Selective Toxicity of Isopropyl Parathion

Metabolism in the Housefly, Honey Bee, and White Mouse

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The metabolism of isopropyl parathion was examined in the housefly, honey bee, and white mouse. Houseflies and honey bees metabolized isopropyl parathion in a qualitatively similar manner. The only water-soluble degradation product isolated and identified in the housefly and honey bee was *O,O*-diisopropyl phosphorothioic acid. Other metabolites appeared to be present in minor quantities but were not identified. Quantitatively, higher levels of organic-soluble material and, in particular, isopropyl paraoxon were found in the housefly than in the honey bee soon after treatment with isopropyl parathion. This, with the greater sensitivity of housefly cholinesterase to isopropyl paraoxon, explains the much greater toxicity of isopropyl parathion to the housefly than to the honey bee. The metabolism of isopropyl parathion in the white mouse was more complex than in the two insects. In addition to isopropyl paraoxon and diisopropyl phosphorothioic acid, evidence indicated the presence of *O*-isopropyl *O-H O*-4-nitrophenyl phosphorothioate and phosphate as major metabolites.

Camp *et al.* (1969) discussed the relative toxicity of *O,O*-diisopropyl *O-p*-nitrophenyl phosphorothioate (isopropyl parathion) and a series of related phosphate, phosphonate, and phosphinate esters to the honey bee, housefly, and white mouse, with their activities as inhibitors of cholinesterase from the honey bee and housefly. Close agreement between anticholinesterase activity and insecticidal toxicity was found with *O,O*-diisopropyl *O-p*-nitrophenyl phosphate (isopropyl paraoxon), which proved to be 36-fold more active in inhibiting housefly than honey bee cholinesterase and 18-fold more toxic to the housefly than to the bee. However, in the case of the other P=O esters, correlation between toxicity and anticholinesterase activity was not so satisfactory. In spite of the reasonable degree of correlation with isopropyl paraoxon, the extremely high level of selectivity shown by isopropyl parathion (>214-fold more toxic to the housefly than to the honey bee) cannot be entirely explained on the basis of selectivity in target enzyme inhibition. This paper is concerned with the comparative metabolism of isopropyl parathion in the honey bee and housefly in an effort to arrive at a biochemical explanation for the selectivity shown by this compound. Because of the relatively low toxicity of isopropyl parathion to the white mouse, metabolism in this animal also was investigated. Although the metabolism of parathion and methyl parathion has been investigated by numerous investigators, isopropyl parathion evidently has not been studied.

MATERIALS AND METHODS

P^{32}-Isopropyl parathion was prepared in the usual manner by the condensation of sodium *p*-nitrophenoxide and P^{32}-diisopropyl phosphorochloridothioate in 2-butanone solvent (Camp *et al.*, 1969). P^{32}-Diisopropyl phosphorochloridothioate was obtained from the Radiochemical Centre, Amersham, England, or prepared from $P_2^{32}S_5$ obtained by radiolabel exchange with P^{32}-phosphoric acid (Casida, 1958) and subsequent reactions according to Fletcher *et al.* (1950). The radioactive isopropyl parathion, recrystallized from ligroin (m.p. 53–55° C.), had a radiochemical purity of >99% and specific activities of 485 and 33,936 c.p.m. per μg. by gas-flow counting.

The sodium salt of *O,O*-diisopropyl phosphorothioic acid was prepared according to Foss (1947) from diisopropyl hydrogen phosphite (McCombie *et al.*, 1945). Numerous attempts were made to prepare sodium *O*-isopropyl *O-p*-nitrophenyl phosphate (monoisopropyl paraoxon) by heating isopropyl paraoxon with sodium iodide or sodium benzenethiolate in refluxing acetone or ethyl alcohol. In all cases the starting material or a compound of unknown composition with unsatisfactory elemental analysis was obtained. Treatment of isopropyl parathion with the same reagents and conditions gave similar unsatisfactory results.

P^{32}-Isopropyl parathion was applied topically to the thoraces of 3-day-old houseflies (S_{NAIDM} strain) and worker honey bees as described previously (Camp *et al.*, 1969). Acetone solutions were radioassayed to determine the amount of material applied to each insect. Following treatment, houseflies were placed in 500-ml. screw-cap jars, mouth-covered with cheesecloth, and the jars were maintained at 15.6° C. (60° F.) for different time intervals. Honey bees were held in a specially prepared metabolism chamber which consisted of a glass tube (8.5 inches long and 3.5 inches in diameter) lined with wire screen. A circle of screen wire was fitted on one end of the tube and attached with Duco cement. After the bees were added, the chamber was inverted into a Petri dish with the sealed end up. This arrangement proved much more effective in maintaining normal behavior in the bees than all-glass

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chambers. In the early phases of this study all-glass chambers were used but abandoned because of high mortality in control replicates. Bees were held at 26.7° C. (80° F.) following treatment. The dosage levels for houseflies were 2.35 and 4.70 μg. per gram and bees were treated at 4.7 and 500 μg. per gram.

The insects were sacrificed 1, 4, 11, and 24 hours after treatment by placing the holding containers in the freezing unit of a refrigerator for 15 to 20 minutes. The procedure used for the extraction of metabolites was essentially identical to that described in detail by Hollingworth *et al.* (1967b). The workup procedure for bees was modified slightly because of the different type of metabolism chamber used. The bee chambers were washed to remove excreta by rinsing with an aerosol sprayer over a large funnel.

The total radioactivity in the external washes, internal extracts, jar holding, and unextractable fractions was determined by evaporation of aliquots in planchets and counting in a gas-flow counter. A 0.1-ml. 5% solution of Carbowax was added to each planchet to minimize loss of radioactive volatiles during evaporation and to aid in dispersing the extracts evenly on the surface of the planchets. Each extract fraction, after removal of the organic solvent in a gentle stream of air, was partitioned with an equal volume of chloroform and water and radioassayed to determine the relative amounts of hydrolyzed and unhydrolyzed material. The chloroform phase was evaporated to dryness and the residue was taken up in 1 to 2 drops of acetonitrile and applied to Absorbosil 1 (Applied Science Laboratories) thin-layer (TLC) plates. Duplicate and triplicate TLC plates were developed in a mixture of 90% chloroform and 10% ethyl acetate (v./v.) and the R_f values of the unhydrolyzed esters were determined by scraping 1-cm. increments into planchets and radioassaying by gas-flow counting.

The R_f values of radioactive isopropyl paraoxon and isopropyl parathion on TLC plates were determined by cochromatography with pure model compounds. These esters were located by spraying the TLC plates with aqueous sodium hydroxide to give yellow spots, formation of blue phosphomolybdate (March *et al.*, 1954), and spraying with 2,6-dibromoquinone-*N*-chloro-*p*-quinoneimine (Menn *et al.*, 1957).

The internal extracts and external holding fractions were also analyzed according to the anion-exchange method described by Hollingworth *et al.* (1967a) after a modification of the method of Plapp and Casida (1958). Dowex 1-X8 (100–200 mesh) was obtained from J. T. Baker Co. and used as described by Hollingworth *et al.* (1967a).

Paper chromatography also was used to a limited extent to confirm the purity of radioactive isopropyl parathion and to characterize water-soluble metabolites. Two paper chromatographic systems were used: a 2-propanol–water–concentrated ammonium hydroxide (75:24:1) system described by Plapp and Casida (1958), and a reversed-phase system consisting of paper impregnated with Silicone 550 (Dow Corning Co.) and developed with absolute ethanol–water–chloroform (Robbins *et al.*, 1959). Radioactivity on paper was determined by the Vanguard Autoscanner 880.

Male Swiss mice were treated orally with P^{32}-isopropyl parathion in olive oil and placed in individual metabolism cages which allowed the separate collection of feces and urine (Hollingworth *et al.*, 1967a). Urine samples were collected 24, 48, and 72 hours after treatment. In some cases, urine samples were collected 4, 8, and 18 hours after treatment and feces after 72 hours. Urinary metabolites were separated by ion-exchange chromatography according to Hollingworth *et al.* (1967a).

METABOLISM IN THE HOUSEFLY AND HONEY BEE

The data for the distribution and metabolism of isopropyl parathion in the housefly (S_{NAIDM}) and honey bee after topical treatment are summarized in Table I.

Table I. Distribution of Isopropyl Parathion and Its Metabolites[a] at Different Time

	Dosage, 4.70 μg./G.							
	1 Hour				4 Hours			
	Fly		Bee		Fly		Bee	
	%	μg./g.	%	μg./g.	%	μg./g.	%	μg./g.
I. Total internal	51.6	2.43	30.3	1.42	59.8	2.81	53.1	2.50
Aqueous	10.9	0.51	12.8	0.60	38.4	1.80	41.8	1.96
Organic	40.7	1.92	17.5	0.82	21.4	1.01	11.3	0.54
Isopropyl parathion	28.7	1.35	—		6.6	0.31	4.9	0.23
Isopropyl paraoxon	12.0	0.57	—		14.8	0.70	6.4	0.31
II. Total external wash	27.9		26.5		5.9		11.8	
Aqueous	0.8		0.4		0.8		0.4	
Organic	27.1		26.1		5.1		11.4	
III. Holding	0.7		1.5		9.9		2.7	
Aqueous	—		—		8.7		0.6	
Organic	—		—		1.2		2.1	
IV. Unextractable	2.6		4.7		5.1		5.9	
V. Total	82.8		63.0		80.7		73.5	

[a] Values expressed in terms of isopropyl parathion equivalents.

Houseflies were treated at 2.35 and 4.7 μg. per gram and honey bees at 4.7 and 500 μg. per gram. The LD_{50} value for isopropyl parathion to the housefly is 4.7 μg. per gram (15.6° C.) and to the honey bee 370 μg. per gram (26.7° C.) (>1000 μg. per gram at 15.6° C.).

At the same dosage level of 4.7 μg. per gram the data in Table I show that isopropyl parathion is absorbed at approximately equal rates by the two insects. This is evident when one compares the values given for total "internal" and total "external wash" for the honey bee and housefly at the various time intervals. Further, the magnitude of the dosage does not seem to affect the penetration rate, since the amounts found internally at 11 hours (only time when comparison may be made) are approximately equal on a percentage basis for bees treated at 4.7 and 500 μg. per gram and houseflies at 2.35 and 4.7 μg. per gram.

On a quantitative basis the results show that houseflies contain internally slightly more isopropyl parathion equivalents than the honey bee at 1 and 4 hours after treatment, but the order is reversed at 11 and 24 hours. Further, the housefly contains internally an almost constant amount of radioactive materials throughout the experimental period of 24 hours, due to the rapid rate of excretion in the housefly, leading to a steady-state situation. In contrast, in bees the levels of radioactivity gradually increased to 70 and 69% of the applied dose at 11 and 24 hours, respectively, because of the reluctance with which bees tend to defecate in captivity. This is evident from examination of the values given in the holding column in Table I. Also throughout the 24-hour period honey bees contained a slightly higher level of water-soluble metabolites of isopropyl parathion than houseflies and houseflies contained a higher level of organic-soluble materials. Figure 1 shows graphically the combined amounts of water-soluble and organic-soluble materials in the internal, external, and holding fractions; the formation of water-soluble degradation products and the disappearance of organic materials in the housefly and honey bee occur at approximately the same rate throughout the 24-hour period.

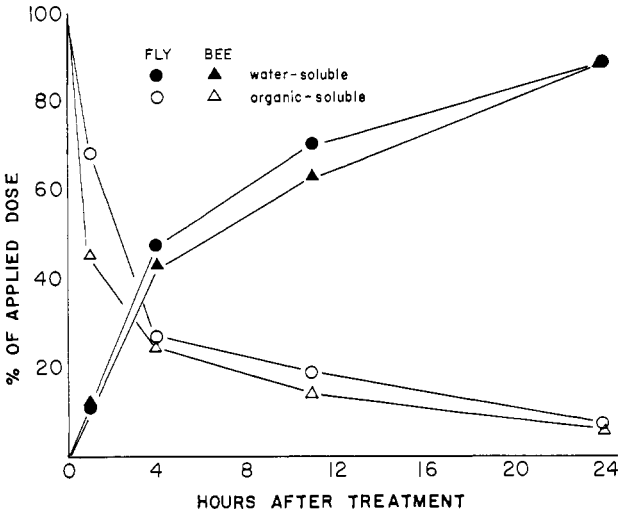


Figure 1. Formation of water-soluble materials and degradation of organic-soluble materials (internal, external, and holding) in houseflies and honey bees after treatment with isopropyl parathion at 4.7 μg. per gram

The most important aspect of this study was to establish and compare the rates of in vivo desulfuration of isopropyl parathion to isopropyl paraoxon in the two insects, in order to determine whether differences in rates of this reaction were in part responsible for the unusual selectivity of isopropyl parathion. These two compounds in the chloroform extract (organic phase) of the internal fraction were separated by thin-layer chromatography (TLC). The TLC procedures described above gave R_f values of 0.55 for isopropyl paraoxon and 0.79 for isopropyl parathion. At the same dosage of 4.7 μg. per gram, the most significant difference in the level of isopropyl paraoxon found internally was at 4 hours, when the honey bee contained 0.29 μg. per gram and the housefly 0.67 μg. per gram. (*Note:* The

Intervals after Topical Treatment of the Housefly (S_{NAIDM}) and Honey Bee

Dosage, 4.70 μg./G.								Dosage, 2.35 μg./G.		Dosage, 500 μg./G.	
11 Hours				24 Hours				11 Hours		11 Hours	
Fly		Bee		Fly		Bee		Fly		Bee	
%	μg./g.	%	μg./g.	%	μg./g.	%	μg./g.	%	μg./g.	%	μg./g.
54.8	2.58	70.0	3.29	48.5	2.28	68.9	3.24	58.9	1.38	57.1	285.5
42.9	2.02	61.2	2.88	44.8	2.11	66.8	3.14	48.6	1.14	27.1	135.5
11.9	0.56	8.8	0.38	3.7	0.17	2.1	0.10	10.3	0.24	30.0	150.0
8.0	0.38	5.1	0.24	1.2	0.05	0.4	0.02	0.7	0.02	27.5	137.5
3.9	0.18	3.7	0.17	2.5	0.12	1.7	0.08	9.6	0.22	2.5	12.5
	(0.17)[b]		(0.16)[b]						(0.21)[b]		(11.9)[b]
4.3		2.5		13.5		5.2		14.5		1.0	
3.4		0.5		12.7		4.4		6.1		0.3	
0.9		2.0		0.8		0.8		8.4		0.7	
30.5		5.5		33.9		21.6		26.7		3.8	
24.2		1.7		31.2		18.5		18.0		0.6	
6.3		3.8		2.7		3.1		8.7		3.2	
2.7		6.2		6.3		9.9		7.1		0.6	
92.3		84.2		102.2		105.6		107.2		62.5	

[b] Values in parentheses indicate actual calculated amounts of isopropyl paraoxon.

values given in Table I are expressed in terms of isopropyl parathion equivalents based on the amount of radioactivity found and the calculated weight of isopropyl paraoxon is given parenthetically.) At 11 hours, the levels of phosphate in the two insects were approximately equal and at 24 hours houseflies contained slightly more than honey bees. At this dosage of 4.7 μg. per gram, 13% of the houseflies were dead after 11 hours and 87% showed signs of hyperirritability, while no symptoms of toxicity were noticeable in the honey bees. The levels of isopropyl paraoxon and parathion found in the internal organic phase are shown graphically in Figure 2. Evidently, at all time intervals the amount of isopropyl paraoxon is greater in the housefly than in the honey bee.

Bees treated at the higher dosage of 500 μg. per gram contained internally after 11 hours 137.5 μg. per gram of isopropyl parathion and 11.9 μg. per gram of isopropyl paraoxon (Table I). The LD_{50} of isopropyl parathion to honey bees is 370 μg. per gram at 26.7° C. (>1000 at 15.6° C.) and at this dosage 83% of the bees were dead after 11 hours. The amount of isopropyl paraoxon present in honey bees at the 500-μg. per gram dosage was approximately 70-fold greater than in the housefly at its LD_{50} dosage of 4.7 μg. per gram. Although direct comparisons at LD_{50} dosages cannot be made, the toxicity of isopropyl parathion to the housefly and honey bee at the respective dosages of 4.7 and 500 μg. per gram is entirely consistent with the relative amounts of isopropyl paraoxon present within the insects and the sensitivity of the respective cholinesterases to this compound. According to our data (Camp *et al.*, 1969), housefly cholinesterase (I_{50} 2.8 × $10^{-7}M$) is approximately 36-fold more sensitive to isopropyl paraoxon than honey bee cholinesterase (I_{50} 1.0 × $10^{-5}M$).

The results obtained with houseflies treated topically at 2.35 μg. per gram (one half of the LD_{50} value) perhaps also deserve comment. At this dosage no mortality was observed at 11 hours after treatment, the time that the flies were worked up. Although the total quantity of absorbed isopropyl parathion is consistent with the applied dosage, the amount of internal isopropyl paraoxon is unexplainably high, slightly higher than the quantity present after treatment at 4.70 μg. per gram. In contrast, the amount of internal isopropyl parathion is substantially less (19-fold) at the lower dosage. In view of the level of isopropyl paraoxon found, the absence of mortality at this dosage is difficult to explain. However, since metabolic analyses were not carried out at earlier time intervals (1 and 4 hours), particularly at 4 hours where the level of isopropyl paraoxon was the highest at the 4.70 μg. per gram dosage, we are not prepared to provide an explanation at this time.

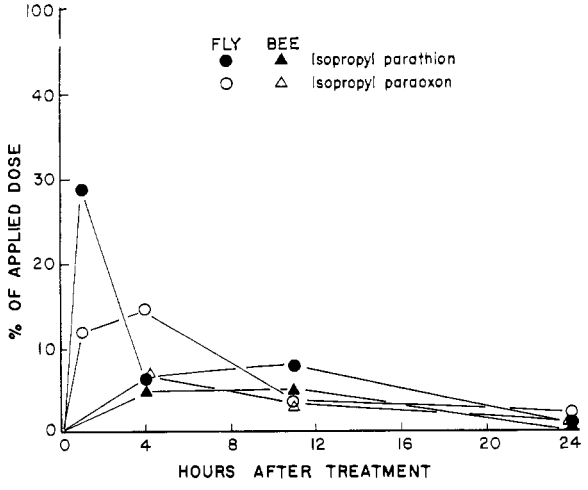


Figure 2. Comparison of quantities of isopropyl parathion and isopropyl paraoxon in internal fraction of S_{NAIDM} and honey bees after treatment with isopropyl parathion at 4.7 μg. per gram

IDENTIFICATION OF METABOLITES

Anion-exchange column chromatography of the "total internal" fractions of the houseflies is shown in Figure 3. The elution pattern for the "total internal" fractions from the honey bee was similar to that of the housefly. Metabolites were identified by TLC and paper chromatography by comparison with known compounds. The R_f values of isopropyl parathion, isopropyl paraoxon, and *O,O*-diisopropyl phosphorothioic acid are presented in Table II. Isopropyl parathion and paraoxon are more soluble in methanol than water and, therefore, were eluted from the column when the first methanol-containing elution fraction was collected (peak I). The eluent containing the intact esters contained the same ratio of isopropyl parathion and isopropyl paraoxon as that found in the "internal" fraction prior to anion-exchange chromatography.

The second of the two peaks (peak II) obtained in the anion-exchange chromatography was identified as *O,O*-diisopropyl phosphorothioic acid. This material eluted immediately after the intact esters and its location agrees favorably with that of *O,O*-dimethyl phosphorothioic acid with respect to methyl parathion and paraoxon (Hollingworth *et al.*, 1967a). TLC analysis of peak II showed that only a single substance, identical to diisopropyl phosphorothioic acid, was present.

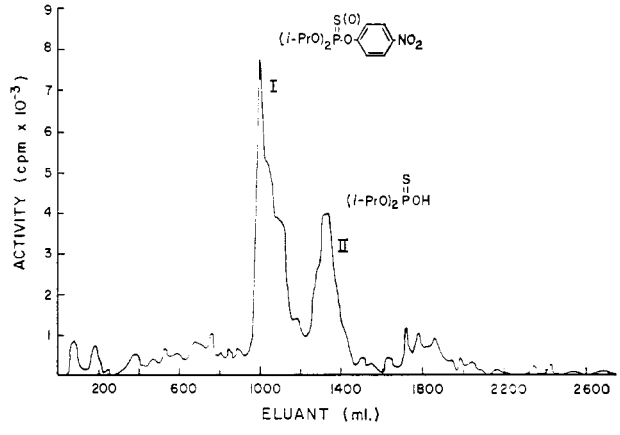


Figure 3. Anion-exchange column chromatography of internal fraction of houseflies 11 hours after treatment with isopropyl parathion at 4.7 μg. per gram

Table II. R_f Values for Paper Chromatography and TLC of Isopropyl Parathion, Isopropyl Paraoxon, and *O,O*-Diisopropyl Phosphorothioic Acid[a]

	Paper		TLC	
	System A	System B	System A	System C
Isopropyl paraoxon	1.00	0.52	—	0.55 ± 0.019
Isopropyl parathion	1.00	0.03	—	0.79 ± 0.019
O,O-Diisopropyl phosphorothioic acid	0.89	1.00	0.85	0

[a] System A. 2-Propanol–ammonia-water.
System B. Silicone-ethanol-water-chloroform.
System C. Ethyl acetate–chloroform.

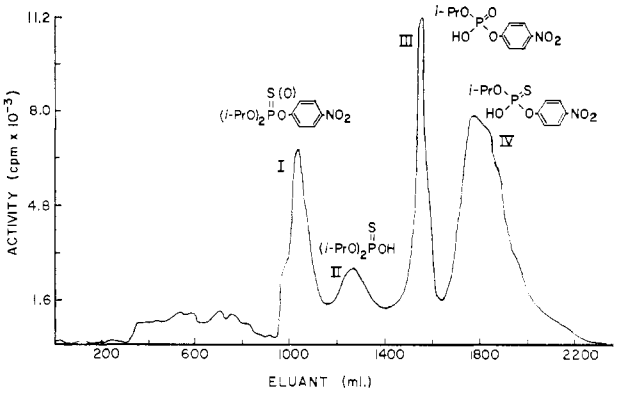


Figure 4. Anion-exchange column chromatography of mouse urine 24 hours after oral treatment with isopropyl parathion at 135.4 mg. per kg.

Evidently, the major pathway for the detoxication of isopropyl parathion in the housefly and honey bee is through the formation of *O,O*-diisopropyl phosphorothioic acid. If other water-soluble metabolites are present in the internal fractions, they are present in only very small quantities. Anion-exchange chromatography and paper chromatography of the "external holding" fractions also showed that the major water-soluble metabolite was in all cases *O,O*-diisopropyl phosphorothioic acid.

The apparent absence of diisopropyl phosphoric acid is puzzling in view of the significant quantities of diisopropyl paraoxon detected in both houseflies and honey bees. Diisopropyl phosphoric acid may be formed in small amounts and then degraded further to isopropyl phosphoric acid and phosphoric acid. The small amount of radioactivity found in an indefinite pattern prior to the elution of isopropyl parathion and paraoxon may be a combination of these acids. However, because of the sample size, serious attempts at positive identification were not made.

METABOLISM IN THE WHITE MOUSE

Because of its secondary importance to this study, the metabolism of isopropyl parathion to the white mouse was investigated in only a preliminary manner. However, because of the rather low order of toxicity of isopropyl parathion to the white mouse (oral LD_{50} 537 mg. per kg.), the problem is one of considerable toxicological interest and importance (Figure 4 and Table III). The metabolic pathway in the detoxication of isopropyl parathion is somewhat more complex in mice than in insects. The major difference is the presence in the urine of substantial quantities of what is believed to be monoisopropyl parathion (*O*-isopropyl *O-H O*-4-nitrophenyl phosphorothioate) and monoisopropyl paraoxon (*O*-isopropyl *O-H O*-4-nitrophenyl phosphate). These two metabolites, peaks III and IV in Figure 4, were not detected in insects. Attempts to synthesize monoisopropyl parathion and monoisopropyl paraoxon by cleavage of one of the isopropyl-*O* bonds in the parent compounds with sodium iodide or benzenethiolate were unsuccessful and our tentative assignment of structures is based on analogy in elution patterns obtained in this study and those obtained by Hollingworth *et al.* (1967a) for the metabolism of methyl parathion in mice in which the same anion-exchange chromatographic system was used.

Table III. Metabolites Found in Urine of Three Male White Mice 24 Hours Following Treatment with Isopropyl Parathion at 135.4 Mg. per Kg.

Metabolite	Fraction	Structure	%[a]
I	Tubes 48–58	$(i\text{-}C_3H_7O)_2P(S)\text{-}OC_6H_4NO_2$ $(i\text{-}C_3H_7O)_2P(O)\text{-}OC_6H_4NO_2$	12.7
II	Tubes 59–71	$(i\text{-}C_3H_7O)_2P(S)OH$	8.9
III	Tubes 72–82	$(i\text{-}C_3H_7O)(HO)P(O)\text{-}OC_6H_4NO_2$	13.7
IV	Tubes 83–111	$(i\text{-}C_3H_7O)(HO)P(S)\text{-}OC_6H_4NO_2$	34.4
Forerun	Tubes 18–44		7.8
Radioactivity not in peaks			1.3
Unrecoverable radioactivity			21.2[b]

[a] Per cent of radioactivity in urine.
[b] 78.8% of radioactivity applied to column recovered.

The chromatographic elution profile in Figure 4 shows without doubt that the mice are able to degrade isopropyl parathion by more than a single pathway. Apparently, mice, unlike the housefly and honey bee, are able to degrade isopropyl parathion and isopropyl paraoxon by dealkylation of the isopropyl moiety in addition to cleavage of the *p*-nitrophenoxide–phosphorus bond. Undoubtedly, this additional degradation pathway contributes in part to the low order of mouse toxicity of isopropyl parathion.

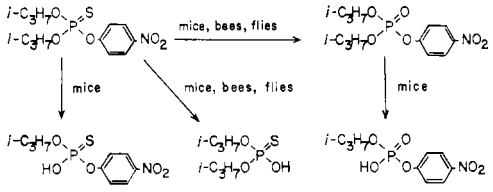
CONCLUSIONS

Qualitatively houseflies and honey bees metabolize isopropyl parathion in a similar manner, but there are quantitative differences. The major difference was a higher level for houseflies of organic-soluble material and isopropyl paraoxon soon after treatment with

isopropyl parathion. The only water-soluble metabolite found in both species was *O,O*-diisopropyl phosphorothioic acid.

The quantitative data indicate that the difference in toxicity of isopropyl parathion to the honey bee and housefly is due in part to differences in rates of in vivo conversion of isopropyl parathion to the active anticholinesterase isopropyl paraoxon. The slower rate of isopropyl paraoxon formation in the honey bee combined with the lower sensitivity of honey bee cholinesterase to this compound (approximately 1/37) adequately accounts for the low honey bee toxicity of isopropyl parathion.

The metabolic scheme below describes the various reactions which were found to take place in this study for isopropyl parathion in the housefly, honey bee, and white mouse.



LITERATURE CITED

Camp, H. B., Fukuto, T. R., Metcalf, R. L., J. AGR. FOOD CHEM. **17,** 243 (1969).

Casida, J. E., *Acta Chem. Scand.* **12,** 1691 (1958).

Fletcher, J. H., Hamilton, J. C., Hechenbleikner, J., Hoegberg, E. J., Sertl, B. J., Cassaday, J. T., *J. Am. Chem. Soc.* **72,** 2461 (1950).

Foss, O., *Acta Chem. Scand.* **1,** 8 (1947).

Hollingworth, R. M., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **15,** 242 (1967a).

Hollingworth, R. M., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **15,** 250 (1967b).

McCombie, H., Saunders, B. C., Stacey, G. J., *J. Chem. Soc.* **1945,** p. 380.

March, R. B., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **2,** 732 (1954).

Menn, J. J., Erwin, W. R., Gordon, H. T., J. AGR. FOOD CHEM. **5,** 601 (1957).

Plapp, F. W., Casida, J. E., *Anal. Chem.* **30,** 1622 (1958).

Robbins, W. E., Hopkins, T. L., Darrow, D. J., Eddy, G. W., *J. Econ. Entomol.* **52,** 214 (1959).

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