Comparative Metabolism of Insecticide Isufenphos in the Housefly (Diptera: Muscidae) and the Cupreous Chafer (Coleoptera: Scarabaeidae)

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The metabolism of ^{14}C -isufenphos was examined in the housefly and the cupreous chafer. Isufenphos penetrated more rapidly into the housefly than into the cupreous chafer. It was rapidly metabolized in the insects and the distribution of ^{14}C -compounds into the aqueous fraction increased gradually. The transformation to water soluble metabolites was faster in the housefly than in the cupreous chafer. No difference in kinds of metabolites was found between the two insects, and as those of isufenphos, isufenphos-oxon, aminoisufenphos, des-*N*-isopropyl isufenphos, desphenyl isufenphos, des-*N*-isopropyl isufenphos-oxon, desphenyl isufenphos-oxon, *O*-ethyl hydrogen phosphoramidate and *O*-ethyl dihydrogen phosphate were identified. The main metabolic pathway was proposed to be the formation of the water soluble metabolites through isufenphos-oxon. Water soluble metabolites were dominantly formed through the cleavage of P-O-aryl linkage.

INTRODUCTION

Isufenphos (*O*-ethyl *O*-2-isopropoxycarbonylphenyl isopropylphosphoramidothioate) has been used for the control of soil insects such as the Japanese beetle, *Popillia japonica* NEWMAN, in turf (LAWRENCE, 1981) and the cupreous chafer, *Anomala cuprea* HOPE, in nursery fields of forest seedlings (HAGIWARA and KOBAYASHI, 1979). As shown previously, the high insecticidal activity of isufenphos to the adzuki bean weevil, *Callosobruchus chinensis* L., and the housefly, *Musca domestica* L., was not dependent on the *in vitro* anti-acetylcholinesterase (AChE) activity of isufenphos-oxon, but the formation of the more potent AChE inhibitor through oxidative activation was suggested (UEJI and TOMIZAWA, 1984, 1986). For the activation mechanism, the metabolism of [ring- ^{14}C] isufenphos was studied in the housefly, and des-*N*-isopropyl isufenphos-oxon was proposed to be an active metabolite (GORDER et al., 1986). The present paper describes the difference of [*O*-ethyl-1- ^{14}C]isufenphos metabolism between the housefly and cupreous chafer.

MATERIALS AND METHODS

Chemicals. [*O*-Ethyl-1- ^{14}C]isufenphos with the specific radioactivity of 3.12 mCi/mmol and radiochemical purity of 99.7% was synthesized by the method reported

previously (UEJI and TOMIZAWA, 1985). Authentic compounds, isufenphos (I), *O*-ethyl *O*-2-isopropoxycarbonylphenyl phosphoramidothioate (II, aminoisufenphos), *O*-ethyl *O*-2-isopropoxycarbonylphenyl isopropylphosphoramidate (III, isufenphos-oxon), *O*-ethyl *O*-2-isopropoxycarbonylphenyl hydrogen phosphorothioate (IV, des*N*-isopropyl isufenphos), *O*-ethyl hydrogen isopropylphosphoramidothioate (V, desphenyl isufenphos), *O*-ethyl *O*-2-isopropoxycarbonylphenyl phosphate (VI, des*N*-isopropyl isufenphos-oxon), *O*-ethyl hydrogen isopropylphosphoramidate (VII, desphenyl isufenphos-oxon), *O*-ethyl hydrogen phosphoramidate (VIII) and *O*-ethyl dihydrogen phosphate (IX) were also prepared according to the procedures described previously. Their chemical structures were confirmed by PMR and MS spectrometry (UEJI and TOMIZAWA, 1984, 1985, 1987).

Thin layer chromatography (TLC). The metabolites were separated on precoated silica gel 20×20 cm 60 F₂₅₄ chromatoplates (Merck) with a thickness of 0.2 mm. The solvent systems and the *R_f* values for the authentic compounds are summarized in Table 1. Radioactive metabolites were detected by autoradiography, using Sakura ³H-type X-ray film, and their radioactivities were determined after scraping respective silica gel regions.

Treatment of the insects with ¹⁴C-isufenphos. A metabolism study was conducted with 2-day-old males of the insecticide susceptible housefly (CSMA strain) and third instar larvae of cupreous chafer (200–290 mg body weight). The insects were kindly supplied by Dr. N. MOTOYAMA of Chiba University and Dr. J. MITSUHASHI of the Laboratory of Insect Pathology, Forestry and Forest Product Research Institute, respectively. Each housefly was topically applied with 0.47 μg of ¹⁴C-isufenphos in 0.4 μl of acetone to a dorsal portion of housefly thorax. One hundred flies were used for each experiment and the treated flies were maintained in a 1 L beaker at 25°C. Ten cupreous chafers were topically applied with 1.0 μl of acetone solution containing 1.02 μg of ¹⁴C-isufenphos per insect and the treated insects were kept in soil at 24–26°C; the soil was volcanic ash collected from the Tsukuba area. Experiments were repeated three times for each insect.

Analysis of ¹⁴C-isufenphos and its metabolites. Houseflies were anesthetized with CO₂ 1, 3, 6 and 24 hr after application. Their body surfaces were rinsed with 10 ml of acetone:methanol (1:1), and radioactivity recovered is referred to hereafter as a body-rinse. The holding beaker was also rinsed twice with 10 ml water, and radioactivity recovered is referred to as a container rinse. Radioactivities in the container rinse were partitioned between benzene and water after adding 20 ml of benzene. The rinsed flies were homogenized with 20 ml of benzene:H₂O (1:1) by a glass homogenizer, and centrifuged. This is referred to as the internal extract. Radioactivities in the internal extract were partitioned between benzene and water. Aliquots of benzene and aqueous layers were put into vials to measure radioactivity on a liquid scintillation spectrometer (Aloka 673). Residues after extraction with both solvents were combusted with a sample oxidizer (Packard 306) and their radioactivities were determined.

The analysis of metabolites in the cupreous chafer was conducted 3, 8, 24 and 48 hr after application. Insects were rinsed with 10 ml of acetone:methanol (1:1) to remove the radioactivity remaining on the surface. The rinsed insects were homogenized with 15 ml benzene, 15 ml water and 3 ml 50% trichloroacetic acid, and the benzene and aqueous layers were separated by centrifugation. Radioactivities in

Table 1. *R_f* values of isofenphos and its metabolites in TLC

Compounds ^b			<i>R_f</i> values in solvent system ^a			
			(A)	(B)	(C)	(D)
I	Isofenphos	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \\ \text{R}_1\text{NH} \end{array} \text{P(S)OR}_2$	0.54	0.82		
II	Aminoisofenphos	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \\ \text{NH}_2 \end{array} \text{P(S)OR}_2$	0.35	0.30		
III	Isofenphos-oxon	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \\ \text{R}_1\text{NH} \end{array} \text{P(O)OR}_2$	0.25	0.20		
IV	Des <i>N</i> -isopropyl isofenphos	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \\ \text{HO} \end{array} \text{P(S)OR}_2$			0.93	0.90
V	Desphenyl isofenphos	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \\ \text{R}_1\text{NH} \end{array} \text{P(S)OH}$			0.87	0.80
VI	Des <i>N</i> -isopropyl isofenphos-oxon	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \\ \text{HO} \end{array} \text{P(O)OR}_2$			0.61	0.50
VII	Desphenyl isofenphos-oxon	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \\ \text{R}_1\text{NH} \end{array} \text{P(O)OH}$			0.54	0.50
VIII	<i>O</i> -Ethyl hydrogen phosphoramidate	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \\ \text{NH}_2 \end{array} \text{P(O)OH}$			0.44	0.25
IX	<i>O</i> -Ethyl dihydrogen phosphate	$\begin{array}{c} \text{C}_2\text{H}_5\text{O} \\ \text{HO} \end{array} \text{P(O)OH}$			0.06	0.08
	Unknown I		0.44	0.71		
	II		0.15	0.12		
	III				0.73	0.66
	IV				0.23	0.14

^a A: hexane: acetone (5:2), B: benzene: MeOH (20:1), C: 2-propanol: NH₄OH:H₂O (75:10:15), D: *n*-BuOH:AcOH:H₂O (80:5:5).

^b R₁: CH(CH₃)₂, R₂:

aliquots of each layer were assayed. The radioactivity in unextractable residues of insects was also determined after combustion with a sample oxidizer. For the determination of ¹⁴C-compounds rubbed off from the body surface of intoxicated cupreous chafer into soil, the soil was immersed in 250 ml of acetone: methanol (1:1) for 48 hr and filtered. The filtrate was concentrated *in vacuo* and radioactivity was partitioned between benzene and water; aliquots of each layer were then put into vials for radioassay.

¹⁴C-Metabolites derived from ¹⁴C-isofenphos were identified by co-chromatography with the authentic compounds, using the solvent systems shown in Table 1. The remainders of body rinse and benzene layer of the insect homogenate were concentrated *in vacuo* and were cleaned up through the Florisil column with ethyl acetate to remove any materials which would interfere with TLC. After neutralizing with 10% sodium

hydroxide, the aqueous fraction was lyophilized and the residues suspended again in 0.1 ml of methanol was applied to TLC.

RESULTS

1. Distribution of radioactivity in the insects applied with ^{14}C -isofenphos

Tables 2 and 3 show the distribution of radioactivity in the housefly and the cupreous chafer. Some overall difference was found. The radioactivity recovered 3 hr after application was greater in the housefly than in the cupreous chafer, and thereafter the recovery percent between the two insects was reversed. ^{14}C -Compounds penetrating into the insects were gradually transformed to water soluble metabolites and excreted. Radioactivity recovered from the container rinse of housefly gradually increased, probably due to the excretion, and this tendency was clear particularly in the water soluble fraction of the container rinse. On the other hand, it was probable that in the soil in which the cupreous chafers were kept, the greater part of the radioactivity was always recovered as benzene soluble compounds due to the direct rubbing off from the body surfaces of intoxicated insects.

Table 2. Distribution of radioactivity in the housefly and container when the insects were applied with [*O*-ethyl-1- ^{14}C] isofenphos

Distribution	Recovery percent of the applied radioactivity (hr)			
	1	3	6	24
Body rinse	11.0	4.9	2.4	1.0
Internal				
Benzene fraction	46.0	36.2	11.0	3.0
Aqueous fraction	26.3	30.2	21.9	10.1
Unextractable residues	4.0	6.4	4.1	3.7
Container rinse				
Benzene fraction	6.6	6.5	5.1	1.6
Aqueous fraction	5.7	13.1	32.9	38.5
Total	99.6	97.3	77.4	57.9

Table 3. Distribution of radioactivity in the cupreous chafer and soil when the insects were applied with [*O*-ethyl-1- ^{14}C]isofenphos

Distribution	Recovery percent of the applied radioactivity (hr)			
	3	8	24	48
Body rinse	5.6	1.0	0.1	0.2
Internal				
Benzene fraction	37.3	29.1	9.2	3.6
Aqueous fraction	8.1	13.8	20.6	16.5
Unextractable residues	4.3	4.0	5.1	2.6
Soil				
Benzene fraction	37.7	32.5	31.2	31.0
Aqueous fraction	1.5	0.9	0.9	1.0
Total	94.5	81.3	67.1	54.9

Table 4. Percentage of metabolites found in houseflies and container when the insects were applied with [*O*-ethyl-1-¹⁴C]isofenphos

Compound ^a	Recovery percent of the applied radioactivity (hr)											
	Body rinse				Internal extract				Container rinse			
	1	3	6	24	1	3	6	24	1	3	6	24
Benzene fraction												
I	10.0	4.2	1.7	0.4	13.6	8.7	1.2	0.6	5.4	5.5	3.8	0.7
II	0.3	<0.1	<0.1	0.1	0.3	0.8	0.5	0.1	<0.1	<0.1	0.1	<0.1
III	0.2	0.1	0.2	0.2	28.3	20.0	7.7	1.8	1.0	0.7	0.5	0.5
Unknown I	<0.1	<0.1	<0.1	<0.1	0.4	0.4	0.1	0.1	0.1	<0.1	0.1	<0.1
II	0.1	0.1	0.1	<0.1	1.8	3.9	0.9	0.2	<0.1	0.1	0.2	0.1
Origin	0.4	0.5	0.3	0.2	1.6	2.4	0.6	0.2	0.1	0.2	0.4	0.2
Aqueous fraction												
IV					2.1	3.1	2.8	0.8	0.5	1.3	2.9	1.7
V					2.4	3.8	2.2	0.4	0.6	2.0	3.8	4.9
VI					0.9	1.9	0.4	0.2	0.1	0.2	0.5	0.5
VII					1.7	2.7	1.0	0.8	0.5	1.4	2.9	4.0
VIII					3.0	3.6	6.3	4.4	1.0	3.1	11.7	12.9
IX					6.6	2.3	2.8	1.5	0.1	0.4	1.7	2.1
Unknown III					2.4	2.4	3.4	0.8	0.3	0.6	0.7	1.7
IV					4.1	9.5	2.6	0.9	2.4	3.9	8.2	9.6
Origin					3.1	0.9	0.4	0.3	0.2	0.2	0.5	1.1

^a See Table 1 for metabolite members.

Table 5. Percentage of metabolites found in the cupreous chafer and soil when the insects were applied with [*O*-ethyl-1-¹⁴C]isofenphos

Compound ^a	Recovery percent of the applied radioactivity (hr)							
	Internal extract				Soil			
	3	8	24	48	3	8	24	48
Benzene fraction								
I	0.9	0.3	0.1	0.1	36.3	28.6	28.8	28.5
II	2.4	1.3	0.8	0.2	0.1	0.5	0.2	0.2
III	30.7	23.0	2.9	0.7	1.0	1.8	1.8	2.0
Unknown I	1.0	0.1	0.1	0.1	<0.1	<0.1	<0.1	<0.1
II	2.0	4.1	3.6	2.4	0.2	0.5	0.3	0.2
Origin	0.3	0.3	1.7	0.1	0.1	1.1	0.1	0.1
Aqueous fraction								
IV	0.9	0.1	0.1	<0.1				
V	0.9	0.3	0.4	0.2				
VI	0.5	1.3	0.9	0.8				
VII	0.5	1.0	1.6	1.0				
VIII	1.1	3.0	4.0	2.9				
IX	1.6	2.5	5.0	4.8				
Unknown III	0.5	0.5	0.4	0.4				
IV	0.6	1.3	6.4	5.2				
Origin	1.5	3.8	1.8	1.2				

^a See Table 1 for metabolite members.

2. Metabolism of ¹⁴C-isofenphos

The kinds of isofenphos metabolites were the same between the housefly and the cupreous chafer. As the benzene soluble metabolites, isofenphos-oxon and amino-isofenphos were identified by co-chromatography with the authentic compounds. Moreover, des*N*-isopropyl isofenphos, desphenyl isofenphos, des*N*-isopropyl isofenphos-oxon, desphenyl isofenphos-oxon, *O*-ethyl hydrogen phosphoramidate and *O*-ethyl dihydrogen phosphate were obtained as the metabolites in the aqueous fraction. The distribution of radioactive metabolites in the housefly and the cupreous chafer treated with ¹⁴C-isofenphos is shown in Tables 4 and 5, respectively. Isofenphos was metabolized more rapidly in the cupreous chafer than in the housefly. The recovery of isofenphos in the container rinse of the houseflies and the soil of the cupreous chafer suggested that it was scraped directly from the body surface of the insects without being metabolized. Among metabolites, amounts of P=O type compounds were always larger than that of P=S type compounds, and the greater part of the metabolites were transformed by the cleavage of P-O-aryl linkage. Main water soluble metabolites were *O*-ethyl hydrogen phosphoramidate and *O*-ethyl dihydrogen phosphate in the housefly and the cupreous chafer, respectively.

DISCUSSION

When the insects were topically applied with ¹⁴C-isofenphos, the penetration rate of the insecticide was different between the two insects, and at 3 hr after application approximately 73% and 50% were recovered as the internal extract of the housefly

and cupreous chafer, respectively. In the penetration study of ^{32}P -crufomate (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate), a wide variation in the amount penetrating the insect body was found among insect species (BRADY and ARTHUR, 1962). More than 80% of the topically applied dose penetrated into the housefly and American cockroach, while less than 25% of the applied crufomate was absorbed 24 hr after application in the boll weevil, *Anthonomous grandis* BOHEMAN and the brown dog tick, *Phipicephalus sanguineus* LATREILLE.

It is likely that the excretion of radioactivity largely corresponded to the aqueous fraction of the container rinse of the housefly and the soil of the cupreous chafer. Based on the distribution of ^{14}C -compounds between benzene and aqueous layers, it is probable that the excretion of these compounds was very slight in the cupreous chafer. It was reported that about 5% of the initial radioactivity was excreted in the housefly applied topically with *S*-[methyl- ^{14}C] *O*-methyl propionylphosphoramidothioate 6 hr after application (KAO and FUKUTO, 1977). It is clear that the excretion of insecticide metabolites differs with insect species and the chemical structure of applied compounds.

Recovery of the same isofenphos metabolites suggested the existence of similar metabolic routes between the housefly and the cupreous chafer, and oxidative desulfuration, dealkylation, dearylation, deamination and hydrolysis might be included in the metabolic pathways. It is probable that isofenphos-oxon as the main metabolite was transformed enzymatically by the mixed function oxidases by analogy with the result of the isofenphos metabolism study with the cupreous chafer microsomal NADPH system (UEJI and TOMIZAWA, unpublished). The water soluble metabolites were formed dominantly by the cleavage of P-O-aryl linkage as shown in the metabolism of ^{14}C -isofenphos with rat (UEJI and TOMIZAWA, 1985, 1987). In the [ring- ^{14}C]isofenphos metabolism in the southern corn rootworm, *Diabrotica undecimpunctata howardi* BARBER, more than 70% of the recovered radioactivity was found at the origin of the TLC plate using the solvent systems of hexane-ethyl acetate (4:1) and acetone-hexane (3:2) (HSIN and COATS, 1986). This result suggested that the formation of conjugates occurred mainly with isopropylsalicylate. Aminoisofenphos-oxon (*O*-ethyl *O*-2-isopropoxycarbonylphenyl phosphoramidate) proposed as an active metabolite of isofenphos (GORDER et al., 1986) was not detected in the present metabolism study. Moreover, no active metabolite(s) was identified in the organosoluble metabolites as shown in

Fig. 1. Proposed metabolic pathway of isofenphos in the housefly and the cupreous chafer.

the *in vitro* rat metabolism (UEJI and TOMIZAWA, 1985). From the above-mentioned results, metabolic pathways of isufenphos in the housefly and the cupreous chafer are proposed as shown in Fig. 1.

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REFERENCES

- BRADY, U. E. and B. W. ARTHUR (1962) Absorption and metabolism of Rulene by arthropods. *J. Econ. Entomol.* **55**: 833–836.
- GORDER, G. W., O. KIRINO, A. HIRASHIMA and J. E. CASIDA (1986) Bioactivation of isufenphos and analogues by oxidative *N*-dealkylation and desulfuration. *J. Agric. Food Chem.* **34**: 941–947.
- HAGIWARA, M. and F. KOBAYASHI (1979) Control of the cupreous chafer with isufenphos. *J. Jpn. For. Soc.* **90**: 387–388 (in Japanese).
- HSIN, C. Y. and J. R. COATS (1986) Metabolism of isufenphos in southern corn rootworm. *Pestic. Biochem. Physiol.* **25**: 336–345.
- KAO, T. S. and T. R. FUKUTO (1977) Metabolism of *O,S*-dimethyl propionyl- and hexanoyl phosphoramidothioate in the housefly and white mouse. *Pestic. Biochem. Physiol.* **7**: 83–95.
- LAWRENCE, K. O. (1981) Japanese beetle: Control of larvae with isufenphos. *J. Econ. Entomol.* **74**: 543–545.
- UEJI, M. and C. TOMIZAWA (1984) Bioactivation of *N*-alkyl substituted phosphoramidothioate insecticides. *J. Pesticide Sci.* **9**: 675–680.
- UEJI, M. and C. TOMIZAWA (1985) *In vitro* metabolism of isufenphos in rat liver. *J. Pesticide Sci.* **10**: 691–696.
- UEJI, M. and C. TOMIZAWA (1986) Insect toxicity and anti-acetylcholinesterase activity of chiral isomers of isufenphos and its oxon. *J. Pesticide Sci.* **11**: 447–451.
- UEJI, M. and C. TOMIZAWA (1987) Metabolism of the insecticide isufenphos in rats. *J. Pesticide Sci.* **12**: 245–251.