Comparative Metabolism of Malathion-C^{14} in Plants and Animals

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The metabolism of malathion-C^{14} was studied in both bean seedlings and rats. The distribution of imbibed or ingested radioactivity in expired CO_2, excrements, and certain tissues was determined. Chromatography was used to determine the distribution of metabolites in extracts of rat urine, rat stomach contents, and plant extracts at various intervals after treatment with the pesticide. The rat eliminated the bulk of ingested radioactivity in the urine within 24 hours; the plant retained the activity primarily in the form of soluble intermediates which were deposited in time into unextractable tissue components.

The effect of pesticides on the environment and particularly upon animals has become not only a national concern but a concern to the scientist as well. Recent advances in the area of pesticides have resulted in the development of less persistent and more selective pesticides. One class of chemicals which has attracted particular attention in this vein is the phosphorothioates. In this class malathion, originally announced in 1950, has been especially attractive because of its extremely low mammalian toxicity.

The effects of malathion on both insects and animals have been well cataloged, and a number of investigations into its biochemical degradation have been reported—for example, its high insect toxicity has been attributed to the "activation" to its oxygen analog malaoxon which is from 2 to 10 times more toxic than malathion itself (Krueger and O'Brien, 1959). Resistance in the case of insects has been attributed to the activity of carboxyesterase(s) with the resulting formation of diacids (Bigley and Plapp, 1962; Dauterman and Matsumara, 1962; Matsumara and Brown, 1961; Matsumara and Dauterman, 1964; Matsumara and Voss, 1964, 1965). Although the major pathway of degradation in the chicken (March *et al.*, 1956), mouse (Krueger and O'Brien, 1959), and rat (Seume and O'Brien, 1960) is via the carboxyesterase(s), there appear to be some limitations, since only the formation of the monoacids has been reported. Soil microorganisms have also been shown to possess carboxyesterase activity (Matsumara and Boush, 1966).

An additional mechanism for malathion degradation, phosphatase(s), has been investigated extensively utilizing P^{32}-labeled substrate (Krueger and O'Brien, 1959; March *et al.*, 1956; Matsumara and Brown, 1961; Matsumara and Voss, 1964). Carboxyesterase action appears to predominate in the mouse, while phosphatase predominates in the housefly. In the cockroach and mosquito the two are about equal (Darrow and Plapp, 1960; Krueger and O'Brien, 1959; O'Brien, 1957). Several excellent reviews have described current knowledge on the mode of action and metabolism of insecticides in insects and animals (O'Brien, 1961, 1966).

The present paper compares the metabolism of malathion (C-^{14}-labeled in the 1,2-succinyl carbon moiety) in plants and rats.

METHODS

Plant Metabolism. Seven-day-old greenhouse-grown red kidney bean seedlings were excised at ground level and placed in small test tubes containing 1 μc. (150 μg.) of *O,O*-dimethyl-*S*[1,2-di(ethoxycarbonyl)ethyl-1,2-C^{14}] phosphorodithioate (malathion-C^{14}) in 0.2 ml. (pH 6.0) of phosphate buffer. The labeled pesticide was obtained from the Nuclear Chicago Corp. and was demonstrated to be chromatographically pure by both paper and thin-layer chromatography. The plants were forced to imbibe the solution by rapidly passing air over the foliage by means of an electric fan. Usually 20 to 30 minutes were required to complete this process. After imbibition, the test tubes were rinsed three times with 0.2 ml. of buffer, then half-filled with nutrient solution. The plants were maintained under lights (14-hour day) and harvested after 1, 2, 3, 4, 7, and 10 days.

Translocation of the carbon-14 label was determined by radioautography in the following manner. The harvested plants were pressed between sheets of filter paper, frozen, and dried in a vacuum desiccator over Dry-rite. Radioautographs of the dried plants were prepared using No-screen medical x-ray film. After exposure, the film was developed in the conventional manner.

Additional plants were subjected to chemical extraction as follows. After removal from the nutrient solution, the stems were thoroughly rinsed with water and blotted dry, and the entire seedling was ground in hexane. The residue was washed twice with hexane and the tissue brought to near dryness under a gentle stream of N_2 at room temperature. The semidry residue was exhaustively extracted with 80% ethanol, filtered on a 1-inch glass fiber filter paper, and dried. The radioactivity in the dried tissue was

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assayed as the carbonate after combustion in a copper oxide train. An aliquot of the ethanol extract was plated directly in a planchet, dried, and counted. An aliquot of the hexane extract was slurried in a planchet with 25 mg. of Nuchar, dried, and counted directly. Standardization of the charcoal counting method with carbon-14-labeled glucose and sucrose showed a comparative efficiency of 31% of the direct plate method. No evidence of loss of malathion from the charcoal could be demonstrated, even after prolonged heating. All radioactivity measurements were reduced to a carbonate counting scale. Samples counted on Nuchar or as direct plates were converted to the carbonate scale using a factor previously determined by combustion experiments.

Extracts were chromatographed on both paper and thin-layer plates. The silica gel thin-layer plates were developed in $CHCl_3$, while the paper strips were developed in 80% phenol, butanol–butyric acid–water (2:2:1, v./v./v.), and ethanol–ammonium hydroxide (28%)–water (80:4:16, v./v./v.). The radioactivity on all the chromatograms was determined with a Packard (Model 7201) chromatogram strip scanner.

$C^{14}O_2$ evolution was measured on a radiometric respirometer (Bourke *et al.*, 1967) which absorbs the respired carbon dioxide in alkali. The plants to be tested were treated in the routine manner and placed in the closed system respirometer. CO_2 measurements were carried out for up to 24 hours using the normal 14-hour day described above.

The radioactivity remaining in the test tubes after imbibition was determined by extracting the nutrient solution with hexane and assaying for radioactivity as described above.

Animal Metabolism. Six male Holtzman rats weighing approximately 200 grams were adapted to the metabolism cages for 3 days before being fed malathion. The cages were of the design used by Roth *et al.* (1948), with the exception of the feeder. This was modified by removal of the stick feeder and addition of a well feeder which would accommodate powdered dry feed mixes.

The basal diet used in this study was composed of: casein, 15%; dextrose, 52.7%; sucrose, 12%; minerals, 4%; nonnutrient fiber, 4%; vitamin mix, 2%; choline dihydrogen citrate, 0.3%; and corn oil, 10%.

At the end of the conditioning period, each rat was fed by stomach tube 5 μc. (25 mg.) of malathion using corn oil as a vehicle. The rats were placed in separate metabolism cages, CO_2-free air was passed through at 50 cc. per minute, and the expired CO_2 was trapped in 0.1*N* NaOH. The CO_2 traps were sampled every 2 hours. The carbon dioxide was precipitated as the barium salt and assayed for radioactivity as previously described. Urine and fecal samples, if present, were collected at the same time as the CO_2. Aliquots of the urine samples were pipetted into planchets, dried, and counted. The feces were burned in a copper oxide combustion train, the CO_2 was collected in alkali and precipitated as the barium salt, and the radioactivity was assayed as for respired CO_2 samples.

At the termination of the experimental period the rats were anesthetized, the chest cavity was opened, and blood samples were taken by heart puncture. The animals were then sacrificed and dissected to remove the heart, lungs, kidneys, liver, spleen, gastrointestinal tract, and stomach contents. All parts were promptly frozen and stored in a freezer for subsequent radiological analysis. For analysis the samples were extracted with hexane, followed by exhaustive extraction with 80% ethyl alcohol. Radioassay was completed as described above. The extracted tissue was dried and burned, and the radioactivity assayed from the carbon dioxide as previously described. All extracts were chromatographed in the solvent systems used in the plant experiments.

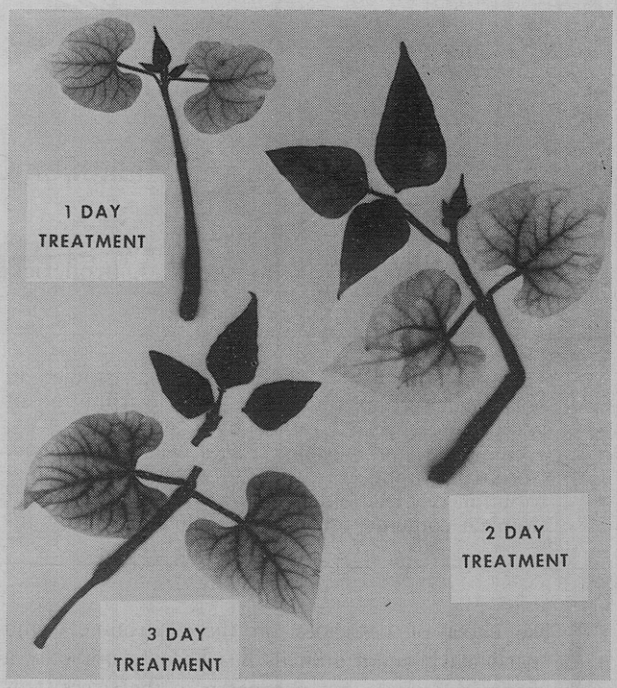

Figure 1. Radioautograms of translocation of carbon-14 in plants which had imbibed malathion-C^{14}

Plants harvested 1, 2, and 3 days after treatment

RESULTS

Plant Metabolism. The radioautograms (Figures 1 and 2) indicate a general distribution of activity in the stem and growing areas, while the older primary leaves show only a slight accumulation. The continued movement with growth suggests that the activity is in some form which is mobile within the plant.

Only a trace of labeled carbon dioxide was evolved from plants which had imbibed malathion. Apparently the complete degradation of the pesticide to carbon dioxide proceeds at an extremely slow rate. The distribution of the activity in the hexane extract, ethanol extract, and tissue is shown in Table I. Appreciable activity was extracted by hexane during the first 2 days, but very little thereafter. The components in the hexane extract which were differentiated by $CHCl_3$–silica gel thin-layer chromatography had R_f values of 0.06 and 0.79 and were present in a 0.04 to 1 ratio. Malathion was found to have an R_f of 0.70. The compound near the origin was found to be identical to

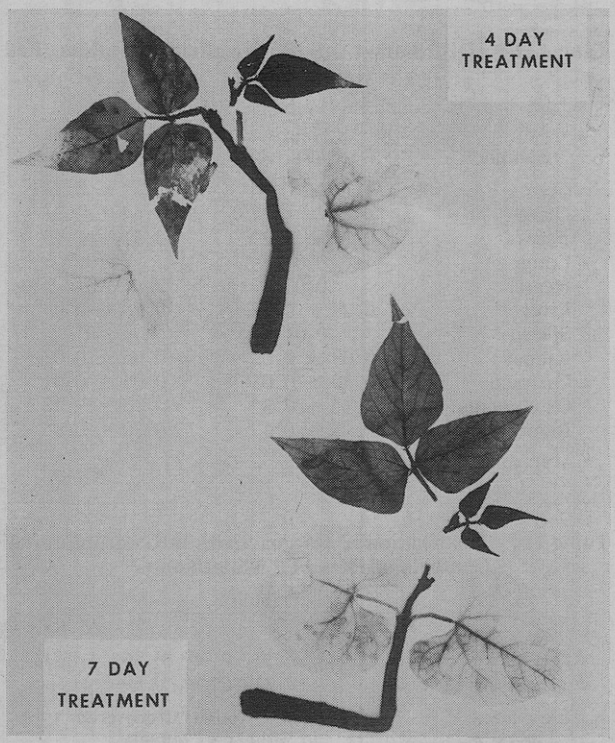

Figure 2. Radioautograms of translocation of carbon-14 in plants which had imbibed malathion-C^{14}

Plants harvested 4 and 7 days after treatment

Table I. Distribution of Carbon-14 from Plants Allowed to Imbibe Malathion-C^{14}

Days from Treatment	% Carbon-14 Added Recovered in			
	Hexane	ETOH-ext.	Residual tissue	Unimbibed
1	7.7	88.7	3.5	
2	1.1	90.4	8.4	Trace
3	0.6	89.2	10.0	Trace
4	0.7	91.2	7.9	0.5
7	Trace	92.0	7.8	Trace
10	Trace	90.9	9.0	Trace

metabolite E present in the ethanol extract. The hexane extract thus appears to contain primarily a single component which disappears rapidly from the plant. Radio gas chromatographic analysis of the hexane extract on a 5-foot by 1/8-inch column (5% Reoplex 400 on Anakrom A, 60-80 mesh) at 185° C. in an Aerograph 204 equipped with a Nuclear Chicago Model 451 proportional detector (inlet tubing temperature 205° C., detector temperature 215° C.) demonstrated the major component to be malathion.

The bulk of the imbibed radioactivity was recovered in the ethanol extract. Paper chromatography of this extract in three different solvent systems followed by radioanalysis on a Packard strip scanner resolved five metabolites. Their R_f values are presented in Table II. Removal of the metabolites from the phenol-water chromatograms and rechromatography in the EAW and BABW

Table II. Distribution of Radioactivity in Ethanol Extract of Plants Treated with Malathion-C^{14}

	Metabolite					Standard Malathion
	A	B	C	D	E	
R_f ϕ^a	0.10	0.34	0.60	0.85	0.85	0.96
R_f EAW^b	0.09	0.10	0.32	0.45	0.73	0.87
R_f BABW^c	0.98	0.07	0.30	0.52	0.78	0.91
Days from Treatment	% of Ethanol Extract Recovered as Metabolite					
1	20.1^d	6.6	19.3	31.0	22.9	
2	18.0	14.0	17.2	28.5	22.3	
5	12.9	10.8	19.9	32.5	23.7	
7	10.7	7.8	31.0	29.0	25.3	
10	8.7	10.4	22.3	30.6	27.8	

[a] 80% phenol in water.
[b] Ethanol–ammonia–water (80:4:16).
[c] Butyric acid–butanol–water (2:2:1).
[d] Average of duplicate determinations.

systems (Table II) confirmed the assignment of the various solvent R_f values. None of these compounds have R_f values similar to that of malathion.

Table II also demonstrates the distribution of radioactivity in the five ethanol metabolites over a 10-day period. Compounds B and D do not appear to change appreciably; however, the per cent of activity in C and E appears to increase slightly, and that in A to decrease. Thin-layer and gas chromatographic analyses of both the metabolites and standards have eliminated the possibility that any of these compounds are monoethyl or diethyl fumarate or diethyl malate. None of the metabolites were volatile enough to be analyzed by gas chromatography. After the attempt at gas chromatography, all of the ethanol extract components were recovered from the first 2 cm. of column packing.

The accumulation of ethanol-unextractable radioactivity in the tissue increased with time (Table I). Although only 3.5% of the total activity was recovered in the tissue after 1 day, this increased with time. This response would indicate some metabolism of the pesticide into ethanol-unextractable material which is deposited within the plant. No attempt to date has been made to determine the identity of these unextracted residues.

The activity remaining in the test tube was assayed throughout the experimental period but after the first day was found only in trace amounts. A summation of all the fractions assayed showed a complete recovery of added activity.

Animal Metabolism. A small but significant amount of ingested malathion was degraded to respiratory CO_2. The over-all rate of CO_2 expiration was constant, showing only small changes due to animal activity (Figure 3, *A*). The total evolution in an 8-hour period amounted to only 1.66%; in a 24-hour period to 2.77%.

The metabolites of malathion appeared in the urine within the first 2 hours, and an increasing rate of excretion rapidly eliminated the ingested (Figure 3, *B*) activity. Almost 45% had been passed within the first 8 hours, and 83.44% during the 24-hour experimental period.

The appearance of activity in the feces was somewhat slower than in the urine (Table III). Only 0.78% had been

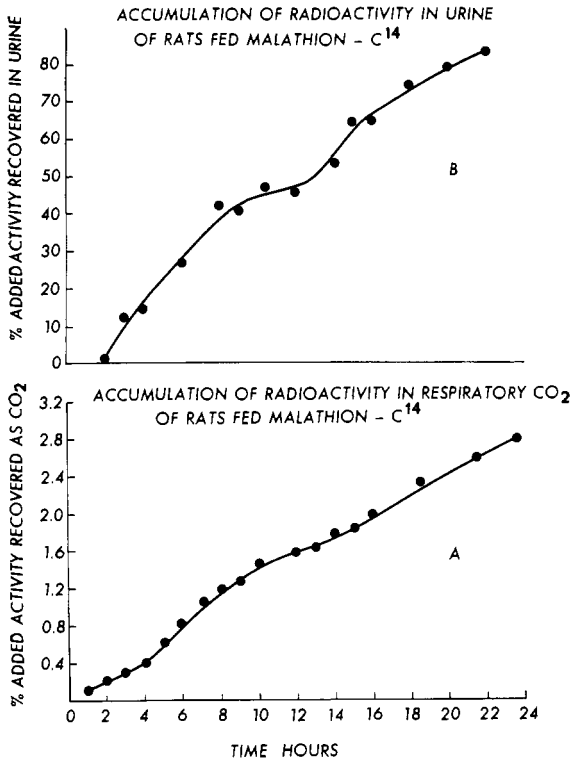

Figure 3. Accumulation of radioactivity in rats fed malathion-C^{14}

A. In respiratory CO_2 *B*. In urine

eliminated within 8 hours; however, 5.51% had been eliminated within 24 hours.

Of the ingested activity, 53.35% had been absorbed from the stomach contents within 8 hours, and only 7.75% remained at the end of 24 hours.

The distribution of carbon-14 in the various tissues examined is also given in Table III. The lung, heart, and spleen all contained less than 0.01% of the ingested activity. The kidney, liver, and blood each contained less than 1% at 8 hours; however, this value had been considerably reduced by 24 hours.

Extraction of the urine with hexane resulted in the removal of only insignificant quantities of radioactivity. Subsequent chromatography of the urine in ethanol-ammonia-water (80:4:16) resolved four radioactive metabolites. The distribution of the four major metabolites with time is shown in Figure 4. Metabolite N represents the largest proportion of total activity in the urine, followed in order by metabolites P, M, and O. The percentage of N and P diminished with time, M remained constant, and O increased.

Paper chromatography of the activity recovered in the stomach contents in butanol–butyric acid–water (2:2:1) resulted in the separation of five metabolites (Table IV). Four had R_f values similar to those found in the urine, while the fifth appeared to be absent from the urine. Of the total activity in the stomach contents the per cent of metabolite V decreased with time, while the per cent of the others increased. The over-all activity in the stomach contents, however, decreased with time.

Table III. Distribution of Radioactivity in Rats Fed Malathion-C^{14}

Sample	Average % Distribution: 8-Hour exposure	24-Hour exposure
CO_2	1.66 ± 0.34[a]	2.77 ± 0.33
Urine	44.12 ± 5.17	83.44 ± 2.21
Feces	0.78 ± 0.22	5.51 ± 1.93
Lung	<0.01	<0.01
Heart	<0.01	<0.01
Liver	0.28 ± 0.07	0.18 ± 0.03
Spleen	<0.01	<0.01
Kidney	0.09 ± 0.03	0.05 ± 0.03
GI tract	0.30 ± 0.03	0.05 ± 0.01
GI contents	46.65 ± 7.32	7.75 ± 2.13
Blood	0.78 ± 0.17	0.20 ± 0.05

[a] Average deviation from mean.

Table IV. Distribution of Radioactivity in Gastrointestinal Contents of Rats Fed Malathion-C^{14}

	Metabolite R	S	T	U	V	Malathion
R_f (BABW)[a]	0.09	0.24	0.34	0.49	0.86	0.92
R_f (EAW)[b]	0.05	0.19	0.31	0.48	0.69	0.86
Exposure, Hours	% of Total Activity Recovered in Stomach Contents					
8	6	9	11	...		73
24	15	11	14	3		53

[a] Butanol–butyric acid–water (2:2:1).
[b] Ethanol–ammonia–water (80:4:16).

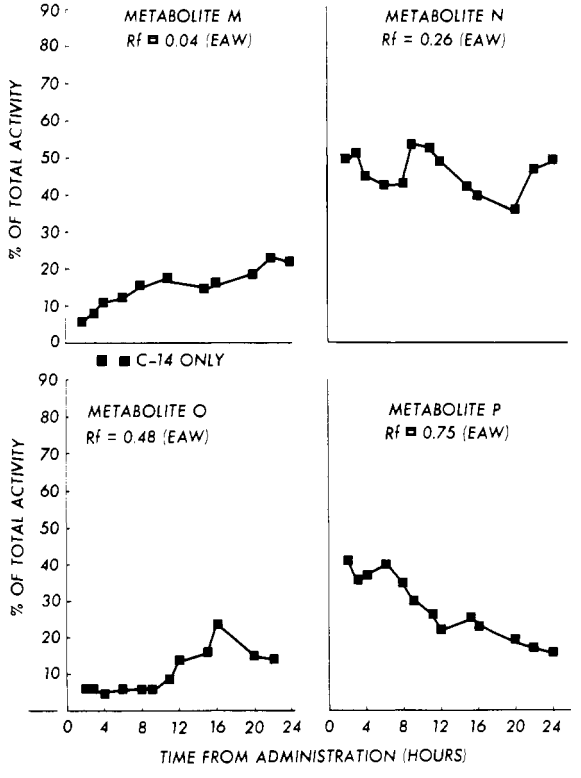

Figure 4. Distribution of radioactive compounds in urine of rats fed malathion-C^{14}

DISCUSSION

Of the radioactivity added, 7.7% was recovered from plants one day after treatment with malathion. In no case was unchanged malathion recovered from the rat.

The evolution of respiratory $C^{14}O_2$ from either plants or rats fed 2,3-succinyl-labeled malathion was insignificant in terms of the total substrate added. The rat did expire about 2% over a 24-hour period; however, only a trace of $C^{14}O_2$ was evolved in the plant experiments. The primary method of elimination of pesticide metabolites in the rat was in the urine. This was rapid; only about 10% of the ingested malathion radioactivitiy was present in the animal after 24 hours. The plant, on the other hand, lacking a system for waste ejection, retained all the radiocarbon absorbed. The deposition of carbon-14 into the tissue appeared to be an important process in the plant. The per cent of carbon-14 recovered in the ethanol-insoluble material (tissue) increased with time to about 9% by the end of the experimental period. The rat, however, being able to excrete waste products, accumulated very little radioactivity in the tissues examined.

The greatest percentage of added activity was recovered as ethanol-soluble intermediates from both plants and animals. The rat apparently degrades malathion shortly after ingestion. The degradation products are then excreted almost immediately in the urine. Some of the metabolites formed in the stomach contents and urine of the rat are similar to those formed in plants, although their distribution may vary.

Generally, plants and animals appear to handle malathion in a similar manner; however, the rate of degradation in animals is somewhat greater. Although the rat may eliminate metabolites in the urine, plants appear to store considerable quantities in the tissue.

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