium chloride (0.25%) in 0.2 N hydrochloric acid or 2,6-dibromo-*p*-benzoquinone imine (0.5%) in cyclohexane and is believed to be *O*-desmethylthiovamidothion.

GSH-Dependent Water-Soluble Metabolites. Glutathione *S*-transferase (soluble fraction enzyme) did not metabolize vamidothion (phosphorothioate compound) whereas it metabolized thiovamidothion (phosphorodithioate). QAE Sephadex A-25 column chromatography of the aqueous phase from a rat liver soluble fraction experiment resulted in elution of two radioactive peaks. The first peak to elute was identified as *S*-methylglutathione on the basis of its elution volume from the column and cochromatography with standard *S*-methylglutathione. The second peak was identified as desmethylthiovamidothion. It also gave a positive reaction when sprayed with palladium chloride solution in hydrochloric acid or 2,6-dibromo-*N*-chloro-*p*-benzoquinone imine in cyclohexane. Since the thiovamidothion used in this study was radiolabeled in the methoxy position, the ratio of the radioac-

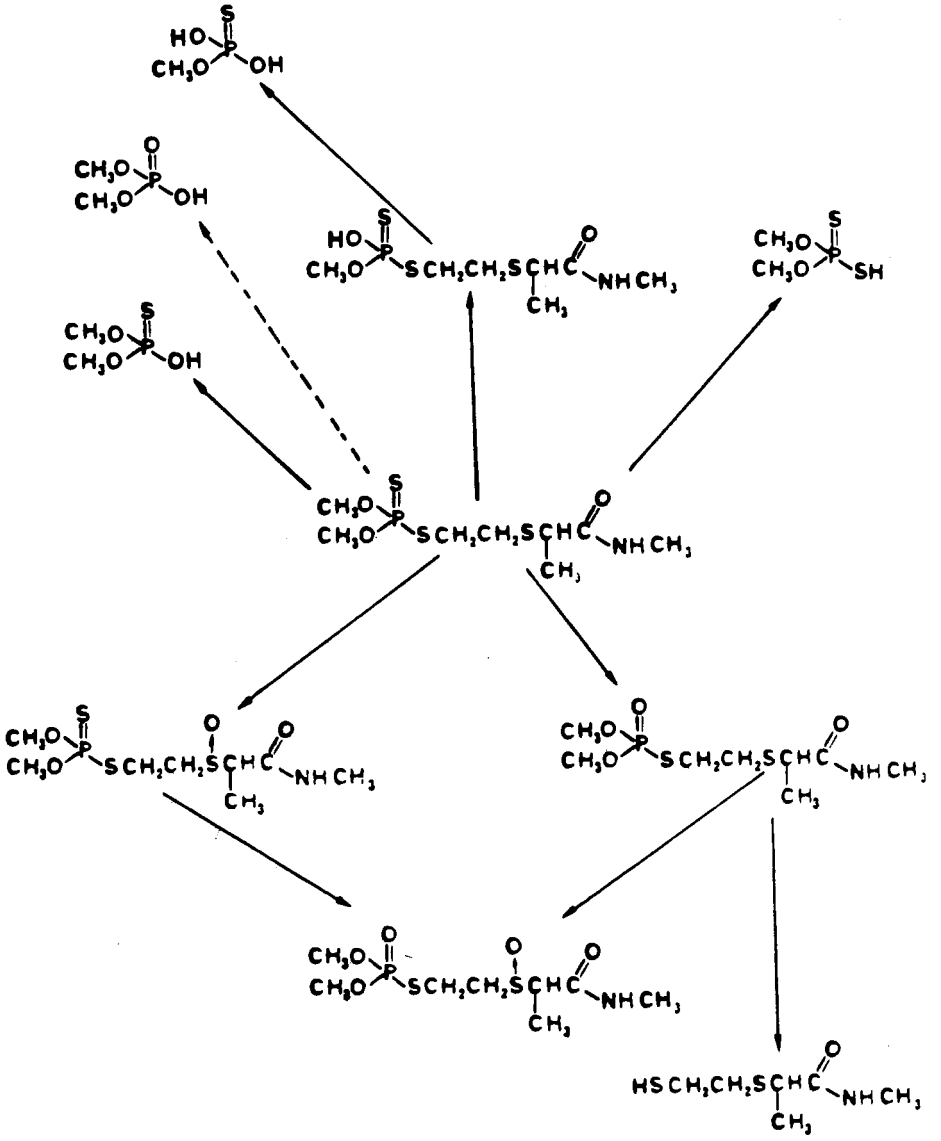

Figure 4. Proposed metabolic pathway of vamidothion and thiovamidothion by rat and mouse liver.

tivity in the two peaks (methylglutathione and desmethylthiovamidothion) was 1:1. On the basis of these findings, the metabolic pathway of vamidothion and thiovamidothion in rat and mouse liver is presented in Figure 4.

Vamidothion was extensively oxidized to the sulfoxide by the cytochrome P-450 dependent monooxygenases system with no further oxidation to the sulfone. Similarly, thiovamidothion was rapidly converted to the sulfoxide, vamidothion, and vamidothion sulfoxide in addition to oxidative hydrolytic products, i.e. cleavage of P–S, S–C, and P–O bonds leading to water-soluble metabolites.

The FAD-containing monooxygenase, which has been reported to catalyze sulfoxidation of the thioether moiety in other sulfur-containing pesticides, including organophosphates (disulfoton, demeton-*S*, demeton-*O*, phorate, fenthion) (Hajjar and Hodgson, 1980, 1982), did not appear to be involved in the oxidation of vamidothion and thiovamidothion.

A small amount of vamidothion hydrolysis can be detected at low substrate concentrations as the result of phosphorotriesterase hydrolysis. Glutathione *S*-transferases, which have been reported to catalyze the *O*-dealkylation reaction of many organophosphorus insecticides, do not play any role in vamidothion metabolism. However, with thiovamidothion as a substrate, glutathione *S*-transferases show moderate activity.

Neither vamidothion nor thiovamidothion appears to be a substrate for amidase. This is in agreement with the findings of Chen and Dauterman (1971a,b) that branched methyl dimethoate and ethylene dimethoate were not substrates for the enzyme. Also, amide-containing phosphates (azodrin, bidrin) and amide-containing phosphorothioates are inhibitors of the enzyme.

ACKNOWLEDGMENT

Paper No. 10341 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC. Work supported in part by PHS Grant ES-00044 from the National Institute of Environmental Health Sciences. We are indebted to Rohne-Poulenc, France, for supplying the chemicals used in this study. M.A.E.-O. is grateful to the Libyan Authorities for financial support during this study and thankful to Dr. Patricia Levi and Dr. Ronald Tynes for providing the purified enzymes and the antibodies.

Registry No. Vamidothion, 2275-23-2; thiovamidothion, 757-87-9; vamidothion sulfoxide, 20300-00-9; thiovamidothion sulfoxide, 17027-39-3; cytochrome P-450, 9035-51-2; monooxygenase, 9038-14-6; glutathione *S*-transferase, 50812-37-8; dimethylphosphonic acid, 868-85-9; 2-[(mercaptoethyl)thio]-*N*-methylpropionamide, 106140-28-7; dimethyl phosphorothioate, 1112-38-5; dimethyl phosphate, 813-78-5; dimethyl phosphoro-

dithioate, 756-80-9; monomethyl phosphorothioate, 106191-34-8; S-methylglutathione, 2922-56-7.

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Received for review February 27, 1986. Accepted August 20, 1986.

Determination and Persistence of Several Fungicides in Postharvest-Treated Apples during Their Cold Storage

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This work describes the storage stability of fungicide residues usually used in postharvest treatment of pome fruits: Benomyl, Carbendazim, Methylthiophanate, Thiabendazole. Fungicide treatments were carried out by immersion of apples, cvs. Starking and Golden Delicious, in aqueous solutions of each fungicide. Fruits were stored at 0 °C (cv. Starking) or +2 °C (cv. Golden Delicious) both in 85–90% relative humidity. Samples were taken monthly, and each fungicide compound was determined in peel, two zones of pulp, and whole fruit by high-performance liquid chromatography. Benzimidazole residues decreased during storage; Thiabendazole, Benomyl, and Methylthiophanate residues found at 160 days of storage were 30–25% (cv. Starking) or 45–55% (cv. Golden Delicious) of the initial amount recovered after treatment. Carbendazim showed the greater storage stability (40–65%) in both cultivars. Most residues were found on the peel, and amounts in the pulp decreased toward the core.

Benzimidazole fungicides, Benomyl [methyl [1-(butylcarbamoyl)-1*H*-benzimidazol-2-yl]carbamate], Carbendazim [methyl 1*H*-benzimidazol-2-ylcarbamate], Methylthiophanate [dimethyl 4,4′-*o*-phenylenebis(3-thioallophanate)], and Thiabendazole [2-thiazol-4-ylbenzimidazole] (Figure 1), have extensively used to control the most important postharvest diseases in pome fruits as *Penicillium expansum* (Spalding and Hardenburg, 1971; Cargo and Dewey, 1970), *Gloesporium spp.* (Bompeix and Morgat, 1970; Burchill and Edney, 1972), and *Botrytis cinerea* (Hardenburg, 1974).

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These compounds are systemic fungicides so they can prevent the development of disease on regions of the plant away from the side of application (Marsh, 1977; Ben-Aziz and Aharonson, 1974; Solel and Edgington, 1973), and their penetration into the fruit was reported on pears (Ben-Arie, 1975).

Systemic fungicides are subject to a number of natural processes that will alter their chemical structure, and the following degradative pathways may be distinguished: first, purely chemical breakdown processes; second, the metabolic systems present in the fruit. Benomyl rapidly loses the butylcarbamate (Carbendazim, MBC; Clemons and Sisler, 1969), and this compound is also formed when benomyl is applied to plants (Baude et al., 1973). Ring cyclization of Methylthiophanate to (benzimidazol-2-yl)-

0021-8561/87/1435-0144$01.50/0