The Selectivity of Sumithion Compared with Methyl Parathion. Metabolism in the White Mouse

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The metabolism of P^{32}-labeled methyl parathion and its 3-methyl analog, Sumithion, has been studied in the white mouse at a series of dosage levels in further investigation of the low mammalian toxicity of Sumithion. The metabolism of the corresponding methylphosphonothionate analogs of methyl parathion and Sumithion also has been investigated. Seven of the eight metabolites of methyl parathion and Sumithion excreted in mouse urine have been identified. All are products of hydrolysis and oxidation. No pronounced differences were observed between these two compounds either in pattern of metabolism or the rate of excretion of metabolites. Evidence is presented that the high selectivity level of Sumithion depends on the ability of the system cleaving the P-O-alkyl bond to play an enhanced role in detoxication as the dosage is increased. This situation is particularly evident with the analogous phosphonothionates, where little desmethylation occurs and the selectivity level of the Sumithion analog is greatly reduced.

In a previous paper (*12*), the authors discussed the comparative toxicity of *O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate (methyl parathion) and *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl)phosphorothioate (Sumithion), their oxygen analogs and their phosphonothioate, phosphonate, and phosphinothioate derivatives to mice and various insects, together with the activity and mode of action of these compounds as inhibitors of mammalian and insect cholinesterases. The relatively low level of mammalian selectivity shown by Sumioxon and the other analogs of Sumithion, when compared with the appropriate analog of methyl parathion, could be attributed to the effect of the *m*-methyl group in reducing the stability of the enzyme-inhibitor complex with mammalian ChE and thus decreasing the over-all rate of inhibition of the enzyme. However, the high level of mammalian selectivity shown by Sumithion itself—mouse oral LD_{50} 54 times greater than that of methyl parathion—cannot be entirely explained on this basis. The present paper continues the examination of the biochemical basis for this selectivity by comparing the metabolism of Sumithion, methyl parathion, and their phosphonothioate analogs in the white mouse.

The metabolism of methyl parathion and related compounds has been studied by many investigators. Diggle and Gage (*5*), in mammals, and Metcalf and March (*18*), in insects, first demonstrated the activation of such phosphorothionates to phosphates by oxidative desulfuration, and Brindley and Dahm (*2*), among others, have unequivocally identified the microsomal oxidation product of methyl parathion as methyl paraoxon. The enzymatic hydrolysis of these compounds is complex, and apparently, distinctly different phosphatases may hydrolyze parathion and paraoxon at the reactive P-O-aryl bond (*16*). In addition, desalkylation has been shown to occur at the P-O-alkyl bonds of both phosphates and phosphorothionates (*8, 29*).

Miyamoto *et al.* (*24*) have investigated the metabolic fate of methyl parathion and Sumithion in rat and guinea pig and were able to identify three of the nine metabolites isolated as dimethyl phosphoric acid, dimethyl phosphorothioic acid, and desmethyl Sumithion The latter compound was produced only by the guinea pig. Comparison of the metabolism of the two compounds is difficult since Sumithion was administered at 10 times the dosage of methyl parathion in each case. In a subsequent paper (*21*), unidentified desmethyl compounds were found in rat tissues. Shishido and Fukami (*31*) have examined the in vitro metabolism of Sumithion, methyl parathion, methyl paraoxon, and parathion by subcellular fractions from rats, insects, and plants. They found five metabolites including dimethyl phosphorothioic acid and the respective desmethyl phosphates and phosphorothionates. In none of these studies could the selectivity of Sumithion be explained realistically on the basis of any metabolic process. Vardanis and Crawford (*32*), on the other hand, using an indirect method, concluded that in mouse liver preparations Sumithion is degraded significantly faster than methyl parathion.

Materials and Methods

P^{32} Sumithion and methyl parathion were prepared from 20 mc. of carrier-free $H_3P^{32}O_4$ (Oak Ridge National Laboratory) by the exchange procedure with P_2S_5—Casida (*3*)—and subsequent refluxing with anhydrous methanol to give *O,O*-dimethyl phosphorodithioic acid, which was distilled and chlorinated to give *O,O*-

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dimethyl phosphorochloridothionate in 44% yield over-all. The condensation with dry sodium *p*-nitrophenoxide or sodium salt of 4-nitro-*m*-cresol (*12*) was carried out with about 0.25 gram of the P^{32}-chloridate and an excess of the phenoxide in dry acetone or methyl ethyl ketone. The reaction mixture was filtered and evaporated and the compounds taken up in benzene, washed twice with 5% Na_2CO_3 and three times with water, dried, and the solvent removed. Methyl parathion crystallized as a white solid after standing in the refrigerator and Sumithion was obtained as a yellow oil. The yields were about 90% from the P^{32}-chloridate, and at the outset of the study, the compounds had specific activities of 0.40 to 0.45 mc. per mmole or 1000 to 1200 c.p.m. per μg. by gas flow counter. These products were 99% radiochemically pure as shown by paper chromatography in the three solvent systems described below.

The P^{32} *O*-methyl methylphosphonate analogs of methyl parathion and Sumithion were prepared from P^{32} *O*-methyl methylphosphonochloridothionate synthesized from $P^{32}Cl_3$ by the method of Perry, Reesor, and Ferron (*26*) and then as described by Hollingworth, Fukuto, and Metcalf (*12*). The P^{32} *O*-methyl *O*-*p*-nitrophenyl methylphosphonothionate or "methyl paraphonothion" formed white crystals, and the P^{32} *O*-methyl *O*-3-methyl-4-nitrophenyl methylphosphonothionate or "sumiphonothion" was a light yellow liquid. After the compounds were washed with 5% NaOH, they were purified by several extractions into cold Skellysolve B to give preparations with specific activities of 0.1 mc. per mmole or 300 to 350 c.p.m. per μg. Paper chromatography showed that they had a radiochemical purity of >95%.

Metabolites of Methyl Parathion and Sumithion

A series of phosphoric acid esters corresponding to anticipated metabolites of these insecticides was prepared as follows. Monomethyl and dimethyl phosphoric acids were prepared from the mixture produced by reacting P_2O_5 and methanol through fractional crystallization of the barium salts—Cherbuliez and Weniger (*4*), Harlay (*10*). The barium salts were converted to the free acids by neutralization with 1*N* H_2SO_4.

O,*O*-dimethylphosphorothioic acid was prepared by the method of Foss (*7*). The free acid was distilled ($b_{1.7}$ 75° to 81° C.) to give a foul-smelling oil, n_D^{25} 1.4652. Methyl *p*-nitrophenyl phosphoric acid (desmethyl methyl paraoxon) and methyl 3-methyl-4-nitrophenyl phosphoric acid (desmethyl Sumioxon) were prepared by methyl carbon-oxygen cleavage of the parent compounds, and methyl paraoxon and Sumioxon were prepared by refluxing in dry acetone with equivalent molar quantities of anhydrous sodium iodide. The products crystallized out upon cooling and were recrystallized from dry acetone–absolute ethanol to give white crystals in yields >90%. Desmethyl methyl paraoxon, sodium salt m.p. 164–6° C. was analyzed: calculated for $C_7H_7NO_8PNa$, C = 33.06%, H = 2.77%; found, C = 33.67%, H = 2.87%.

O-methyl *O*-*p*-nitrophenyl phosphorothioic acid or desmethyl methyl parathion and *O*-methyl *O*-(3-methyl-4-nitrophenyl)phosphorothioic acid or desmethyl Sumithion were prepared as the sodium salts by the action of benzenethiol as described by Miller (*20*). They were recrystallized from $CHCl_3$–*n*-hexane. The sodium desmethyl methyl parathion m.p. 99° to 102° C. was analyzed: calculated for $C_7H_7NO_5PNaS \cdot H_2O$, C = 28.74, H = 3.22; found, C = 29.07, H = 3.14%. Attempts to prepare these desmethyl phosphorothionates by cleavage with NaI resulted in the production of isomers such as *S*-methyl *O*-*p*-nitrophenyl phosphoric acid as was clearly shown by NMR in D_2O which separated the CH_3O protons, a doublet at 3.55 and 3.75 p.p.m. from the TMS standard, and CH_3S protons, a doublet at 2.00 and 2.25 p.p.m.—Martin and Besnard (*15*). The isomerization appears to be the result of the ability of the thiolate anion formed in the initial cleavage reaction to react further with methyl iodide formed in the reaction between the parent compound and sodium iodide. Thus, to dealkylate such a triester, a nucleophile which will give a product which does not react with the thiolate anion is required. Such a nucleophile is found in $C_6H_5S^-$, which produced 90% of the desmethyl compound to 10% of the *S*-methyl isomer as judged by NMR.

Metabolites of Phosphono-Analogs of Methyl Parathion and Sumithion

Methylphosphonic acid was prepared by the hydrolysis of methylphosphonic dichloride and recrystallization from ethyl acetate, m.p. 104.5–105° C. (*13*). Methyl methylphosphonic acid was obtained by the hydrolysis of methyl methylphosphonochloridate—n_D^{25} 1.4275, literature value 1.4248 (*9*). *O*-methyl methylphosphonothioic acid was prepared by the method of Hoffmann, Kagan, and Canfield (*11*) through hydrolysis of the acid chloride. After distillation in a falling film molecular still the product was purified by recrystallization as the dicyclohexylamine salt, m.p. 174–176.5° C.—literature value 179.5–181° C. (*11*), free acid n_D^{25} 1.5021, literature value 1.5005.

The desmethyl derivatives, *p*-nitrophenyl methylphosphonic acid and 3-methyl-4-nitrophenyl methylphosphonic acid were prepared as the sodium salts exactly as described for the corresponding desmethyl phosphate. The sodium salt of *O*-*p*-nitrophenyl methylphosphonothioic acid was prepared as a mixture with other hydrolysis products by treatment of the parent phosphonothionate with 95% alcoholic KOH at 38° C. for 6 hours. The sodium salt of *O*-(3-methyl-4-nitrophenyl)methylphosphonothioic acid, m.p. 67–71° C., was prepared by treatment of the parent phosphonothionate with benzenethiol as described for desmethyl Sumithion. The purity of all the potential metabolites was characterized by paper chromatography with the use of color tests for functional groups.

Male Swiss mice were fed the radiotracers in 0.15 ml. of olive oil (*12*), placed in individual metabolism cages for the separate collection of urine and feces, similar to those described by Roth *et al.* (*30*), provided with food and water, and maintained at constant temperature of 80° F., and at 30% R.H. Urine samples were collected at 6, 12, 18, 24, 48, and 72 hours after dosage and

frozen immediately, until analysis. Feces were collected after 72 hours only.

Chromatographic Analysis of Metabolism

Paper chromatography was used to determine purity and compare identities of the synthetic metabolites and eluates from column chromatography and to determine radioactivity by strip counting (Vanguard 880). Strips of Whatman No. 1, 30 cm. long and tapering from 23 to 18 mm., were used in 30 × 2.5-cm. test tubes containing 2 ml. of one of the following solvent mixtures: (*a*) ethanol-water-chloroform (38 to 60 to 2) used with paper impregnated with 5% (w./v.) Silicone 550 in Skellysolve B and air dried (*19*); (*b*) Skellysolve B, toluene (4 to 1), saturated with propylene glycol and used with paper impregnated with a mixture of propylene glycol and ethanol (1 to 1), and dried at 80° C. for 12 minutes; and (*c*) 2-propanol–water–concentrated NH_4OH (75 to 24 to 1) used with untreated paper (*27*). R_f values for the various compounds investigated are given in Table I.

The presence of phosphorus was determined by the perchloric acid–ammonium molybdate method of Hanes and Isherwood described by March, Metcalf, and Fukuto (*14*); *p*-nitrophenol liberation by spraying with 10% alcoholic KOH and warming at 80° C. in which relative rates of hydrolysis were a useful guide to structure since triesters hydrolyzed faster than desmethyl esters and phosphates faster than phosphorothionates; P=S groups were detected by treatment with 1% 2,6-dibromo-*N*-chloro-*p*-benzoquinone imine in acetone and heating for 7 minutes at 110° C. as described by Menn, Erwin, and Gordon (*17*).

Ion exchange chromatography was used to separate the urinary metabolites collected from mice over a 24-hour period by essentially the same method as described by Plapp and Casida (*28*). A column of Dowex-1-X8 anion exchange resin 3 × 30 to 35 cm. was prepared and the urine sample, plus a mixture of candidate metabolites for identification by cochromatography, was added to the column followed by 50 ml. of distilled water. Gradient elution was then begun with the following series of mixtures: 0.01 to 0.1*N* HCl, 450 ml. each; 0.1*N* HCl–CH_3OH (1 to 3) to 1*N* HCl–CH_3OH (1 to 3), 300 ml. each; 1*N* HCl–CH_3OH (1 to 3) to concentrated HCl–H_2O–CH_3OH (1:1:6), 200 ml. each; concentrated HCl–H_2O–CH_3OH (1:1:6) to concentrated HCl–H_2O–acetone (1:1:6), 200 ml. each; concentrated HCl–H_2O–acetone (1:1:6), 200 ml. Pressure was applied to the column to maintain a flow

Table I. R_f Values for Paper Chromatography of Phosphorothionates, Phosphonothionates, and Their Metabolites

Compound	System (*a*)	System (*b*)	System (*c*)
Sumithion (II)[a]	0.12 (0.02)[b]	0.82 (0.01)	1.00
Methyl parathion (I)	0.17 (0.01)	0.83 (0.02)	1.00
Sumioxon (VI)	0.90 (0.02)	0.19 (0.08)	1.00
Methyl paraoxon (V)	0.92 (0.02)	0.15 (0.04)	1.00
Desmethyl Sumithion	0.95 (0.02)	0.0	0.80 (0.04)
Desmethyl methyl parathion	0.94 (0.02)	0.0	0.77 (0.03)
Desmethyl Sumioxon	0.97 (0.01)	0.0	0.75 (0.01)
Desmethyl methyl paraoxon	0.96 (0.02)	0.0	0.74 (0.05)
p-Nitrocresol	0.88 (0.02)	0.0	0.75 (0.02)
p-Nitrophenol	0.90 (0.02)	0.0	0.72 (0.03)
O,O-dimethyl phosphorothioic acid	1.00	0.0	0.65 (0.01)
Dimethyl phosphoric acid	1.00	0.0	0.53 (0.05)
Monomethyl phosphoric acid	1.00	0.0	0.16 (0.06)
Orthophosphoric acid	1.00	0.0	0.05 (0.02)
Sumiphonothion (IX)	0.15 (0.02)	0.89 (0.02)	1.00
Methyl paraphonothion (VIII)	0.27 (0.03)	0.65 (0.04)	1.00
Sumiphono-oxon (XI)	0.83 (0.07)	0.17 (0.05)	1.00
Methyl paraphono-oxon (X)	0.92 (0.01)	0.11 (0.02)	1.00
Desmethyl sumiphonothion	...	...	0.78 (0.01)
Desmethyl methyl paraphonothion	...	...	0.80 (0.01)
Desmethyl sumiphono-oxon	...	...	0.68 (0.02)
Desmethyl methyl paraphono-oxon	...	...	0.65 (0.03)
Methyl methylphosphonothionic acid	1.00	0.0	0.57 (0.02)
Methyl methylphosphonic acid	1.00	0.0	0.49 (0.03)
Methylphosphonic acid	1.00	0.0	0.09 (0.02)

[a] Numbers refer to formulas shown in (*12*).
[b] Indicates standard deviation.

rate of 7 to 10 ml. per minute and fractions of 19.5 ml. were collected. The results (Figures 2, 3, and 5) were extremely reproducible and 90 to 100% recovery of applied radioactivity was obtained.

Estimation and Identification of Metabolites

Duplicate 1-ml. samples of each fraction were evaporated in planchets and counted by a gas flow counter to determine the total P^{32} in each fraction of the eluate. The identity of the peaks was confirmed by: analogy with published work on related compounds, coincidence of P^{32} peaks with those of cochromatographed metabolites as established by phosphorus determinations on the eluate, and evaporation or extraction of eluate samples and rechromatography of both P^{32} and nonradioactive compounds on paper. Phosphorus was determined in the eluate samples by the phosphomolybdenum blue methods of Allen (*1*) or Fiske and Subbarow (*6*).

Discussion

Metabolism in the Mouse. The metabolism of P^{32} methyl parathion and Sumithion was compared in the white mouse at the same dosage level and over a series of dosage rates since previous investigations suggested that the dosage level strongly affected the balance of metabolites produced. However, the dosage levels were chosen also so that the two insecticides could be compared at equitoxic levels—i.e., 17 mg. per kg. for methyl parathion and 850 mg. per kg. for Sumithion, the minimum lethal dosages where relatively severe cholinergic symptoms occurred. At the next lower dosages—i.e., 3 mg. per kg. for methyl parathion and 200 mg. per kg. for Sumithion—only very slight symptoms of intoxication were observed.

Rate of Excretion. The appearance of P^{32} metabolites in mouse urine is both rapid and complete (Figure 1). With Sumithion, at the three lowest dosages, more than 75% of the amount administered was recovered in the urine within 24 hours and, even at the highest dosage (850 mg. per kg.), 55% was recovered in the urine within 24 hours. In all four dosages of Sumithion, the recovery in urine plus feces was >90% after 72 hours. Although the initial rate of excretion was almost identical to that with Sumithion, the recovery of methyl parathion in the urine was substantially less at both dosage levels and total recovery in urine and feces seemed to be complete at about 85%. In every case the amount of radioactivity in the feces was low and tended to increase with the dosage level, but never exceeded 10% of the dose administered. Accurate estimation was not possible owing to the difficulty of extracting the P^{32} from the feces with common solvents.

Nature of Metabolites. All the radioactive metabolites found in mouse urine were positively identified (Figures 2 and 3 and Table II), with the exception of the minor amounts of metabolite 5, which after hydrolysis gave a positive test for *p*-nitrophenol and may be the completely dealkylated phosphate. Both P-O-alkyl and P-O-aryl bonds of the phosphorothionates and of the activated phosphates are split readily. The radioactivity in the urine is fully accounted for by hydrolysis products and small amounts of P=O activation products. There is no evidence that such reactions as reduction of the nitro group to an amino group, oxidation of the ring methyl group, or hydroxylation of the ring are important in detoxication of these compounds. The presence of appreciable amounts of the activated phosphates methyl paraoxon and Sumioxon (metabolite 4) is of particular interest. The identity of this fraction was confirmed by cochromatography on the column and by rechromatography on paper with systems (*a*) and (*b*). The lipophilicity of methyl paraoxon and Sumioxon is apparently low enough to permit their excretion in the urine, and although this does not appear to be true of the parent phosphorothionates, this excretion of the true toxicant in the urine is a detoxication mechanism of some significance. The H_2O–$HCCl_3$ partition values for the urine samples showed that from 0.6 to 3.8% of the total P^{32} partitioned into chloroform, and this is consistent with the urinary content of the chloroform partitioning phosphates. The results obtained indicate the metabolic scheme presented in Figure 4.

Comparative Metabolism of Methyl Parathion and Sumithion. The metabolism of the two insecticides is directly comparable at the dosages of 3 and 17 mg. per

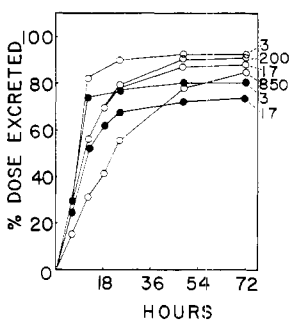

Figure 1. Rates of excretion of radioactivity in the urine of mice dosed orally with Sumithion, O, and methyl parathion, ●, at indicated dosages

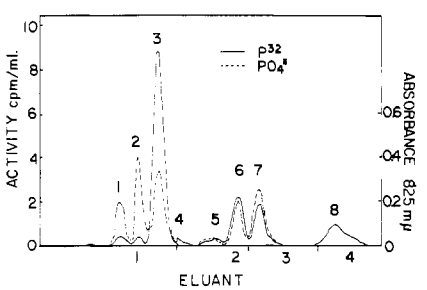

Figure 2. Ion exchange chromatogram of methyl parathion metabolites in mouse urine following 3.0 mg. per kg. oral dosage

– – – – – Unlabeled known metabolites added

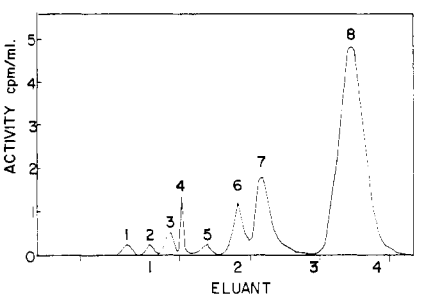

Figure 3. Ion-exchange chromatogram of Sumithion metabolites in mouse urine following 850 mg. per kg. oral dosage

Table II. Metabolites in Mouse Urine within 24 Hours after Dosage

Metabolite		Dose, Mg./Kg. 3	17	3	17	200	850
		Total Dose, Mg. 0.64	3.19	0.54	3.31	37.5	173
		Methyl Parathion		Sumithion			
1	%[a]	2.0	5.8	2.0	2.4	1.9	1.2
Phosphoric acid	μg.	9.8	124	9.7	58.0	547	1130
2	%	1.7	2.0	1.5	2.5	2.4	1.1
Methyl phosphoric acid	μg.	8.4	42.6	7.3	60.5	691	1034
3	%	53.1	31.9	32.2	21.4	5.8	3.0
Dimethyl phosphoric acid	μg.	260	680	157	628	1670	2823
4	%	0.6	2.4	2.7	1.6	3.3	2.5
Phosphate	μg.	3.0	51.1	13.1	38.7	950	2353
5	%	1.9	3.1	2.2	2.5	2.4	2.4
Unknown	μg.	9.3	66.0	10.7	60.5	691	2258
6	%	14.9	12.9	12.8	20.3	8.7	6.6
Dimethyl phosphorothioic acid	μg.	73.3	275	62.5	491	2540	6208
7	%	14.1	23.1	26.1	28.4	24.6	17.1
Desmethyl phosphate	μg.	69.3	492	127	687	7085	16090
8	%	11.7	18.8	20.5	20.1	50.9	66.1
Desmethyl phosphorothioate	μg.	57.5	400	100	486	14640	62200

[a] Per cent of radioactivity in the urine.

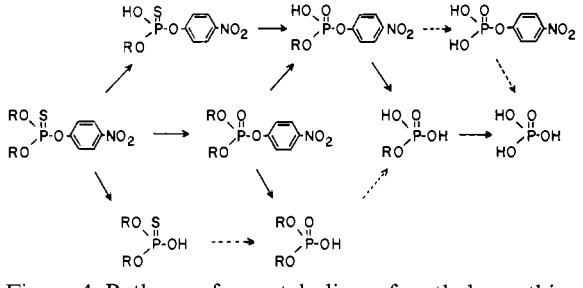

Figure 4. Pathways for metabolism of methyl parathion and Sumithion

– – – – – Hypothetical pathways

kg. as shown in Table II. The recoveries of Sumithion at each dosage are greater than those of methyl parathion because about 15% of the latter is not recovered in the excreta. Two consistent differences were found: There was more dimethyl phosphoric acid following the methyl parathion treatments, and more desmethyl phosphorothionate and desmethyl phosphate were produced following Sumithion treatments. However, there appear to be no gross differences in the way that the two compounds are metabolized at the same dosage level. On the other hand, there may be differences in the rate of detoxication of the two compounds, especially in individual organs, which would only be revealed by more detailed studies than those attempted here.

Comparisons at equitoxic dosages show that the metabolic patterns are entirely different for the two insecticides. Clearly the pattern of metabolism varies with dosage in a similar way for both compounds. It appears that systems, which play a critical role in detoxication at lower dosage levels—e.g., at 17 mg. per kg., where methyl parathion is toxic and thus selectivity first appears—may be relatively unimportant at the highest dosage and thus not involved in determining the final level of selectivity which will be reached by Sumithion.

Metabolism at Different Dosages. The data in Table II give a clear-cut picture of the variation in metabolism with dosage. Metabolites which are secondary breakdown products, such as phosphoric acid (1) and methyl phosphoric acid (2), the unhydrolyzed phosphate (4), and the unknown (5) make relatively constant contributions over the 3 to 850 mg. per kg. range. The desmethyl phosphate (7) and the dimethyl phosphorothionic acid (6) are relatively constant at lower dosage rates but decrease at the highest levels. The most dramatic changes are shown by dimethyl phosphoric acid (3) and the desmethyl phosphorothionates (8). The former is the major metabolite for both methyl parathion and Sumithion at the 3 mg. per kg. dosage, but comprises only 3% of the urinary metabolites at 850 mg. per kg. The 320-fold increase in dosage of Sumithion causes only a 17-fold increase in the amount of this metabolite in the urine after 24 hours. On the other hand, the desmethyl phosphorothionate increases in

importance with dosage and is by far the major metabolite at the 200 and 850 mg. per kg. levels. In this case, the 320-fold dosage increase results in a 620-fold increase in the amount of desmethyl Sumithion in the urine despite the lower level of excretion in 24 hours at the highest dosage. Similar results were observed with ronnel (*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate) by Plapp and Casida (*28*) and a competitive situation exists between the various detoxication mechanisms so that the desmethylating enzymes are not reached at low substrate levels but at higher levels. Because of the saturation of the alternative pathways, an increasingly large proportion of the dosage is degraded by desmethylation, which then assumes increasing importance in protective metabolism.

The remarkable decrease in the level of dimethyl phosphoric acid is more difficult to explain since this compound can be produced by hydrolysis either before or after oxidation (Figure 4). However, a substantial decrease in the importance of dimethyl phosphoric acid (3) occurs even on increasing the dosage from 3 to 17 mg. per kg., although the production of desmethyl phosphate (7) increases. Therefore, the oxidation system is not likely to be saturated at these levels, and the decrease in production of dimethyl phosphoric acid is the result of saturation of the enzyme hydrolyzing the phosphate.

Metabolism of Methyl Paraphonothion and Sumiphonothion. The metabolism of these compounds should be particularly informative because of the importance of desmethylation as a detoxication process. In these phosphonates with only a single methyl ester group the response to desmethylation is likely to be substantially different and may throw some light on the importance of this process in selectivity and on the very high relative toxicity of the phosphonothionate analog of Sumithion. The results with these compounds (Table III and Figure 5) are different in many respects from those of the corresponding phosphorothionates. The urinary excretion of P^{32} was never as complete as with the phosphorothionates, and this was true especially for methyl paraphonothion where it was only about 60% at 72 hours. As was found with the phosphorothionates, the total recovery in urine and feces was much lower with methyl paraphonothion than with sumiphonothion.

A wide range of metabolites was discovered by ion-exchange chromatography. Of the eight compounds detected, five were positively identified and one tentatively. All those identified were hydrolysis products and there was no evidence of degradation of the P-CH_3 bond. The unidentified compounds were metabolite 1, a small but constant initial peak, and metabolite 4, present in small amounts at 2.5 to 2.9 mg. per kg., but not at 10 mg. per kg. Metabolite 5 behaved like the unhydrolyzed phosphonate in its chromatographic properties, but although the levels of this metabolite ranged from 3.0 to 7.7% of the P^{32} in the urine, the amount of P^{32} partitioning into $HCCl_3$ was very low, making this identification questionable.

The most striking aspect of the phosphonothionate metabolism is the very high and constant proportion of

Table III. Metabolites in Mouse Urine within 24 Hours after Dosage

		Dose, Mg./Kg.		
		2.5	2.9	9.7
		Total Dose, Mg.		
		0.41	0.52	1.7
Metabolite		Methyl Paraphonothion	Sumiphonothion	
1	%[a]	1.3	0.7	0.8
Unknown	μg.	2.8	2.5	10.5
2	%	21.3	24.9	31.4
Methyl phosphonic acid	μg.	45.8	89.9	411
3	%	60.5	57.5	53.6
Methyl methylphosphonic acid	μg.	130	207	702
4	%	0.9	1.2	0.0
Unknown	μg.	1.9	4.3	0.0
5	%	6.4	7.7	3.0
Phosphonate	μg.	13.8	27.8	39.3
6	%	7.5	4.2	4.8
Methyl methylphosphonothioic acid	μg.	16.1	15.2	62.9
7	%	1.2	2.6	4.0
Desmethyl phosphonate	μg.	2.6	9.4	52.4
8	%	0.9	1.2	2.4
Desmethyl phosphonothionate	μg.	1.9	4.3	34.1

[a] Per cent of radioactivity in the urine.

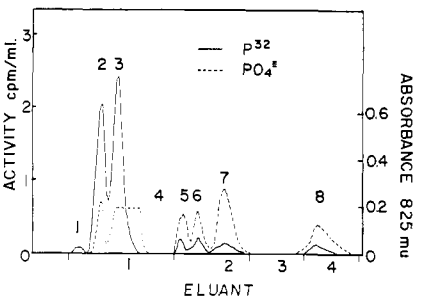

Figure 5. Ion exchange chromatogram of sumiphonothion metabolites in mouse urine following 10 mg. per kg. oral dosage

– – – Unlabeled known metabolites added
Peak 1 contains urinary phosphate

methyl phosphonic acid (metabolite 2) and its methyl ester (metabolite 3). At all three dosages, these two compounds form 80 to 85% of the total metabolites, with methyl methylphosphonic acid forming over 50%. Small amounts only of methyl methylphosphonothionic acid (metabolite 6) are present and the desmethyl compounds are present in very low levels (Table III) as

compared to the substantial levels of the corresponding desmethylphosphates (Table II). This evidence, together with the toxicological behavior of the phosphonates suggests that the hydrolysis of P-O-aryl bond is the principal pathway leading to formation of methyl phosphonic acid although at least some of it must arise from further degradation of desmethyl compounds. If desmethylation is indeed of minor importance as a detoxication mechanism with these phosphonothionates, a suitable explanation for the high toxicity and low degree of selectivity of these compounds would be provided since, as has already been shown with the phosphorothionates, desmethylation is able to take up the burden of detoxication as the other systems fail to keep pace with the increasing dosage. This general argument applies equally well to the corresponding phosphinothionates where the Sumithion analog has only a low level of selectivity (*12*). In this case, desmethylation is not possible since no P-O-alkyl bonds are present.

No major differences are apparent in the metabolism of methyl paraphonothion and sumiphonothion, although the rate of output of hydrolysis products from methyl paraphonothion was slower over the first 24 hours, suggesting that detoxication may proceed at a slower rate than with sumiphonothion. Increasing the dosage of sumiphonothion about threefold had little effect on the pattern of metabolism observed.

One other fact emerges from Table III, namely that more than 90% of the urinary metabolites are P=O compounds. A comparison with the data in Table II shows that the phosphonothionates are subject to more extensive oxidation than the analogous phosphates. Although no one has ascertained whether this oxidation occurs before or after hydrolysis, the authors suggest that the phosphonothionates themselves are not readily hydrolyzed and thus accumulate at the site of oxidation. This would help explain the high toxicity of these compounds and the slower appearance of their urinary metabolites.

The Selectivity of Sumithion. The data given in (*12*), together with the results of the present investigation and the studies of other workers (*21*, *22*, *24*, *31*, *32*), should make it possible to draw certain conclusions as to the biochemical basis for the selectivity of Sumithion. Any satisfactory explanation must account not only for the 50-fold selectivity of Sumithion over methyl parathion to the mouse orally, intraperitoneally, and dermally, but also the sharply reduced (5.7-fold) selectivity of the oxygen analog Sumioxon and the slight (3.8-fold) selectivity of the phosphonothionate sumiphonothion over methyl paraphonothion. Apparently, Sumithion and methyl parathion behave in similar fashion with regard to penetration, activation by oxidation, metabolism, transport, and excretion. The one clearcut difference is the reduced anticholinesterase activity of Sumioxon, which is only 0.2 as active as methyl paraoxon as an inhibitor of mouse brain and bovine erythrocyte cholinesterases. This difference in the critical biochemical lesion very logically explains the difference in toxicity between Sumioxon and methyl paraoxon and also might largely account for the selectivity of Sumithion in the guinea pig where the selectivity level of Sumithion is less than tenfold and about the same as the selectivity level of Sumioxon (*23*). However, in the mouse and rat, a single mechanism cannot explain the selectivity of Sumithion which clearly seems to relate to an integrated sequence of biochemical events in which several steps may favor higher toxicity of methyl parathion. In addition to the difference in anticholinesterase activity already noted, Miyamoto (*22*) reported that methyl paraoxon penetrates into the brain considerably faster than Sumioxon. This is a curious observation in that Sumithion appears to penetrate at least as well as methyl parathion. In fact, if penetration of the blood-brain barrier is related to oil-water partitioning, as is often suggested, Sumithion should penetrate noticeably faster than methyl parathion since the olive oil–water partition coefficient for Sumithion is 4950, while that of methyl parathion is 1010. The partition coefficients for the *n*-octanol–water system are 1990 and 520, respectively. Presumably, the lipophilic *m*-CH_3 group produces a similar relationship between the partition coefficients of methyl paraoxon and Sumioxon.

With regard to metabolism, two additional factors appear to be of importance in the selectivity of Sumithion over methyl parathion. Desmethylation has been stressed in the present work, especially with respect to its protective action at the high dosage levels of 200 and 850 mg. per kg., where desmethyl Sumithion becomes the major metabolite (Table II). In addition, more desmethyl compounds are produced from Sumithion than from methyl parathion at the comparable dosage levels of 3 mg. per kg., where 26% of the urinary metabolites are desmethyl compounds with methyl parathion and 47% with Sumithion (Table II). The markedly decreased selectivity shown by the methylphosphonate and diethyl analogs of Sumithion, as compared with the corresponding derivatives of methyl parathion (*12*, *25*), also strongly supports this idea as the opportunity for desalkylation is considerably reduced in both of these analogs (*31*). Further, desmethylation is of importance in the selectivity of Sumithion, but not of Sumioxon, because of the delay factor provided by P=S to P=O oxidation which permits the desmethylation mechanism to function.

A second point of significance is the consistently higher proportion of total P=O metabolites found with methyl parathion, 71.5% of the total at 3 mg. and 65.2% at 17 mg., as compared with Sumithion, 64.5% at 3 mg. and 56.3% at 17 mg. per kg. In particular, the materially increased proportion of dimethyl phosphoric acid found with methyl parathion (Table II) suggests that Sumithion is less readily activated by P=S oxidation than methyl parathion. This is in accord with in vitro studies of activation by mouse liver slices and microsomes showing that methyl parathion is more rapidly oxidized than Sumithion (*32*).

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