Thomas, J. A., Morris, D. R., Hager, L. P., *J. Biol. Chem.* **245,** 3135 (1970b).

Vogel, A. I., "Textbook of Practical Organic Chemistry," Longmans Green, London, 1948, p 630.

Walling, C., "Free Radicals in Solution," Wiley, New York, 1957, pp 590, 595.

Yamazaki, I., Piette, L. H., *Biochem. Biophys. Acta* **50,** 62 (1961).

Yamazaki, I., Mason, H. S., Piette, L. H., *J. Biol. Chem.* **235,** 2444 (1960).

Received for review August 19, 1971. Accepted December 3, 1971. Work described in this paper of the Journal Series, New Jersey Agricultural Experiment Station, was supported in part by the R. R. F. NE-53. The senior author is grateful to the Canadian Department of Agriculture for financial support.

Fate of Formothion on Bean Plants in the Greenhouse

Horst H. Sauer

The fate of formothion is studied on bean plants following foliar application. In general, the distribution pattern between the vapor phase, surface, and subsurface area is similar to that of dimethoate. The half-life of formothion breakdown amounts to 1.2 days. Hydrolytic attack causes rapid formothion degradation to approximately equal amounts of dimethoate and *O,O*-dimethyl dithiophosphorylacetic acid. Further breakdown products are dimethoxon, *O,O*-dimethyldithiophosphoric acid, and bis(*O,O*-dimethylthiophosphoryl) disulfide. Only dimethoate and dimethoxon are insecticidally active metabolites. Although their residual amounts are considerably lower after application of Anthio than of dimethoate, and although formothion itself dissipates very fast, the insecticidal efficacy of Anthio is equal to dimethoate. It is concluded that the initial biological action after formothion treatment is caused by formothion itself. The long-term efficacy, however, is generated by potentiation of the insecticidal activity of the Anthio metabolites dimethoate and possibly dimethoxon by synergistic action of *O,O*-dimethyldithiophosphorylacetic acid and bis-(*O,O*-dimethylthiophosphoryl) disulfide.

Anthio is one of the least toxic systemic organophosphorous insecticides with an LD_{50} of 370–400 mg/kg (Klotzsche, 1966). By systemic and contact action it controls a wide range of sucking, mining, and some biting pests on various crops. Its active ingredient is formothion [*O,O*-dimethyl *S*-(*N*-methyl *N*-formylcarbamoylmethyl) phosphorodithioate], which is closely related in its molecular structure to dimethoate. Comparative biological trials in the field under conditions of good agricultural practice as well as in the greenhouse revealed almost identical data of performance of both formothion and dimethoate (Staub, 1964; Wood and Tyson, 1965; Almeida and Cavalcante, 1966; Damiano, 1967; Thompson, 1967; Bassand and Klotzsche, 1970; Jalloul, 1968). Residue investigations, however, analyzing for formothion, dimethoate, and dimethoxon, yielded consistently lower residues in plants treated with formothion (van Hoek, 1966, 1967). Therefore the following study was undertaken to determine quantitatively the fate of formothion applied to bean plants under semi-controlled conditions in comparison to dimethoate. Its aim was to search for an explanation for the almost identical insecticidal action of both formothion and dimethoate, based upon their residual behavior.

MATERIALS AND METHODS

Syntheses of Radio-Labeled Compounds. FORMOTHION-*carbonyl*-^{14}C. To prepare formothion-*carbonyl*-^{14}C, ^{14}C-$BaCO_3$ was treated with concentrated H_2SO_4. The evoluting ^{14}C-CO_2 was converted to ^{14}C-carboxyl acetic acid by a Grignard reaction with CH_3MgI in ether. After dilution with unlabeled acetic acid, bromine was added to yield ^{14}C-carboxylbromoacetic acid, followed by conversion into the corresponding acid chloride by adding phthaloylchloride. The acid chloride was dissolved in trichloroethylene and refluxed with *N*-methylformamide for 2 hr. The solvent was removed at 50°C in vacuum. The crude ^{14}C-bromoacetic acid *N*-methylformamide was dissolved in dioxane and reacted with the sodium salt of dimethylphosphorodithioic acid in water. Purification was done by silica gel column chromatography using ethyl acetate as eluting solvent. The total yield based upon ^{14}C-$BaCO_3$ was 50%. Radiochemical purity was 100% and specific activity was 5.53 mCi/mmol. The material was stored in benzene at 5°C.

Purity of the described labeled compounds was determined by tlc chromatography on silica gel G with ethyl acetate. Visualization of the compounds was done by spraying with potassium iodoplatinate or by treatment with I_2 vapor. Radiochemical purity was determined by tlc–radioscanning on a Berthold Scanner No. 2 (Berthold Frieseke GmbH, 75 Karlsruhe-Durlach, Germany) and by scratching off the silica gel layer in 0.5-cm zones which were transferred into counting vials, extracted with the scintillator solution, and counted as described later.

FORMOTHION-*methoxyl*-^{14}C. ^{14}C-Methanol obtained from ^{14}C-$BaCO_3$ (specific activity 54 mCi/mmol) by reduction of liberated ^{14}C-CO_2 with $LiAlH_4$ in tetrahydrofurfuryloxytetrahydropyrane was reacted with P_2S_5 in shellsol R for 2 hr at 60°C and an additional 2 hr at 80°C. Gaseous reaction products (H_2S) were removed in vacuum. The remaining solution was diluted with benzene and extracted with 1 *N* NH_4OH to yield the ammonium salt ^{14}C-dimethylphosphorodithioic acid. The synthesis of formothion-*methoxyl*-^{14}C was completed by reacting an aqueous solution of the ammonium salt with chloroacetic acid *N*-methylformamide in dioxane for 3 hr at 35°C. Water-soluble by-products were removed from the reaction mixture dissolved in benzene by partition into 2 *N* $KHCO_3$. Purification of the material diluted with unlabeled carrier was done as described for

Agrochemical Research Division, SANDOZ A.G., Basel, Switzerland.

Table I. Common Name, Chemical Name, R_f Values, and LD_{50} (Oral, Male Rat) of Formothion and Its Metabolites

Common name	LD_{50}, mg/kg	R_f values on SiO_2G tlc solvent systems						Chemical name
		1	2	3	4	5	6	
Formothion	370–400	0.56	0.07	0.70	0.62	0.64	0.75	*O,O*-Dimethyl *S*-(*N*-methyl *N*-formylcarbamoylmethyl) phosphorodithioate
Dimethoate	247	0.20	0.00	0.46	0.33	0.34	0.62	*O,O*-Dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate
Dimethoxon	55	0.00	0.00	0.17	0.00	0.00	0.41	*O,O*-Dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothioate
Formothion oxygen analog		0.19	0.00	0.45	0.25	0.27	0.61	*O,O*-Dimethyl *S*-(*N*-methyl-*N*-formylcarbamoylmethyl) phosphorothioate
Carboxylic acid	2500–3000	0.38	0.00	0.48	0.00	0.00	0.63	*O,O*-Dimethyldithiophosphorylacetic acid
Disulfide	10,000	0.71	0.59	0.78	0.78	0.76	0.82	Bis(*O,O*-dimethylthiophosphoryl) disulfide
DDPA		...	0.00	...	0.00	0.00	0.25	*O,O*-Dimethyldithiophosphoric acid

Tlc solvent systems: 1, benzene–ethyl acetate–acetic acid 50:48:2. 2, benzene. 3, benzene–ethyl acetate–acetone–acetic acid 50:25:25:2. 4, benzene–methylenchloride–acetonitrile 50:25:25. 5, benzene–ethyl acetate–acetonitrile 60:15:25. 6, benzene–ethanol–formic acid 70:30:1.

^{14}C-*carboxyl*-formothion. The total yield based upon ^{14}C-$BaCO_3$ was 46%. Radiochemical purity was 99% and the specific activity was 26.2 mCi/mmol. The material was stored in benzene at 5°C.

DIMETHOATE-*carbonyl*-^{14}C. Synthesis was carried out using the same procedure as described for formothion-*carbonyl*-^{14}C starting with ^{14}C-$BaCO_3$ (5 mCi/mmol), except using methylamine dissolved in chloroform instead of *N*-methylformamide dissolved in trichloroethylene. The total yield based upon ^{14}C-$BaCO_3$ was 30%. Radiochemical purity was 99.5% and specific activity was 4.9 mCi/mmol. The material was stored in benzene at 5°C.

DIMETHOATE-*methoxyl*-^{14}C. The same procedure was used as outlined for formothion-*methoxyl*-^{14}C, except using bromoacetic acid *N*-methylamide dissolved in acetone instead of chloroacetic acid *N*-methylformamide in dioxane. The total yield based upon ^{14}C-$BaCO_3$ was 28%. Radiochemical purity was 99% and specific activity was 18.5 mCi/mmol. The material was stored in benzene at 5°C.

OTHER CHEMICALS. Unlabeled standards and potential metabolites employed in this study are summarized in Table I. They have been synthesized in the SANDOZ laboratories with the exception of the dimethoxon, which was purchased from Siegmund and Irmengard Ehrenstorfer, Fritz-Hintermayrstrasse, D 89 Augsburg/Germany. Disulfide (Table I) was synthesized according to U.S. patent 2,523,146 (1950) and Sasse (1964).

PLANTS. Bean plants, *Phaseolus vulgaris* (variety Ohnegleichen), were grown from soil TKS-1 : sand-3 : 1 (TKS-1 was purchased from Floratorf, Torfstreuverband GmbH, 29 Oldenburg) in the glasshouse under long day conditions, 16 hr light and 8 hr dark, with artificial light (Philips tubes, TL 40W-29 and 40W-33, 2500 lux). Temperature was 16°C and humidity ranged from 60 to 70%. Plants of uniform size were treated 2 weeks after sowing when they were in the two-leaf stage and kept in the glasshouse after treatment until harvest.

Penetration Studies. The treatment was carried out by foliar application. Formothion-*carbonyl*-^{14}C and dimethoate-*carbonyl*-^{14}C were formulated as 20% emulsifiable concentrates (20% a.i., 1% acetic anhydride, 7% emulsifier, 10% shellsol R, and 62% xylene) which were diluted with water to a concentration of 0.1% of active ingredient.

The foliar application consisted of spotting 50 times 1 μl of the emulsions by means of an Arnold Micro Applicator [Burkard Scientific (Sales) Ltd., Rickmansworth, Hertfordshire/England] all over the upper surface of each of two leaves per plant, administering an approximate dosage of 100 μg of active ingredient of 5.2×10^6 dpm (formothion) and 3.6×10^6 dpm (dimethoate) per plant. During the course of the application procedure, four radiocounting standards were prepared by spotting the same amount on standard glass microscope slides in the same manner.

Four plant replicates each were sampled at appropriate times by cutting the two leaves of each plant. The plant remainder was discarded, since preliminary results (Wagner, 1970) as well as results on dimethoate (de Pietri-Tonelli, 1965) had shown the absence of significant amounts of radioactivity in the remaining plant parts. Each replicate (two excised leaves) was washed carefully with 25 ml of a 1% aqueous detergent solution (lauryldiglycolethersulfate sodium salt). This solution did not alter subcuticular plant tissues. A 0.5-ml aliquot of the washing solution was taken for radiocounting. The two washed leaves were carefully rinsed twice with detergent solution and with tap water, and extracted with acetonitrile containing 0.25% acetic acid by means of a Potter homogenizer. The homogenate was centrifuged at 4000 rpm for 5 min in a centrifuge Vismara Model 920, rotor 5244 (Tecnomara AG. Zürich, Switzerland). The supernatant was carefully transferred with a pipette into a 25-ml volumetric flask. The remainder was reextracted three times. An aliquot of the combined extracts was taken for radiocounting.

The total radioactivity of the remainder was determined by the oxygen flask combustion method of Kalberer and Rutschmann (1961).

A Packard liquid scintillation counter (Model 3375) was used for assaying radioactivity. The standard counting solution consisted of 6 g of butyl-PBD, [2-(4-*tert*-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole], in 1000 ml of toluene–ethanol (6:4).

For comparative purposes, standard glass microscope slides were treated exactly as described for the foliar application, each with 25 times 1 μl of the emulsions, and kept under the same conditions in the glasshouse. One replicate each was sampled at the same time intervals and assayed for remaining radioactivity by washing with 25 ml of the 1% detergent solution and radiocounting of a 0.5-ml aliquot.

Metabolism Studies. Foliar applications of formothion-*methoxyl*-^{14}C and dimethoate-*methoxyl*-^{14}C were carried out

Table II. Recrystallization of Disulfide to Constant Specific Radioactivity

Compounds	Solvent used for crystallization	counts/min/μmol
Disulfide		$2.53 \pm 0.12 \times 10^5$
	Methanol	$2.37 \pm 0.12 \times 10^5$
	Methanol	$2.45 \pm 0.12 \times 10^5$
	Benzene–petroleum ether	$2.30 \pm 0.12 \times 10^5$

Table III. Percentage of Applied Dose of Compounds Recovered from Acetonitrile Extract (Table V)

Compounds	Days after application					
	1/48	1/4	1	3	7	14
Formothion	92.0	75.1	58.1	16.5	3.3	1.9
Formothion oxygen analog	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Dimethoate	2.3	3.5	4.9	7.0	4.3	1.8
Dimethoxon	0.4	1.6	2.8	4.8	3.9	3.5
Carboxylic acid	3.3	3.0	2.3	3.0	2.8	2.6
Disulfide[a] 0.5×	0.4	2.2	4.8	9.2	5.4	4.4
DDPA	0.3	1.0	2.4	4.9	3.4	3.2
Polar metabolites	<0.5	<0.5	<0.5	<0.5	<0.5	3.0
Total identified of the applied dose	99.3	87.0	75.9	46.0	23.7	20.5
Identified of the acetonitrile extract (Table V)	99	96	93	79	76	85

[a] Disulfide % values are based on percent of the applied radioactivity. To obtain % equivalent of formothion, the values have to be divided by 2, since disulfide contains two of the $(^{14}CH_3O)_2PS$ groups of formothion.

Table IV. Percentage of Applied Dose of Compounds Recovered from Acetonitrile Extract (Table VI)[a]

Compound	Days after application					
	1/48	1/4	1	2	7	14
Dimethoate	92.0	91.9	76.2	50.9	30.3	12.0
Dimethoxon	0.2	0.4	0.8	2.4	3.5	4.9
Carboxylic acid	0.6	0.4	0.4	0.4	0.3	0.3
Disulfide	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
DDPA	0.2	0.3	0.8	1.3	0.7	2.7
Total identified of the applied dose	93.2	93.1	78.3	55.1	34.9	20.1
Identified of the extract (Table VI)	93	97	92	89	82	70

as described for the penetration studies. The dosages applied per plant were approximately 100 μg of active ingredient, yielding 20.3×10^6 dpm (formothion) and 18.9×10^6 dpm (dimethoate). For reference purposes ten standards were prepared during the course of the foliar application procedure by spotting 50 times 1 μl of the emulsions upon standard glass microscope slides. By washing the glass plates with 25 ml of the detergent solution, the applied insecticide was removed and its radioactivity determined by counting a 0.5-ml aliquot to yield the standard counts.

Five plant replicates each were sampled at appropriate time intervals by cutting off the two treated leaves of each plant. The plant remainder was discarded. Each replicate (two excised leaves) was extracted at 0°C and further treated as described for the penetration studies. All extracts were stored at −25°C, when not used.

Identification and Quantitative Evaluation of Compounds. A 20- or 100-μl aliquot of each extract was cochromatographed with standard materials of formothion, formothion oxygen analog, dimethoate, dimethoxon, carboxylic acid, disulfide, and DDPA (for chemical names, see Table I) on silica gel G thin-layer plates (0.25 mm thickness), using the appropriate solvent system as indicated in Table I. The standard materials were visualized by using the potassium iodoplatinate spray reagent of MacRae and McKinley (1963). Quantity and localization of radioactivity zones were determined by scratching off the silica gel G layer in 0.5-cm zones. The silica gel G zones were transferred into counting vials and extracted by shaking vigorously for 2 min with the scintillator solution before counting.

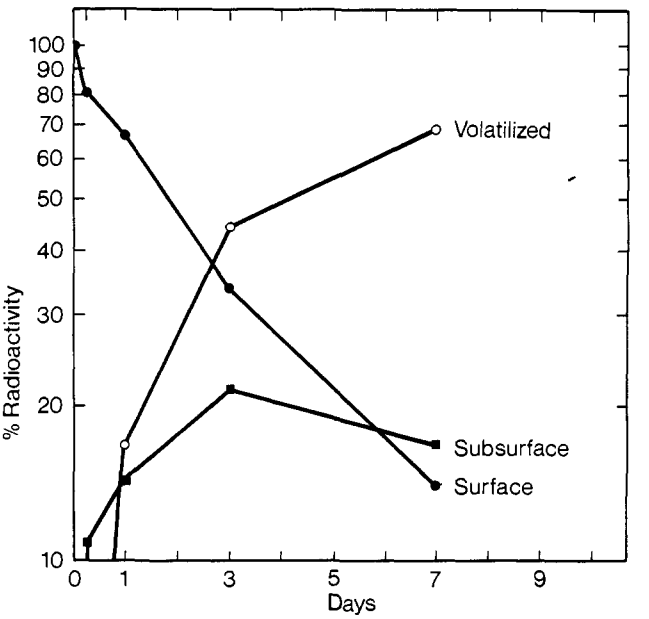

Figure 1. Percentage of applied dose recovered from bean leaves treated with 50 μg of formothion-*carbonyl*-^{14}C

For further identification and evaluation of radiochemical purity of formothion and its metabolites, except DDPA, 5 ml of the extract solutions (combined replicates of the 7 days sample) were (after addition of standard material) subjected to preparative thin-layer chromatography on five silica gel G plastic sheets 20 × 20 cm (purchased from Macherey-Nagel & Co., Düren, Germany). The extract solution was spotted automatically upon the silica gel G layers by means of a Desaga Autoliner (Mod. 121000, DESAGA GmbH, Heidelberg). The developing solvent was benzene–ethyl acetate–acetic acid 50:48:2, except for dimethoxon, where benzene–ethyl acetate–acetone–acetic acid 50:25:25:2 was used. Of each plastic sheet three small vertical zones, each 1 cm wide, were cut and sprayed with potassium iodoplatinate reagent to localize the standard material. The untreated sheets were cut horizontally corresponding to the zones revealed by potassium iodoplatinate treatment. Each compound was extracted from the untreated silica gel G plastic sheets with methanol.

Corresponding extracts were combined and concentrated by evaporation at room temperature in a rotary evaporator to a small volume. Identification and confirmation of radiochemical purity was done by cochromatography of an aliquot with standard material on silica gel G thin-layer plates using appropriate solvent systems (Table I). Localization of standard materials and radiocounting was carried out as described previously.

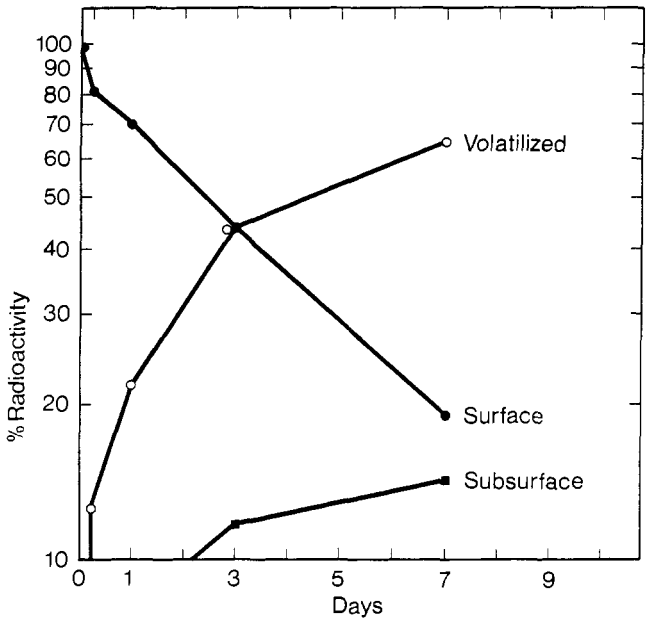

Figure 2. Percentage of applied dose recovered from bean leaves treated with 50 μg of dimethoate-*carbonyl*-^{14}C

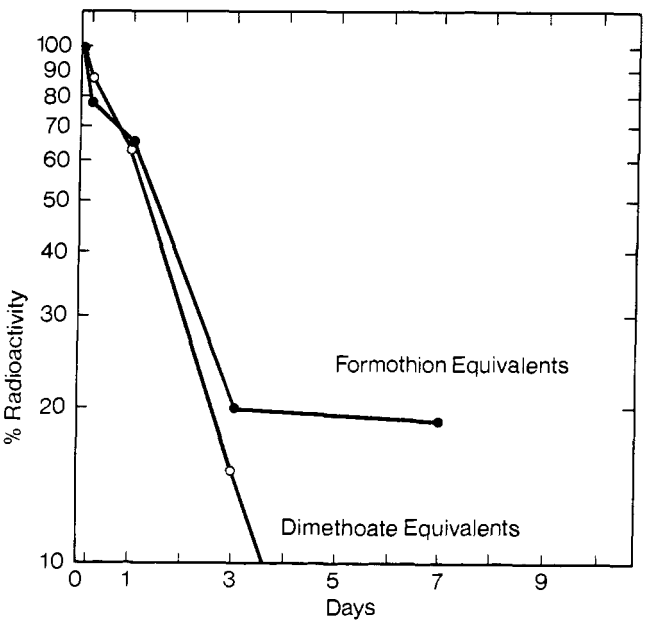

Figure 3. Percentage of applied dose recovered from glass plates treated with formothion-*carbonyl*-^{14}C and dimethoate-*carbonyl*-^{14}C

Additional verification of the disulfide was obtained by adding 100 mg of pure standard to the purified extract and recrystallization to constant specific radioactivity (inverse isotope dilution analysis), as indicated in Table II.

RESULTS AND DISCUSSION

Penetration and Volatilization. The dissipation of radioactivity (applied as formothion-*carbonyl*-^{14}C and dimethoate-*carbonyl*-^{14}C, respectively) from the surface of bean leaves, as shown in Figures 1 and 2, approached first-order kinetics. One day after treatment 67% and after 7 days 14% of the total radioactivity of applied formothion remained on the surface. The corresponding dimethoate values were 70 and 19%, respectively. Dissipation occurred due to penetration and volatilization of the active ingredients and their metabolites.

Penetration, which depends largely on the lipophilic character of the applied pesticide (Tietz, 1954; Linskens *et al.*, 1965; Hull, 1970), occurred rather slowly (Figures 1 and 2) compared, *e.g.*, to the more lipophilic Systox isomers (Tietz, 1954). One day after treatment 14.5% and 7 days after treatment 16.6% of the applied radioactivity of formothion were recovered as penetrated residues. The corresponding dimethoate values were 8.0 and 14.2%, respectively. Subsurface radioactivity increased, especially after application of formothion, mostly within the first few days after treatment to reach its maximum level after approximately 3 days. The applied radioactivity of formothion obviously penetrated slightly faster than the applied radioactivity of dimethoate, which is in agreement with the rule of lipophilic dependency of the penetration behavior, since formothion shows a water solubility of 2600 ppm againt 25,000 ppm of dimethoate (Gunther *et al.*, 1968). Radioactivity, which was not recovered from the bean leaves neither as surface nor subsurface radioactivity, was assumed to be *volatilized.* This seems to be justified since the analytical procedure did not involve any operations which could lead to significant losses of radioactivity, nor significant amounts of radioactivity migrated to untreated plant parts (Wagner, 1970; de Pietri-Tonelli, 1965). The calculated values, as indicated in Figures 1 and 2, show that within 3 days nearly half of the radioactivity applied as formothion or dimethoate had volatilized 69 or 66%, respectively, after 7 days.

Volatilization from glass plates occurred at higher rates than from leaves (Figure 3). After 3 days 80% of the total applied radioactivity of formothion and 85% of dimethoate had volatilized, after 7 days 82 and 98%, respectively, indicating that on leaf surfaces volatilization was retarded possibly by adhesion of the molecules to the lipic layer and by penetration into subcuticular areas. Metabolic action also might have changed the volatilization pattern. Loss of formothion from glass plates showed first-order kinetics only up to 3 days after treatment, suggesting the interference of breakdown products. Comparing with the loss rate from bean leaves, which was nearly of first-order through the whole experiment, one might speculate that on bean leaf surfaces no accumulation of breakdown products occurred because of penetration into the leaf and because of stabilization of the active ingredient by the lipic layer. Loss of dimethoate from glass plates followed first-order kinetics up to 7 days, as was previously found by Dauterman *et al.* (1960). We determined the half-life, however, to be only 30 hr.

Generally the results show that on bean leaves the distribution pattern of the applied radioactivity of formothion between vapor phase, surface, and subsurface up to 7 days after application did not differ significantly from the distribution pattern of dimethoate.

Metabolism. Dissipation of the active ingredient formothion itself and the occurrence of metabolites in and on treated bean leaves was found to be as indicated in Table III. The corresponding dimethoate values are shown in Table IV.

Both formothion and dimethoate dissipation followed first-order kinetics, as indicated in Figure 4. The half-life time of formothion was 1.2 days and of dimethoate was 3.1 days. Since volatilization rates were shown to be almost identical, the 3 times faster dissipation rate of formothion was caused by more rapid breakdown.

Previous publications on dimethoate metabolism in plants have shown hydrolytic, oxidative (Dauterman *et al.*, 1960; Santi and de Pietri-Tonelli, 1959), and *N*-demethylation

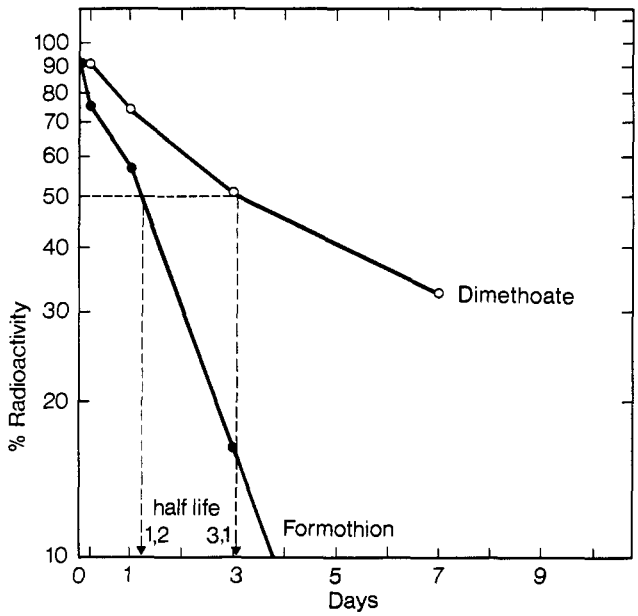

Figure 4. Percent loss of formothion-*methoxyl*-^{14}C and dimethoate-*methoxyl*-^{14}C from bean leaves

(Lucier and Menzer, 1970) mechanisms being responsible for dimethoate breakdown. Hydrolysis led to less toxic metabolites, oxidation and *N*-demethylation to more toxic ones.

The present studies on formothion breakdown demonstrated that hydrolysis was the initial and most predominant degradation pathway. Thirty minutes after application 2.3% dimethoate was found, increased up to 7.0% after 3 days, and declined to 1.8% after 14 days. Also by a hydrolytic mechanism, carboxylic acid was formed at constant level of approximately 3% up to 14 days. The formation of more polar hydrolysis products like DDPA and similar ones (Dauterman *et al.*, 1960; Lucier and Menzer, 1968) occurred at slower rates, reaching maximum levels at 3 and 14 days, respectively.

The only oxidation metabolite detected was dimethoxon, which reached its maximum level after 3 days and declined to 3.5% equivalents after 14 days.

Formothion oxygen analog could not be detected at a limit of detection of 0.1% formothion equivalent.

N-Demethylation metabolites, as have been found by Lucier and Menzer (1968) and identified by the same authors (1970) in the dimethoate metabolism, were not detected. Although we did not possess the standard materials, we feel we may draw this conclusion, since no significant radioactivity was detected using the tlc solvent system 1 for those compounds, as described by Lucier and Menzer (1968).

However, a new metabolite, which has not been reported yet in the dimethoate metabolism, could be isolated from the plants treated with formothion, and was identified as disulfide by cochromatography on silica gel G thin-layer plates using six different solvent systems (Table I) and by inverse radioisotope dilution analysis, as indicated in Table II. It increased up to 4.6% equivalents after 3 days and declined to 2.2% after 14 days. It was not detected in the dimethoate metabolism (Table IV).

Possibly a radical mechanism was involved in its formation, since model tests with uv radiation (272 nm) on glass and silica gel G thin-layer plates showed disulfide originating from both formothion and carboxylic acid (Laroche, 1967). The treatment of bean plants with unlabeled carboxylic acid

Table V. Percentage of Applied Dose Recovered from Bean Plant Leaves Treated with 100 μg of Formothion-*methoxyl*-^{14}C[a]

	Days after application					
Recovered in	1/48	1/4	1	3	7	14
Acetonitrile extract	100	91	82	58	31	24
Plant remainder	0.5	0.8	1.6	3.1	5.4	4.9
Unrecovered[b]		8.2	16.4	38.9	63.6	71.1

[a] All values are means of five replicates (rel S.D. 15%). [b] Calculated value 100% minus percent recovered in extract and plant remainder.

Table VI. Percentage of Applied Dose Recovered from Bean Plant Leaves Treated with 100 μg of Dimethoate-*methoxyl*-^{14}C[a]

	Days after application					
Recovered in	1/48	1/4	1	3	7	14
Acetonitrile extract	99.7	96.4	84.9	61.7	42.5	28.9
Plant remainder	0.2	0.4	0.7	2.1	4.5	3.3
Unrecovered[b]	0.1	3.2	14.4	36.2	53.0	67.8

[a] All values are means of five replicates (rel S.D. 15%). [b] Calculated value 100% minus percent recovered in extract and plant remainder.

and the subsequent detection of disulfide (van Hoek, 1970) confirmed our assumption that disulfide is formed from formothion in plants at least partially *via* carboxylic acid.

Recovery of the applied total radioactivity amounted to approximately 100% after 30 min and approximately 92% after 6 hr, as indicated in Table V. Since the analytical procedure did not involve any operations which could lead to significant losses of radioactivity, and recovery at 30 min was 100%, the loss of radioactivity with increasing time was assumed to be caused exclusively by volatilization.

This rate of loss was almost identical to the rate of loss (volatilization) of the ^{14}C-*carbonyl*-labeled compounds discussed earlier.

The recovered radioactivity at 30 min was practically quantitatively identified (see Table III). The percent radioactivity not being identified in the extracts afterwards consisted possibly of methanol originating from hydrolysis of the methoxyl groups.

Compounds more polar than DDPA have only been detected in the 14-days sample but have not been identified, since they are very likely of the same structure as those identified by Dauterman *et al.* (1960) and Lucier and Menzer (1968). They are of no toxicological and biological importance.

The radioactivity recovered by combustion of the extracted plant remainder could not be identified. Doman and Romanova (1962) had shown the incorporation of methanol, formaldehyde, formic acid, and CO_2 by photosynthetic assimilation into natural plant constituents of bean leaves. Consequently we anticipate that the labeled methoxyl group entered the biosynthetical pathway of natural plant constituents *via* phosphoric esters and methanol. Based upon present findings, formothion breakdown in bean plants is proposed to occur as shown in Figure 5.

CONCLUSION

From the present results it is obvious that formothion metabolism differs significantly from dimethoate metabolism by the immediate hydrolytic attack of the C–N–C bonds, leading to approximately equal amounts of dimethoate and carboxylic acid. Both of the degradation pathways are highly important.

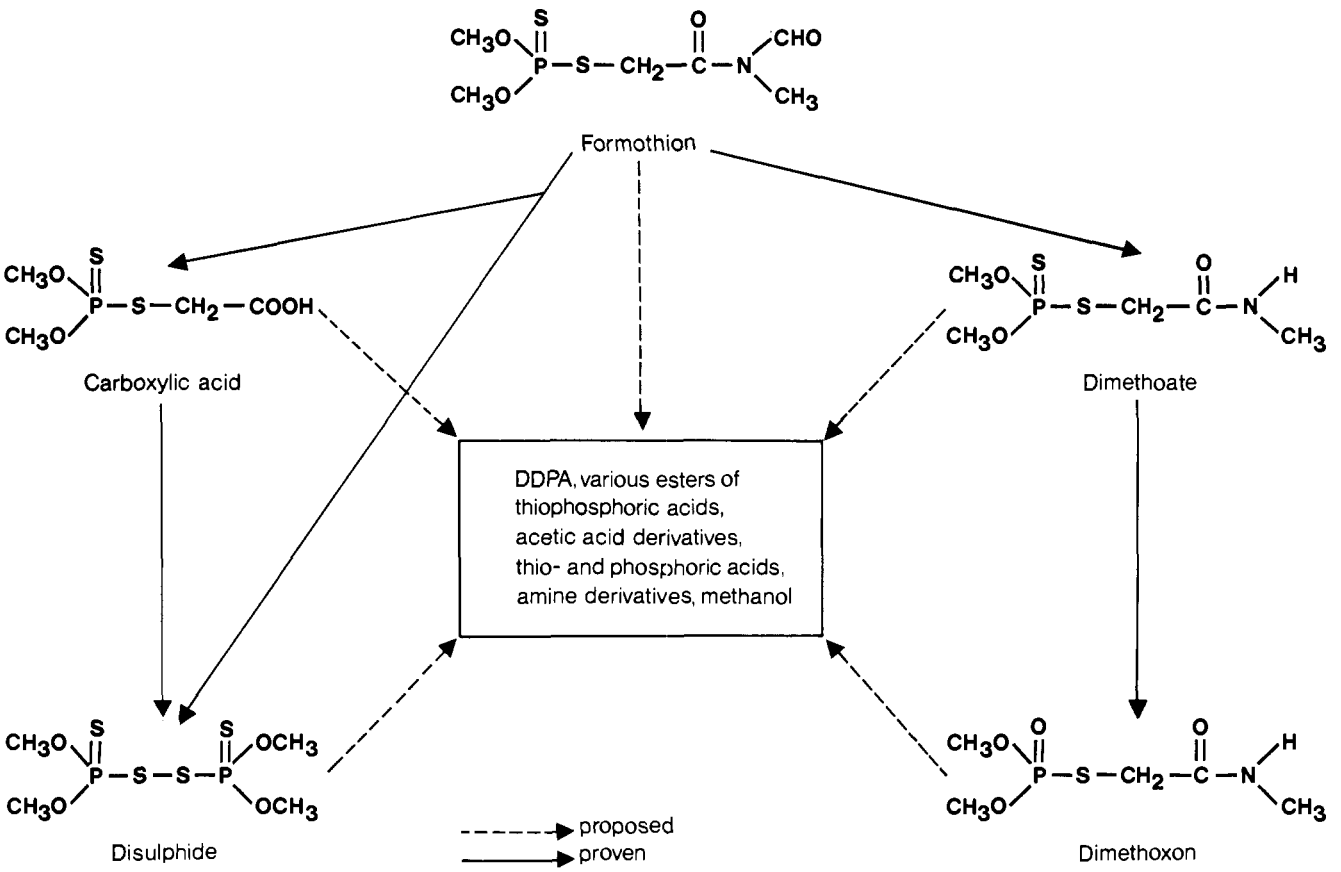

Figure 5. Proposed metabolic pathway of formothion applied to bean leaves

Pathway I leading to dimethoate and consequently to its P=O metabolite provides metabolites which are carrier compounds of insecticidal activity. Their toxicity to mammals is relatively high (Edson and Noakes, 1960; Dauterman *et al.*, 1959) and their residue level in treated plants ought to be determined by routine residue analyses.

Pathway II leading to carboxylic acid and disulfide provides residues, which were shown by Bassand and Klotzsche (1970) to act as synergists upon dimethoate. Their toxicity to mammals is low (Bassand and Klotzsche, 1970). They need not be determined by routine analyses.

Consequently the present findings explain clearly the previously mentioned discrepancy between the identical biological action and the low residue level of plants treated with formothion analyzed for formothion, dimethoate, and dimethoxon in comparison to plants treated with dimethoate. The initial biological action after treatment with formothion is caused by formothion itself. With increasing time the long-lasting insecticidal efficacy is caused by relatively small amounts of dimethoate and dimethoxon, potentiated by synergistic action of carboxylic acid and disulfide.

Ruzicka *et al.* (1968) found that the degradation rate of dimethoate was enhanced under field conditions compared with laboratory conditions. This is in agreement with our results on formothion, which moreover show even greater discrepancies of residue levels between formothion and dimethoate treatment than encountered by the presently described laboratory experiment.

ACKNOWLEDGMENT

The author is grateful to Gustav Thomann and Karl Lutz for their support throughout the program, to Emil Schreier for his synthetical contribution, and to Marcel Schaechtele for his skilled manual assistance.

LITERATURE CITED

Almeida, P. R., Cavalcante, R. D., *O Biologico* **32,** 199 (1966).
Bassand, D., Klotzsche, C., presented at the 7th Intern. Plant Protect. Congr., Paris, Sept. 1970.
Damiano, A., Internat. Pest Control, May–June and November–December (1967).
Dauterman, W. C., Casida, J. E., Knaak, J. B., Kowalczyk, T., J. AGR. FOOD CHEM. **7,** 188 (1959).
Dauterman, W. C., Viado, G. B., Casida, J. E., O'Brien, R. D., J. AGR. FOOD CHEM. **8,** 116 (1960).
Doman, N. G., Romanova, A. K., *Plant Physiol.* **37,** 833 (1962).
Edson, E. F., Noakes, D. N., *Toxicol. Appl. Pharmacol.* **2,** 523 (1960).
Gunther, F. A., Westlake, W. E., Jaglan, P. S., *Res. Rev.* **20,** 65 (1968).
Hull, H. M., *Res. Rev.* **31,** 82 (1970).
Jalloul, A., *FAO Rept.* **34,** 3 (1968).
Kalberer, F., Rutschmann, J., *Helv. Chim. Acta* **44,** 1956 (1961).
Klotzsche, C., *Arch. Gewerbepathol. Gewerbehyg.* **22,** 246 (1966).
Laroche, B., Report No. 6, 1967, Agrochemical Research Division, SANDOZ A. G., Basel, Switzerland.
Linskens, H. F., Heinen, W., Stoffers, A. L., *Res. Rev.* **8,** 161 (1965).
Lucier, G. W., Menzer, R. E., J. AGR. FOOD CHEM. **16,** 936 (1968).
Lucier, G. W., Menzer, R. E., J. AGR. FOOD CHEM. **18,** 698 (1970).
MacRae, H. F., McKinley, W. P., J. AGR. FOOD CHEM. **11,** 174 (1963).
de Pietri-Tonelli, P., *Advan. Pest Control Res.* **6,** 31 (1965).
Ruzicka, J. H., Thomson, J., Wheals, B. B., *J. Chromatogr.* **33,** 430 (1968).
Santi, R., de Pietri-Tonelli, P., *Nature* **183,** 398 (1959).
Sasse, K., *Houben-Weyl, Meth. Org. Chem* **12**(II), 811 (1964).
Staub, A., *Schweiz. Zr. Obst. Weinbau* **73,** 141 (1964).
Tietz, H., *Höfchen-Briefe* **7,** 1 (1954).
Thompson, L. S., *J. Econ. Entomol.* **60,** 716 (1967).
van Hoek, C., Report Nos. 5/66 (1966), 8/67 (1967), 11/67 (1967), Agrochemical Research Division, SANDOZ A.G., Basel, Switzerland.
Wagner, H., Report 5.3.1970 (1970), Pharma-Chemical Research Division, SANDOZ A.G., Basel, Switzerland.
Wood, C. H. P., Tyson, T., presented at the 3rd Brit. Ins. and Fung. Conf., Brighton, 1965.

Received for review September 28, 1971. Accepted December 6, 1971.