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The Metabolism of Abate in Mosquito Larvae and Houseflies

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Abate (*O,O,O',O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate), a selective mosquito larvicide, and a series of its oxidation products were examined for toxicity to mosquito larvae (*Aedes aegypti*) and adult houseflies (*Musca domestica*). These compounds showed variable toxicity to mosquito larvae but were generally nontoxic to houseflies. Studies on the behavior of Abate in mosquito larvae and houseflies indicate that the rate of absorption of this material into mosquito larvae from a water medium is extremely rapid compared to the rate of penetration into the housefly after topical application. Further, mosquito larvae were able to convert substantially more of the applied Abate to PO esters than houseflies. The selective action of Abate to mosquito larvae may be attributed to penetration and metabolism rates.

INTRODUCTION

Abate (*O,O,O',O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate) is currently being used for the control of aquatic larvae. Based on extensive toxicological evaluations, Abate has proved to be highly effective against a variety of mosquito larvae (1–3) but it is relatively nontoxic to other insects, fish (4), and mammals (5, 6). The reported LD_{50} of Abate to rats and mice is approximately 4000 mg/kg (5).

With increasing emphasis being placed on integrated pest management as a means of pest control, the need for the development of selectively toxic insecticides which affect only, or virtually only, the target pest is becoming increasingly imperative. While substantial progress has been made in elucidating the mechanisms responsible for the selective activity of materials which are toxic to insects but safe to mammals, relatively little information is available concerning differences in toxicity between insect species. Abate, because of its peculiar specificity toward aquatic larvae, provides an interesting example of selectivity between insects. This report is concerned with the absorption and metabolism of Abate in the larvae of the mosquito (*Aedes aegypti* L.) and in the common housefly (*Musca domestica* L.) in an effort to account for its selective toxicity. The metabolism of Abate in the rat has been described by Blinn (7).

MATERIALS AND METHODS

Synthesis

O,O-Dimethyl phosphorochloridothioate (8), dimethyl phosphorochloridate (9), and 4,4'-dihydroxydiphenyl sulfoxide (10) were prepared according to literature methods.

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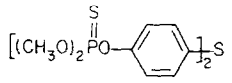
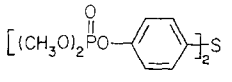
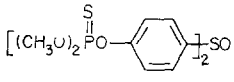
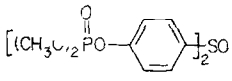
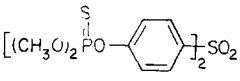
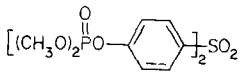
TABLE 1

The chemical and toxicological properties of Abate and its analogs

	Compound structure	Designated symbol	mp (°C)	Elemental analysis		Insect toxicity	
				Calcd	Found	Housefly LD_{50} (μg/g)	Mosquito larvae LC_{50} (ppm)
1		PS(S)	36	—	—	205	6.3×10^{-3}
2		PO(S)	1.5522[a]	C 44.22 H 4.60	44.50 5.56	>500	6.3×10^{-2}
3		PS(SO)	41–42	C 39.65 H 4.14	39.53 4.18	>500	3.3×10^{-2}
4		PO(SO)	32–33	C 42.60 H 4.44	42.46 4.24	>500	2.4×10^{-1}
5		PS(SO_2)	61–62	C 38.52 H 4.03	38.38 4.07	>500	3.9×10^{-2}
6		PO(SO_2)	85–87	C 41.15 H 4.29	41.36 4.52	>500	2.9×10^{-1}

[a] Liquid, refractive index.

4,4′-Dihydroxydiphenyl sulfide was prepared according to a procedure supplied by the American Cyanamid Co., Princeton, NJ, and 4,4′-dihydroxydiphenyl sulfone was purchased from the Aldrich Chemical Co.

The analogs of Abate listed in Table 1 were prepared by reacting the sodium salt of the appropriate phenol and chloridate. The following description for the synthesis of the sulfoxide analog of Abate (3) is typical. 4,4′-Dihydroxydiphenyl sulfoxide (0.92 g, 0.0042 mole) was converted to its sodium salt by treatment with 0.345 g (0.0084 mole) sodium hydroxide in ethanol. Toluene was added to the mixture, the ethanol was removed by azeotropic distillation and 1.42 g (0.0097 mole) dimethyl phosphorochloridate was added. The mixture was heated at reflux for 1 hr, cooled, washed twice with 5% sodium bicarbonate solution, followed by water until the wash was neutral. The toluene solution was dried over sodium sulfate and compound 3 was obtained as a crystalline solid upon the addition of hexane and cooling to −5°C.

Ring-labed [^{3}H] Abate (300 mCi/mmole) was provided by the American Cyanamid Co., Princeton, NJ. The tritium label was *ortho* to the thio–ether linkage. This material was purified by means of thin-layer chromatograpny (TLC) using plates prepared from Silica Gel G (American Optical) and HF-254 (Merck), (1:1) and toluene as the developing solvent. The entire purification procedure, including spotting of the plates and development, was carried out under nitrogen to avoid air oxidation.

The band containing Abate (R_f = 0.24) was removed and extracted with acetone. Removal of the acetone gave [^{3}H] Abate of 99% purity.

Bioassay

The toxicity of the various compounds listed in Table 1 were determined against the susceptible S_{NAIDM} strain of houseflies (*Musca domestica* L.) and the 4th-instar mosquito larvae (*Aedes aegypti* L.) according to usual procedures (11, 12). Anticholinesterase activity, i.e., bimolecular inhibition constant k_i, was determined against housefly-head cholinesterase according to previous methods (13–15). For anticholinesterase determination against mosquito larvae, 4th-instar larvae were weighed in a Duall grinder with 5–10 ml of phosphate buffer (pH 7.9) and 2–3 drops of a saturated aqueous solution of phenyl thiourea to retard melanization. The larvae were homogenized at 2–10°C in an ice bath and centrifuged at 10,000 relative centrifugal force in a Sorvall RC-2 centrifuge for 1 hr at 2–10°C. The supernatant fraction was diluted with buffer to give an enzyme preparation equivalent to 0.3 larva/ml which was used for assay of anticholinesterase activity. The enzyme solution was prepared fresh every 2 days and kept under nitrogen in the refrigerator when not in use.

Metabolism

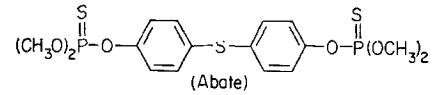
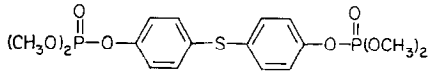
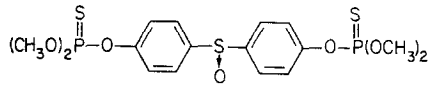
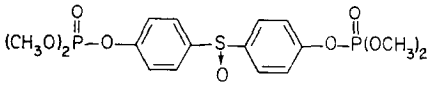
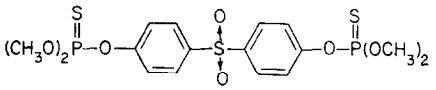
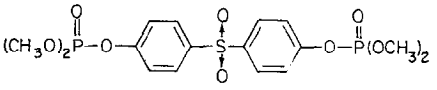
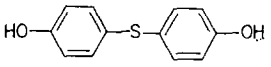
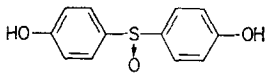
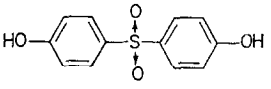
Susceptible 3-day-old female houseflies (S_{NAIDM}) were treated topically on the dorsal surface of the pro- and mesothorax using a 50-μl Hamilton syringe equipped with a 1.0-μl dispenser. [^{3}H]Abate in 1.0 μl acetone solution was applied to each female housefly at the dosage levels of 4.23 μg/g and 53.8 μg/g. Each replicate consisted of 10 flies which were placed in 20-ml glass vials without food or water and closed with cheesecloth. The treated flies were kept at 72°F for predetermined periods ranging from 15 min to 13 hr and sacrificed by placing the vials in a Dry-Ice cabinet. The procedures used to determine rates of penetration and for the extraction of the metabolites have been described (16, 17).

TLC plates used for the separation and identification of the metabolites were prepared according to Blinn (18) from equal amounts of Silica Gel G and Silica Gel HF-254. The plate was spotted on the lower left corner of the plate (3.0 cm from each edge) with the metabolic extract, followed by 3–4 drops of a mixture of 0.1% solutions containing compounds 1–6 (Table 1), 4,4′-dihydroxydiphenyl sulfide (7), sulfoxide (8), and sulfone (9). The plate was developed sequentially using first toluene (A), followed by a toluene (95)–chloroform (95)–methanol(10) mixture (B) in the other direction. The plate was then returned to the original position and developed with a toluene(165) – acetonitrile(98) – nitromethane(37) mixture (C). The individual R_f values for the various compounds for each of these solvent systems are given in Table 2. The position of each compound after sequential development by the three solvent systems is shown graphically in Fig. 1.

After the final development, the plate was air dried and the various spots consisting of compounds 1–9 were visually detected under ultraviolet light. The nine spots and origin spot were marked using a dissecting needle and removed from the plate using a Kontes spot remover. After each spot was removed the gel was placed into a scintillation vial and the gel adhering to the spot remover was rinsed into the vial by forcing two 1.0-ml portions of acetone through the spot remover under air pressure. Each vial containing gel was then placed under a very gentle stream of nitrogen to remove the acetone and the vials were each filled with 10.0 ml of the scintillation fluid and counted on a Packard Tri-Carb 3002 liquid scintillation spectrometer. The radioactivity of each chromatographed compound

TABLE 2

The TLC-chromatographic characteristics of Abate and its analogs in three solvent systems

No.	Compound	$R_f \times 100$		
		System 1[a]	System 2[b]	System 3[c]
1	 (Abate)	24	68	71
2		0	29	17
3		0	54	54
4		0	14	3
5		5	61	66
6		0	21	12
7		0	9	51
8		0	2	13
9		0	4	38

[a] System 1 = toluene.
[b] System 2 = toluene:chloroform:methanol (95:95:10 by vol).
[c] System 3 = toluene:acetonitrile:nitromethane (165:98:37 by vol).

then was corrected for background and, based on the original radioactivity, the amount of each metabolite was calculated. "Trace amounts" were reported for metabolites yielding radioactivity between 0.2–0.4 times background. All TLC procedures were carried out under a nitrogen atmosphere.

At the low-dosage level (4.23 μg/g) the radioactive material remaining at the origin of each plate after TLC of the fly extract was subjected to acid hydrolysis by gentle reflux for 24 hr under nitrogen. The acid hydrolysates were extracted with ether, concentrated to approximately 0.2 ml, and fortified with 7, 8, and 9. The final fortified extract was spotted on TLC plates, developed with solvent C, and the compounds detected under ultraviolet light. The spots were marked and scraped into scintillation vials as described for the other metabolic products obtained from the internal fraction.

Fourth-instar *Aedes aegypti* mosquito

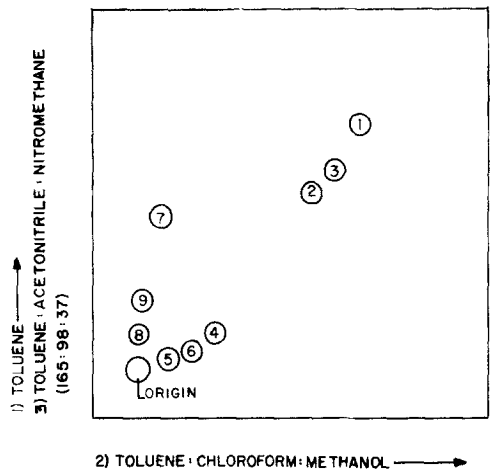


FIG. 1. *Two-dimensional TLC separation of Abate and its metabolites using three solvent systems.*

larvae were weighed on a tared plastic screen after allowing air to evaporate most of the water while the larvae were in a Büchner funnel. From the average wet weight (1.76 mg/larva) the number of mosquito larvae (approx 140) required to make 0.25 g of larvae were counted, transferred into a 5.0-ml beaker containing 3.0 ml distilled water (72°F) and 0.0565 ppm [^{3}H]Abate. After predetermined time periods, the larvae were removed from the Abate water medium using a sintered-glass funnel and the water was collected in a graduated centrifuge tube. The holding beaker and larvae were washed with three 2- to 3-ml portions of water to remove any activity remaining on both the beaker and larvae. The washes were combined with the original water solution and analyzed as the water medium. The larvae were removed from the sintered-glass funnel and immediately frozen under Dry Ice and stored for short periods of time in a freezer until they were extracted according to the procedure used for houseflies. The water medium was acidified with 1 drop of concentrated hydrochloric acid and immediately extracted with three 4-ml portions of ether and the combined ether extracts were assayed for radioactivity and examined by TLC in the usual manner. The mosquito larvae extracts were analyzed in the same manner as described for the housefly extracts.

RESULTS

Toxicity

The toxicity data in Table 1 show that Abate and its oxidation products are relatively nontoxic to the housefly. Only Abate showed appreciable toxicity (205 μg/g) and all of the oxidation products were nontoxic at the highest dose tested. Against the 4th-instar mosquito larvae, *Aedes aegypti*, Abate also was more effective than its oxidation products and the order of effectiveness may be summarized as follows:

$$PS(S) > PS(SO) > PS(SO_2) > PO(S) > PO(SO) > PO(SO_2).$$

Evidently, the order of toxicity to mosquito larvae is directly related to the oxidation state of the molecule. Although this order may be purely fortuitous, i.e., decreasing toxicity with increasing oxidation state (and also polarity), it nevertheless suggests that absorption of the toxicant into the larvae from the aqueous phase may strongly influence the effectiveness of the compound. PS esters, being more lipophilic than PO esters, should be able to penetrate the hydrophobic epicuticular wax layer of the larvae at a faster rate compared to PO esters. The same reasoning also applies to the relative toxicities of the sulfide, sulfoxide, and sulfone. In a similar study with *Culex pipiens quinquefasciatus*, the various intact oxidative metabolites of fenthion {*O,O* - dimethyl - *O* - [4 - (methylthio) - *m* - tolyl] phosphorothioate}, a compound closely related in structure to Abate, also were found to be less toxic than fenthion (19).

No obvious relationship was evident between toxicity and anticholinesterase activity of the PO esters (4–6) and it appears that sensitivity of fly-head and mosquito larvae cholinesterase toward these

TABLE 3

Bimolecular rate constants (k_i) for the inhibition of housefly-head and mosquito larvae cholinesterase by PO analogs of Abate

	Compound	$k_i(M^{-1}\ min^{-1}) \times 10^{-6}$	
		Housefly	Mosquito larvae
4	PO(S)	6.99	0.37
5	PO(SO)	0.59	1.10
6	$PO(SO_2)$	1.20	1.56

compounds has little to do with the selective properties of Abate. Against fly-head cholinesterase (Table 3) the PO(S) ester (4) was significantly more effective as an inhibitor than the oxidation products and the sulfoxide (5) was least effective. In comparison, the pattern of anticholinesterase activity against mosquito larvae cholinesterase was in the order expected, i.e., $PO(SO_2) > PO(SO) > PO(S)$, based on the estimated effect of the bridging linkages on the reactivity of the phosphoryl moieties (20). Overall, however, the series of PO esters were equally effective in inhibiting fly-head and mosquito larvae cholinesterase.

Metabolism in the Housefly

The distribution of radioactivity after topical application of [^{3}H]Abate at the two dosages of 4.23 and 53.8 μg/g is summarized graphically in Fig. 2. Recovery of total radioactivity was quite good at the higher dosage, ranging from 84–100%, but was less satisfactory at the lower dosage which had an average recovery of only 60%.

As expected, the fraction of applied Abate penetrated into the housefly (estimated from amounts found on the external surface of the fly) was much greater at the lower dosage and was approximately equal to that found previously for phoxim (21) and isopropyl parathion (17). At the higher dosage the amount of penetrated Abate relative to the applied dose was quite low and the rate of penetration was approximately zero-order, indicating that the penetration process was near saturation at this dosage. Because of slow penetration, the amount of radioactivity found internally after treatment at the higher level never exceeded 16% of the applied dosage. However, the actual amount of radioactivity present in the fly was 3- to 9-fold greater at the higher than at the lower dosage.

Virtually all of the expected metabolic products were found internally in the fly, either as the intact ester, or as hydrolyzed material. Table 4 gives the various amounts, in terms of microgram equivalents of Abate per gram of fly for each metabolite found in the internal fraction after treatment with Abate. The data show that there is a significant lag in the accumulation of total intact esters (1–6) at the higher dosage relative to the amount of applied Abate. For example, at the point where approximately maximum accumulation occurs (5 hr) and where comparison can be made, the

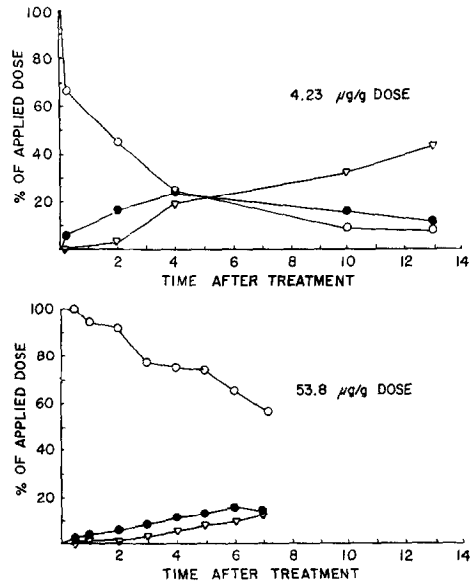


FIG. 2. *Distribution of radioactivity in houseflies after topical application at two dosages; ○—% found externally on fly, ●—% found internally in fly, △—% remaining in vial.*

TABLE 4

Amounts of Abate and its metabolites found internally in the fly after treatment with Abate

Compound	Abate dosage (μg/g)	Amount (μg Abate equiv/g) of recovered radioactivity at indicated time intervals (hr)								
		0.25	0.5	1.0	2.0	4.0	5.0	6.0	7.0	10.0
1 PS(S) (Abate)	53.8	0.62	0.98	1.37	1.90	4.25	4.67	5.16	4.55	—
	4.23	0.22	—	—	0.50	—	0.68	—	—	0.46
2 PS(SO)	53.8	0.04	0.04	0.07	0.13	0.46	0.61	0.70	0.85	—
	4.23	0.01	—	—	0.04	—	0.08	—	—	0.04
3 PS(SO_2)	53.8	0.02	Tr[a]	ND[b]	0.02	0.10	0.20	0.19	0.21	—
	4.23	ND	—	—	0.01	—	0.04	—	—	0.02
4 PO(S)	53.8	ND	Tr	0.01	0.04	0.11	0.14	0.13	0.12	—
	4.23	Tr	—	—	0.01	—	0.02	—	—	0.01
5 PO(SO)	53.8	ND	ND	0.01	0.04	0.12	0.14	0.22	0.18	—
	4.23	Tr	—	—	0.01	—	0.02	—	—	0.02
6 PO(SO_2)	53.8	ND	ND	Tr	0.02	0.09	0.12	0.10	0.11	—
	4.23	Tr	—	—	Tr	—	0.01	—	—	0.01
7 HO(S)	53.8	ND	ND	ND	ND	ND	Tr	Tr	Tr	—
	4.23	Tr	—	—	Tr	—	Tr	—	—	Tr
8 HO(SO)	53.8	ND	0.01	0.04	0.19	0.18	0.24	0.32	0.14	—
	4.23	0.01	—	—	0.02	—	0.03	—	—	0.02
9 HO(SO_2)	53.8	ND	ND	ND	ND	ND	Tr	0.02	0.02	—
	4.23	Tr	—	—	Tr	—	0.01	—	—	0.01
Conjugates (R_fO)	53.8	0.09	0.11	0.33	0.51	1.10	1.14	1.83	1.34	—
	4.23	0.02	—	—	0.19	—	0.11	—	—	0.01
Total PS esters	53.8	0.68	1.02	1.44	2.05	4.81	5.48	6.05	5.62	—
	4.23	0.23	—	—	0.55	—	0.80	—	—	0.52
Total PO esters	53.8	ND	Tr	0.02	0.10	0.32	0.40	0.45	0.41	—
	4.23	Tr	—	—	0.02	—	0.05	—	—	0.04

[a] Trace.

[b] Not detectable.

total amount of intact esters at the high dosage was 5.88 μg/g compared to 0.85 μg/g at the low dosage. Thus, the ratio of intact ester present internally at the two dosages is 6.8, a significantly smaller number than the 12.3-fold difference in the applied dosages. At the earlier time periods, the ratio was even smaller. These results serve to point out the limiting effect that penetration has on housefly intoxication by Abate.

The largest amount of radioactivity present in the fly was in the form of unchanged Abate at all time intervals after treatment, ranging from 75–90% of the total intact esters, indicating that the metabolism of Abate to oxidation products is quite slow in the housefly. Of the various oxidative metabolites, Abate sulfoxide (2) was present in substantially larger amounts than any of the other intact esters. Significant amounts of the PO esters (4–6) were not found until 1 hr after treatment and the amount reached a maximum at around 5–6 hr.

The total amount of PO esters (4–6) accumulating in the housefly was much lower than that found for PO esters generated from other phosphorothionates previously studied in this laboratory. For example, Camp *et al.* (17) found 0.57 and 0.70 μg/g isopropyl paraoxon in flies 1.0 and 4.0 hr, respectively, after treatment with 4.70 μg/g isopropyl parathion. In

comparison, houseflies contained only 0.013 and 0.39 μg/g equiv of PO esters at the same time intervals after treatment with 53.8 μg/g Abate. Since intoxication of houseflies probably occurs through the PO esters, i.e., by inhibition of cholinesterase, the low level of PO esters present after Abate treatment is consistent with the high tolerance of houseflies toward Abate.

A small amount of the total radioactivity found internally in the housefly was in the form of free phenols (7–9) of which the sulfoxide (8) was present in greatest abundance. Based on the nature of the TLC system the amount of material remaining at the origin of each chromatogram of the internal extract was presumed to be made up largely of conjugates of the phenols. However, the possibility that some of this material may consist of other highly polar metabolites, e.g., demethylated Abate and related metabolites or the monophosphate and monophosphorothioate derivatives, may not be excluded. At the lower dosage of 4.23 μg/g, each origin spot was hydrolyzed with hydrochloric acid and the hydrolysate examined by TLC. At each post-treatment time the amount of Abate phenol (7) was the principal hydrolytic product, e.g., 5 hr after treatment 68% of the hydrolysate consisted of 7, followed by 34% of the sulfone (9). These results may explain why very little 7 or 9 was found free in the fly. Evidently, these two phenols or their equivalents, especially 7, are rapidly conjugated in flies.

Metabolism in Mosquito Larvae

For the study of Abate metabolism in mosquito larvae (*A. aegypti*) a single dose of 0.0565 ppm in 3.0 ml water containing 0.25 g larvae was used. On a weight basis the level of Abate present in the water was equivalent to 0.678 μg/g larvae. Because of its aqueous habitat it was necessary to examine the water medium for metabolites as well as the larvae. It became apparent early in our studies that the larvae absorbed Abate at a rapid rate and that metabolites appear both internally in the larvae and externally in the water medium after short intervals following exposure. This absorption and desorption of Abate and its metabolites shall be referred to as "recycling."

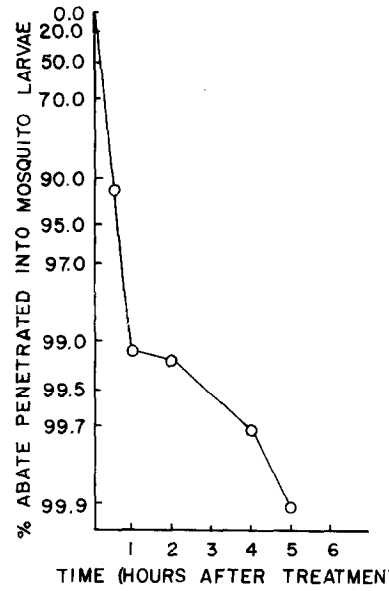


FIG. 3. *The percentage of applied dosage penetrated into mosquito larvae at various time intervals after treatment with Abate.*

As shown in Fig. 3, the absorption (or penetration) of Abate into mosquito larvae was extremely rapid, over 99% being absorbed within 1 hr after exposure. In calculating the rate of absorption, the amount of Abate remaining in the water was assumed to be unabsorbed material, although there was the possibility of recycling of Abate itself.

In spite of the recycling phenomenon, substantial amounts of radioactivity remained inside the larvae. Data for the distribution of Abate and its metabolites in larvae and the water medium are presented graphically in Fig. 4. The amount present in water was divided into an ether-extractable and an ether-unextractable fraction to

differentiate between metabolites and conjugates since compounds 1–9 all partitioned in favor of ether. The total recovery of radioactivity including that found in the larvae, ether-extractables of the water medium and ether-unextractables in the water, ranged from 80–91%. The results show that substantial amounts of radioactivity accumulate in the larvae, even as early as ¼ hr after exposure (42%), rising to a maximum after 1 hr (55%). Concurrently, there was a rapid decrease in ether-soluble material and a gradual increase in ether-insoluble material in the water medium. Evidently, Abate or its metabolites accumulate in mosquito larvae at a rate some 2–3 times faster than in houseflies.

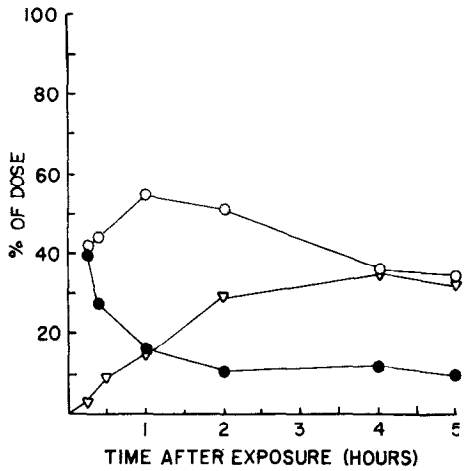


FIG. 4. *Distribution of Abate and its metabolites in mosquito larvae and the water medium;* ○*—internal in larvae,* ●*—ether extract of water medium,* △*—water medium after ether extract.*

Table 5 provides information on the nature and amounts (in terms of μg equiv Abate per g larvae) of Abate and its metabolites at various times after exposure. As in the case of houseflies, the largest amount of radioactivity found internally in mosquito larvae was in the form of Abate. However, in contrast to houseflies Abate appeared to be more rapidly metabolized in mosquito larvae; the internal amount of Abate being reduced from 0.211 μg/g at 0.5 hr to 0.053 μg/g at 5.0 hr. In relation to

TABLE 5

Amounts of Abate and its metabolites in mosquito larvae and water medium (ether extract) after exposure to 0.678 μg/g Abate

Compound	Amount (μg Abate equiv/g) of recovered radioactivity at indicated time intervals (hr)									
	0.25	0.5		1.0		2.0		4.0		5.0	
	larvae	larvae	water	larvae	water	larvae	water	larvae	water	larvae	water
1 Abate	0.183	0.211	0.065	0.120	0.009	0.071	0.011	0.049	0.004	0.053	0.002
2 PS(SO)	0.004	0.002	0.111	0.003	0.059	0.004	0.026	0.004	0.016	0.003	0.011
3 PS(SO_2)	0.002	0.001	0.002	0.009	0.010	0.008	0.002	0.001	0.004	Tr	0.014
4 PO(S)	0.003	0.001	0.002	0.001	0.010	0.001	0.003	0.001	0.007	Tr	0.003
5 PO(SO)	0.005	0.002	0.001	0.026	0.004	0.005	0.003	0.001	0.005	0.001	0.001
6 PO(SO_2)	0.001	0.001	0.001	0.005	0.002	0.002	0.001	0.001	0.005	0.001	0.003
7 HO(S)	Tr[a]	0.001	Tr	ND[b]	ND	0.001	ND	ND	ND	Tr	Tr
8 HO(SO)	0.023	0.019	0.001	0.022	0.005	0.048	0.009	0.021	0.012	0.033	0.002
9 HO(SO_2)	0.003	0.003	0.002	Tr	0.002	ND	0.005	0.003	0.017	0.007	0.017
Conjugates (R_fO)	0.061	0.059	0.003	0.184	0.010	0.205	0.013	0.170	0.016	0.144	0.016
Total PS esters	0.189	0.214	0.178	0.132	0.078	0.083	0.039	0.055	0.024	0.056	0.027
Total PO esters	0.009	0.004	0.004	0.032	0.016	0.008	0.007	0.003	0.017	0.002	0.007

[a] Trace.
[b] Not detectable.

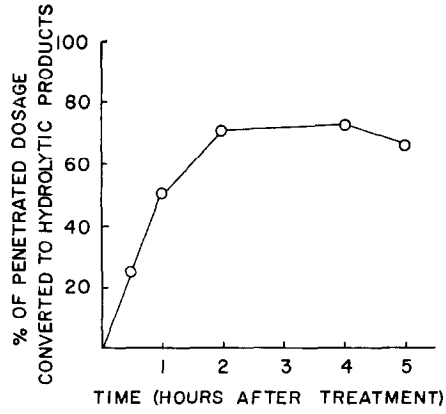


FIG. 5. *The percentage of the penetrated Abate converted to hydrolytic products by mosquito larvae after various time intervals.*

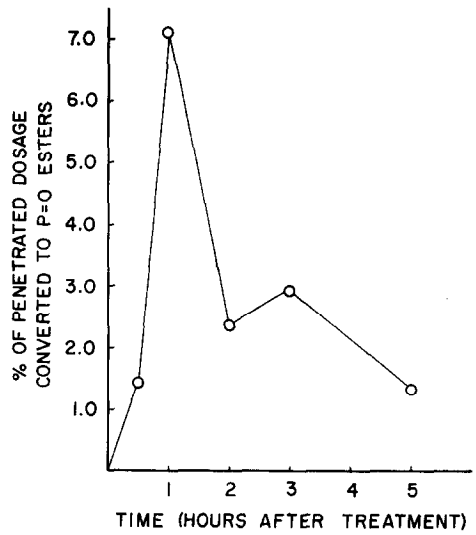


FIG. 6. *The percentage of the penetrated Abate converted to PO esters by mosquito larvae after various time intervals.*

Abate the amount of the other PS esters (2 and 3) present in the larvae was small at all posttreatment times. On the other hand, 2 was the principal constituent present in the water medium.

Mosquito larvae evidently are able to degrade Abate and its intact ester metabolites by hydrolysis more rapidly than houseflies. This is apparent from the amounts of phenols (7–9) and conjugates listed in Table 5 and the relative amounts of ether-insoluble radioactivity present in the water medium as indicated in Fig. 4. The total accumulation of hydrolytic products (larvae + water medium) is shown graphically in Fig. 5. As early as 1 hr after initial exposure, approx 50% of the applied Abate was in the form of hydrolyzed products, increasing to 70% after 2 hr.

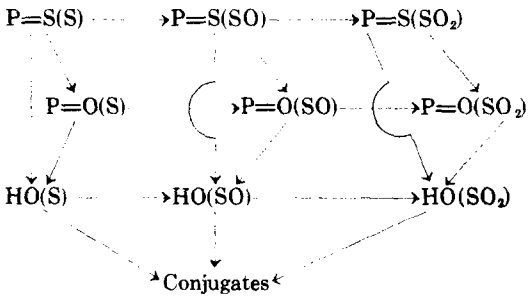
The amount of PO esters found at any time after treatment is the result of the difference in formation and degradation of these esters. Since mosquito larvae are able to degrade Abate and its intact metabolites to hydrolytic products faster than houseflies, it appears that the conversion of PS to PO esters takes place more rapidly in mosquito larvae than in houseflies. For example, at 1 hr after treatment approximately 7.1% (0.048 μg/g) of the applied dose of 0.678 μg/g was present as PO esters (4–6) in the total system (larvae and water medium). In comparison, houseflies treated at 4.23 μg/g, a dosage some 16-fold greater than for mosquito larvae on a weight basis, contained 0.02 μg/g (0.59% of applied dosage) at 2 hr following treatment. The relative amounts of total PO esters found in the combined larvae and water medium at measured intervals after treatment are shown graphically in Fig. 6.

The data in Table 5 show that significant amounts of intact esters are present in the water at all time periods, the largest fraction consisting of the PS(SO) derivative (2). These results indicate that Abate, after absorption in the larvae, is rapidly converted to metabolic products and a substantial portion of the metabolites is eliminated into the water medium, including intact esters. These materials, then, are available to the mosquito larvae for reabsorption and further metabolism.

DISCUSSION

The pathways for the metabolism of Abate in the housefly and mosquito larvae

are qualitatively similar as indicated in the scheme below.



No gross difference in metabolic pathways or products was notable which would readily explain the selective action of Abate. It should be pointed out that assay was not made for the presence of any of the mono-oxono metabolites, i.e., intact esters in which only one of the thiono sulfurs was converted to oxygen. These compounds were not prepared owing to difficulties involved with their synthesis. However, no evidence was obtained in our analysis which confirmed the presence of any of these mono-oxono products. The presence of the mono-oxono ester of the sulfone has been reported on bean leaves after treatment with Abate but it apparently was found in minute amounts since no figures were given (18).

Evidence also was not obtained for the presence in either houseflies or mosquito larvae of the monophosphates or monophosphorothioates. It is possible that they were in the form of conjugates and, therefore, would have remained at the origin of the thin-layer chromatograms. In the study of the fate of Abate in bean plants, only trace amounts of the monophosphate ester of the sulfone was reported to be present in leaves (18).

In spite of the overall similarity in the behavior of Abate in houseflies and mosquito larvae, there are distinct quantitative differences which may account, at least in part, for the toxicity of Abate to these insects. A notable difference is the apparently greater rate of Abate absorption into mosquito larvae compared to flies. At the dosage of 0.678 μg/g (0.3 ml of 0.0565 ppm Abate containing 0.25 g larvae), the penetration of Abate into mosquito larvae was extremely rapid and virtually all of the Abate in the water medium was absorbed into the larvae in less than 1 hr after exposure. The dosage of 0.678 μg/g is approx 27-fold less than the calculated LC_{50} value of 18 μg/g Abate to larvae (from an LC_{50} value of 0.0063 ppm Abate in 100 ml water containing 20 larvae). The calculation assumes that all of the Abate in the water medium is absorbed by the larvae during the bioassay, although this may be an overestimation and the value of 18 μg/g should be regarded as a maximum.

The lower dosage of 4.23 μg/g is approximately 48-fold less than the LD_{50} value of 205 μg/g to houseflies. At this dosage, the percentage of applied Abate penetrated after 1 hr was considerably less than the percentage of Abate penetrated into mosquito larvae after treatment at 0.678 μg/g. In terms of actual amounts penetrated, houseflies absorbed only 2.6 times as much Abate in 1 hr compared to mosquito larvae, although the dosage applied to flies (4.23 μg/g) was more than 6-fold greater than that applied to mosquito larvae. The relative rate of penetration in houseflies after treatment at 53.8 μg/g, a dosage approx 4-fold less than the LD_{50} value, was much slower and may be the limiting step in the intoxication process.

Unfortunately, it is not possible to compare penetration rates at equivalent dosages since absorption into mosquito larvae were not determined at any other concentration and because of the complication arising from the difference in the mode of application. However, the results indicate that at comparable dosages relative to their respective LD_{50} values, Abate is absorbed

faster by mosquito larvae than by houseflies. Recent studies by Henry *et al.* (22) have shown that mosquito larvae exposed to Abate in ponds were concentrating this material 100 times over the bulk pond concentration.

The accumulation of intact PO esters produced by desulfuration of PS esters also was greater in mosquito larvae compared to houseflies, particularly in the early stages after treatment where as much as 8-fold more PO ester was present in the larvae. The rapid buildup of PO esters in mosquito larvae may be attributed in part to rapid initial absorption and the recycling process which allows reabsorption of previously eliminated intact esters from the water medium. The recycling phenomenon particularly applies to the PS(SO) ester which was present in relatively large amounts in the water medium shortly after larvae exposure. Rapid absorption of Abate and conversion to intact PO esters in the larvae undoubtedly are important factors which contribute to the peculiar specificity of Abate to mosquito larvae.

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