the R_f values of the metabolites in butanol–ammonia–water indicated that the minor metabolite had been converted to dicamba. In the case of the major metabolite, the saponification yielded a compound which differed from the original. This suggested that the aromatic ring had been hydroxylated.

By the microcoulemetric method for chlorine determination, the ratio of ^{14}C to chlorine for the major metabolite was approximately 80% of dicamba. This indicated that if the benzoic acid structure still was intact, the major metabolite, contained two chlorine atoms per molecule. By trapping the $^{14}CO_2$ from the combustion of the major metabolite, the chlorine, measured by the microcoulometric gas chromatography, was found to come from this metabolite.

The identification of the minor metabolite was afforded further by comparing its R_f values, as detected on a strip scanner, with those of nonlabeled 3,6-dichlorosalicylic acid, which was detected via a phenolic spray reagent. This reagent consisted of mixing equal volumes of 1% ferric chloride solution with a 1% potassium ferricyanide solution. The ^{14}C-methoxy-labeled dicamba gave no labeled minor metabolite at the R_f's expected for dichlorosalicylic acid on the four chromatography systems, which indicated that the methoxy group was present in the major metabolite.

The intermediate product in the synthesis of 5-hydroxy dicamba from dicamba was isolated and elemental analysis obtained. Methyl-5-amino-2-methoxy - 3 - 6 - dichlorobenzoate gave pale yellow plates, m.p 108° C., from ethanol. Found 43.2;3.7. $C_9H_9O_3NCl_2$ requires C = 43.2 and H = 3.6.

Gas liquid chromatography, infrared spectrophotometry, and mass spectrometry were used to identify the major metabolite as being 5-hydroxy-2-methoxy-3, 6-dichlorobenzoic acid. The retention times of the methylated metabolite and methylated 5-hydroxy dicamba are found in Table III. To obtain an IR spectrum, a sufficient amount of the methylated metabolite was purified further by means of a GLC fraction collector. This spectrum was compared with the IR spectrum of the methylated 5-hydroxy dicamba (Figure 1).

Figure 2 gives the mass spectra for the methylated metabolite and methylated 5-hydroxy dicamba.

References

(1) Dryder, R. D., Whitehead, C. W., *Res. Rept. Natl. Weed Comm. Sec.* **9,** 147 (1962).
(2) Faulkner, J. K., Woodcock, D., *J. Chem. Soc.* **1965,** p. 1187.
(3) Fertig, S. N., Furrer, Armin, H., Jr., *Proc. Northeast. Weed Control Conf.* **17,** 340 (1963).
(4) Jaworski, E. G., J. AGR. FOOD CHEM. **12,** 33 (1964).
(5) Jaworski, E. G., Fang, S. C., Freed, V. H., *Plant Physiol.* **30,** 272 (1955).
(6) Leafe, E. L., *Nature* **193,** 485 (1962).
(7) Montgomery, M. L., Freed, V. H., J. AGR. FOOD CHEM. **12,** 11 (1964).
(8) Switzer, C. M., *Res. Rept. Natl. Weed Comm. Eastern Sec.* **7,** 107 (1962).

Received for review May 2, 1966. Accepted July 18, 1966. Technical Paper No. 2132, Oregon Agricultural Experiment Station. Work supported by Velsicol Chemical Corp. and the U. S. Public Health Service (Grant No. ES0004-02.)

INSECTICIDE ABSORPTION

Parathion Absorption, Translocation, and Conversion to Paraoxon in Bean Plants

ALI EL-REFAI[1] AND T. L. HOPKINS

Department of Entomology, Kansas State University, Manhattan, Kan.

Parathion deposits on bean plant leaves, *Phaseolus vulgaris,* had half lives of 1 day on leaf surfaces and 2.4 days for the total plant under controlled environmental conditions. Disappearance was initially more rapid from leaves than from glass surfaces owing to combined evaporation and absorption, but later the cuticular residues became more persistent. Leaf absorption of parathion occurred rapidly with about 30% of the original deposit within the plant by 2 days. Parathion degradation products accumulating on both glass and leaf surfaces were similar and included paraoxon, *p*-nitrophenol, and possibly *S*-ethyl parathion. Total paraoxon residues remained small at approximately 1% of the original application. Parathion was readily absorbed from nutrient solution by the roots, but less than 2% was translocated to the foliage. Small paraoxon residues also accumulated in roots and foliage.

THE BEHAVIOR OF PARATHION deposits on roots and foliage and the mechanisms of loss from plants have not been elucidated clearly, although many data exist on parathion residues in various crops. Parathion apparently penetrates into living plants to a limited extent, and volatilization from foliage surfaces has been suggested as a major loss factor, as well as hydrolysis in or on the plant (*6, 17*). Translocation of parathion in plant tissue has been indicated by several studies showing mortality of insects feeding on plants grown in parathion-treated soil. However, volatilization of parathion from the soil to the foliage was not ruled out as a possible cause of toxicity (*5, 17*). David and Aldridge (*7*) demonstrated that the guttation fluid from wheat plants treated with parathion by root application was toxic to mosquito larvae and contained an anticholinesterase with properties similar to paraoxon. They concluded that parathion was oxidized in the roots close to the site of absorption as no parathion could be detected in the foliage by bioassay techniques. Zeid and Cutkomp (*18*) also showed by bioassay that paraoxon was translocated in plants, but evidence for parathion movement in plant tissue was inconclusive.

[1] Present address, Insect Control Section, Ministry of Public Health, Cairo, U. A. R.

Oxidation of phosphorothionates in plants has been demonstrated primarily for the systemic insecticides, with very few studies on the weak or nonsystemic organophosphorus compounds (*4, 12*). In vitro experiments indicate that plant peroxidases may catalyze the oxidation and hydrolysis of parathion and other related insecticides (*13*).

The present study was to determine the rate and quantity of parathion absorption by both leaf and root applications under controlled environmental conditions and its possible translocation and rate of loss from bean plants. Residues remaining on the leaf surfaces as well as extracts of the plant tissues were analyzed for paraoxon and other possible anticholinesterases to determine their significance as degradation products of parathion.

Materials and Methods

Garden beans, *Phaseolus vulgaris* (Bountiful variety) were germinated in vermiculite in the greenhouse, and the seedlings transferred to a nutrient solution supplemented with iron (Hyponex plant food, Hyponic Chemical Co., Copley, Ohio) 1 week after the beans sprouted. The plants were grown under fluorescent lights with a 16-hour photoperiod until maximum primary leaf growth was reached in about 17 days, when they were ready for insecticide treatment.

The parathion (99.7%) was an analytical grade standard supplied by American Cyanamid Co., Princeton, N. J. Purity checks by thin-layer chromatography revealed only parathion when 10 μg. of the standard was chromatographed and potassium hydroxide used to detect the *p*-nitrophenyl compounds. Traces of paraoxon, however, were detected in the standard when the very sensitive cholinesterase spray technique was used (*8*). Parathion emulsions for treatment of leaf and glass surfaces were prepared in distilled water with 0.2% v./v. of a 1 to 1 mixture of Triton X-155 and Triton B-1956 (Rohm and Haas Co., Philadelphia, Pa.) emulsifiers.

Leaf and Glass Surface Treatment. Rates of loss of surface deposits of parathion from primary leaves of bean plants and glass plates (10 × 10 cm.) were compared in a semicontrolled environment.

Each primary leaf and a comparable surface area on each glass plate were treated with 100 μl. of a freshly prepared emulsion containing 450 μg. of parathion. The emulsion was applied topically with a micropipet and spread uniformly over the entire surface area. The zero-time samples were collected immediately after the surface deposit was dry and each leaf or plate was rinsed with 10 ml. of chloroform to remove the insecticide deposit. The treated glass plates were held horizontally at the same height as the leaves under fluorescent lights with a 16-hour photoperiod, a light intensity of approximately 800 footcandles, and an ambient temperature of 26–30° C. Surface residues were recovered for analyses from four leaves and four glass plates at 2-day intervals by the preceding rinsing technique. The rinsed leaves and the remainder of the plant then were weighed, homogenized, and extracted to determine the amount of absorbed parathion and organosoluble degradation products.

Root Treatment. Saturated solutions of parathion in nutrient solution were prepared by shaking 30 mg. in 1 liter for 5 minutes, and the excess parathion then was removed by centrifugation. Roots of individual plants were immersed on 100 ml. of supernatant, which contained approximately 20 p.p.m. of dissolved parathion. The jar containing the solution was covered then with aluminum foil close to the plant stem to reduce evaporation and possible fumigant contamination of the foliage. Untreated plants interspaced among the treated plants throughout the study showed no evidence of contamination by either parathion or paraoxon. The plants were grown in the same manner and treated at the same age as those used in the leaf studies and were held under the same experimental conditions of temperature and light.

At each posttreatment interval, six or more plants were removed by clipping the stem above the foil cover and then were weighed, homogenized, and extracted to determine internal concentrations of translocated parathion. The roots were rinsed with ethyl acetate to remove any external residues and then were analyzed to determine absorbed parathion.

Contents of the jars were transferred quantitatively to separatory funnels and extracted with two equal volumes of ethyl acetate and the root rinses were pooled with the contents to determine the quantity of parathion or metabolites remaining in the nutrient solution.

Analytical Procedures. Parathion and paraoxon were extracted from homogenized plant tissue with acetonitrile, and the interfering pigments and lipids were removed by the charcoal–magnesium oxide–Celite column reported by Storherr *et al.* (*15*). Recovery from bean plants fortified with parathion at 1 or 4 p.p.m. was 92 and 94%, respectively. Paraoxon recovery was less quantitative at 86%.

Parathion was determined quantitatively by two different analytical methods as follows:

Colorimetric analysis was made with the *p* - nitrobenzyl pyridine reagent (PNBP) of Getz and Watts (*9*), reported to be specific for organophosphorus triesters and capable of detecting both parathion and paraoxon.

Gas-liquid chromatography was made with an Aerograph Model A-600-B (Wilkins Instruments, Walnut Creek, Calif.) and hydrogen flame detector modified for increased sensitivity to organophosphorus compounds with a sodium thermionic detector (*10*).

A 5-foot × 1/8-inch i. d. borosilicate glass column was packed with 3% QF-1 on 60- to 80-mesh silanized Gas Chrom P (Applied Science Lab., State College, Pa.) and was conditioned for 24 hours at 225° C. prior to use. Operating temperature was 185° C. with nitrogen and hydrogen flow rates of approximately 25 ml. per minute and air 400 ml. per minute. Borosilicate glass injector liners were used to minimize sample decomposition. Under those conditions, retention times were 2.2 minutes for parathion and 2.8 minutes for paraoxon. However, paraoxon residues were never sufficiently large in the experimental extracts to use GLC for quantitation.

Partition thin-layer chromatography with chromogenic and cholinesterase detection methods was used for qualitative analyses of parathion, paraoxon, and other degradation products in plant extracts and surface rinses (*8*). Paraoxon residues were estimated quantatively on the chromatoplate with the cholinesterase method by comparing spot size and color density with a series of spots from paraoxon standard solutions.

Results

Leaf and Glass Surface Residues. The half life of parathion on the surface of both leaves and glass plates were slightly more than 1 day under the environmental conditions used (Figure 1). The rate of loss from leaves was initially more rapid than from glass with a maximum difference of 8% at 2 days. A second and diminished loss rate occurred thereafter, and the total parathion residue eventually became less on the glass surfaces than on the leaves. The disappearance rate from glass was essentially a linear first-order decay curve throughout the 10 days. Less than 10% of the initial deposit remained on either surface after 4 days.

Analyses of the surface rinses by gas-liquid chromatography and by the colorimetric PNBP gave comparable results until the 4-day posttreatment interval when the latter method showed higher recovery of parathion. Examination of all the surface rinses by thin-layer chromatography demonstrated detectable quantities of paraoxon at 2 days and *p*-nitrophenol on both leaf and glass surfaces at 4 days. The 6- to 10-day rinses showed accumulation of additional degradation products that contained the *p*-nitrophenyl moiety as detected on thin-layer plates with the KOH reagent (Figure 2), which may have accounted for higher recoveries by the colorimetric method. One compound tentatively was identified as isoparathion—*O*-ethyl *S*-ethyl *O*-*p*-nitrophenyl phosphorothiolate—as it corresponded to the R_f value of the major heat isomerization product of parathion (*14*). The two other degradation products detected were unidentified. Quantitative estimation of paraoxon on leaf and glass surfaces is discussed later.

Absorption of Parathion by Bean Leaves. Five per cent of the parathion deposit was absorbed within a few minutes after being applied to bean leaves and could not be recovered by rinsing leaf surfaces and recovering the cuticular waxes (Table I). Highest concentrations of parathion accumulating in plant tissues (about 30% of the initial deposit) occurred at 2 days and declined to 9% by 10 days. The rate

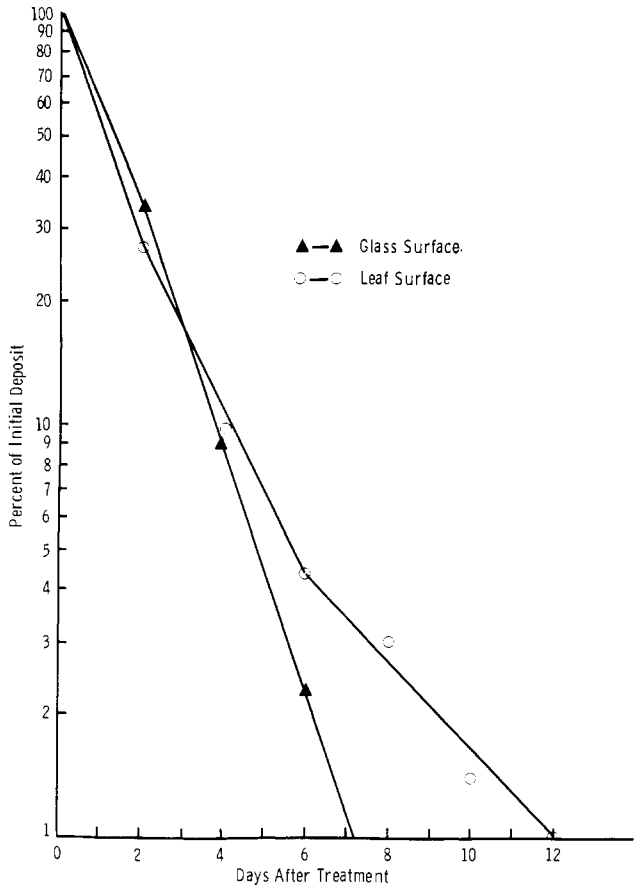

Figure 1. Disappearance behavior of parathion residues on glass and bean leaf surfaces

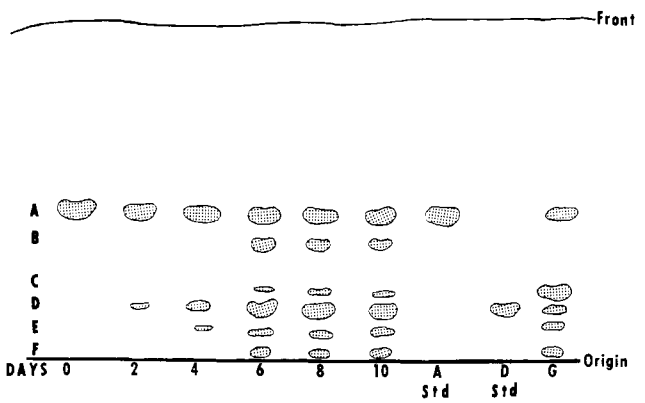

Figure 2. Thin-layer chromatography (normal phase) of surface residues from bean leaves treated with parathion

Alcohol KOH chromogenic agent. *A*, parathion; *B*, unknown; *C*, S-ethyl parothion; *D*, paraoxon; *E*, *p*-nitrophenol; *F*, unknown; *G*, heat-isomerized parathion

of loss of total parathion, including both surface and internal residues, was uniform for 6 days with an 82% combined loss by degradation and evaporation at that time. The half life of the total parathion residue was 2.4 days. Only an additional 10% of the parathion disappeared within the next 2 days, indicating a persistence phase and a diminishing loss rate. Analyses of the tissue extracts by gas-liquid chromatography and by the PNBP colorimetric method agreed very closely at all intervals.

Paraoxon Accumulation. Traces of paraoxon could be detected in the zero-time parathion deposits by the cholinesterase detection method on thin-layer chromatograms, which was expected since similar trace contamination of the analytical standard was observed by the method that has a detection limit of approximately 0.025 nanograms of paraoxon. No paraoxon, however, could be detected at zero time by the less sensitive potassium hydroxide reagent. Paraoxon residues arising from either chemical or enzymatic oxidation of the parathion application, therefore were established by comparing the zero-time extracts with those of subsequent intervals by both KOH and cholinesterase detection methods on thin-layer plates. A quantitative estimate of the amount of paraoxon in the surface rinses and tissue extracts also was made by comparing size and intensity of inhibition spots with a series of spots from standard concentrations of paraoxon (Table II).

Paraoxon accumulated on both glass and leaf surfaces in quantities sufficient to be detected by the KOH reagent (Figure 2). The amounts were quantitatively similar on both surfaces as determined by the cholinesterase assay and never exceeded 1% of the original parathion deposit. Amounts of paraoxon considered in relation to the remaining parathion residues at each time interval represented an increasingly larger percentage throughout the 10 days. Approximately 26% of the residue on glass and 13% on leaves was paraoxon at the 10-day interval (Table II).

Paraoxon also was detected in the plant tissue extracts in increasing concentrations and was equivalent to about 1% of the original deposit by 8 to 10 days. The combined surface and internal paraoxon remained relatively constant from 4 to 10 days, however, because the progressively declining amounts on the leaf surface were compensated by a rising concentration in the tissue (Table II). About 10% of the combined external and internal plant residue at 10 days was ascribed to paraoxon.

Trace amounts of other degradation products were detected in the tissue extracts in increasing amounts throughout the 10-day interval. They had R_f values similar to those detected on the leaf surface and may have been absorbed along with the parent insecticide.

Root Absorption. Parathion was absorbed rapidly by the roots of bean plants immersed in a nutrient solution containing 20 p.p.m. of the insecticide (Table III). Most of the uptake or about 37% of the original parathion occurred in the first 2 days and increased to 50% by 10 days. Parathion was found in the aerial foliage of the plant, demonstrating that translocation occurs, but quantities accumulating there were low, ranging between 1 and 2% of the original insecticide.

Unlike its action in leaf applications, parathion remained relatively stable in nutrient solution with quantitative recoveries during the first 4 days and a loss of only 36% by 10 days. Parathion would be expected to be very stable at the slightly acid pH (6.6) of the nutrient solution, and apparently, hydrolysis in the roots occurs at a very slow rate.

Paraoxon was detected in the root extracts at all time intervals, and its concentration in p.p.m. is compared with

Table I. Absorption and Loss of Parathion Applied to Bean Plant Leaves[a]

Days	Leaf Surface GLC[b]	Leaf Surface PNBP[c]	Plant Tissue GLC	Plant Tissue PNBP	Total Residue GLC	Total Residue PNBP	Loss, %
0	94.6	95.9	4.9	5.4	99.5	101.3	0
2	26.8	23.1	28.0	29.2	54.8	52.3	46
4	9.8	13.5	18.2	17.6	28.0	31.1	60
6	4.4	8.2	12.5	11.9	16.9	20.1	82
8	3.0	4.9	9.0	8.8	12.0	13.7	87
10	1.4	2.5	8.7	8.9	10.0	11.4	89

[a] Figures are percentages of original deposits of 450 μg./leaf.
[b] Gas-liquid chromatography.
[c] *p*-Nitrobenzyl pyridine colorimetric method.

Table II. Paraoxon Accumulation on Glass and Bean Leaf Surfaces Treated with Parathion and in Plant Tissues[a]

	Original Deposit (PNBP) %[b]				Remaining Residue (PNBP) %[b]			
		Bean Plants				Bean Plants		
Days	Glass	Leaf surface	Tissue	Total	Glass	Leaf surface	Tissue	Total
0	Trace	Trace	0	Trace	Trace	Trace	0	Trace
2	0.1	0.1	0.3	0.4	0.3	0.5	0.9	0.7
4	0.2	0.8	0.4	1.2	2.2	5.6	2.5	3.8
6	0.6	0.6	0.6	1.2	8.4	7.9	5.0	6.2
8	0.5	0.3	1.0	1.3	12.2	6.6	11.0	9.4
10	0.5	0.3	0.9	1.2	26.3	12.8	9.7	10.4

[a] Original deposit of 450 μg. per plate or leaf. Thin-layer chromatography and cholinesterase detection. Paraoxon estimated by spot size and intensity compared with a series of standard concentrations.
[b] Deposits or residues determined colorimetrically by the *p*-nitrobenzyl pyridine method which includes both parathion and paraoxon.

Table III. Absorption and Translocation of Parathion by the Roots and Foliage of Bean Plants from Nutrient Solution Containing 20 P.P.M. Parathion[a]

Days	Nutrient Solution	Roots	Foliage	Total	Loss
0	100.0	0	0	100.0	0
2	61.2	37.1	1.2	99.5	0
4	55.2	45.0	1.8	102.0	0
6	36.2	43.8	1.3	81.3	18
8	29.8	45.8	1.0	76.6	23
10	10.5	50.8	1.3	62.6	36

[a] Figures are percentages of total parathion originally in solution as determined by gas-liquid chromatography.

corresponding parathion residues in Table IV. Paraoxon concentrations remained relatively constant with 4 to 5 p.p.m. in the roots between 4 and 10 days and 0.5 to 0.9 p.p.m. in the foliage. The ratio of paraoxon to parathion in the foliage was much lower than the corresponding ratio in the roots, possibly reinforcing evidence that paraoxon is much more readily translocated than is parathion.

Analysis of the nutrient solution, in which plants were grown for root absorption studies, revealed paraoxon, parathion, and *p*-nitrophenol on the chromatoplates as well as traces of other degradation products. To determine whether paraoxon accumulating in the nutrient solution was due to chemical oxidation in the aqueous medium or to oxidation of parathion in the roots and diffusion of paraoxon into the medium, a set of control solutions containing 20 p.p.m. of parathion were held under the same conditions except without plants. Paraoxon accumulated in the bottles at about the same rate and concentration, indicating possible photochemical oxidation processes in the aqueous medium.

Stem Injection Studies. Undiluted parathion was injected with a microsyringe directly into the stems of bean plants in the vascular bundle region to effect translocation of parathion in the tissue without prior exposure to atmosphere. The injection site was cleaned thoroughly outside by wiping the acetone and then wrapped with cotton. After approximately 2 weeks the plants were homogenized, extracted, and analyzed by TLC for paraoxon and other possible degradation products.

Chromatoplates sprayed with KOH reagent revealed parathion, small amounts of paraoxon, and a metabolite with the same R_f value as isoparathion.

Table IV. Concentrations of Parathion and Paraoxon in the Roots and Aerial Parts of Bean Plants[a]

Days	Plant Tissue	Parothion, P.P.M. PNBP[b]	Parothion, P.P.M. GLC[c]	Paraoxon, P.P.M. TLC[d]
2	Roots	705.0	690.0	1.6
	Foliage	6.8	6.0	0.2
4	Roots	697.0	697.0	3.9
	Foliage	11.4	9.8	0.5
6	Roots	785.0	808.0	4.6
	Foliage	7.7	7.0	0.4
8	Roots	763.0	786.0	5.6
	Foliage	6.5	5.6	0.9
10	Roots	629.0	616.0	4.0
	Foliage	6.7	5.9	0.7

[a] Grown in nutrient solution containing 20 p.p.m. parathion for 10 days.
[b] *p*-Nitrobenzyl pyridine colorimetric method.
[c] Gas-liquid chromatography.
[d] Thin-layer chromatography.

Discussion

Parathion residues on both glass and bean leaf surfaces disappeared rapidly with half lives of 1 day or slightly longer. The nature of the substrate affected disappearance rate, with parathion losses from glass occurring at approximately the same rate throughout the study, whereas disappearance from leaf surfaces occurred at progressively diminishing rates, similar to the degradation and persistence curves described by Gunther and Blinn (*11*). Volatilization was the primary factor in parathion loss from glass plates, with degradation products becoming significant at 6 days when the surface residues were less than 5% of the original deposit. The initially faster disappearance from leaf surfaces may be explained by the combined volatilization and absorption losses. At 2 days nearly 30% of the original parathion deposit was found in plant tissues. At the same interval, 46% of the original deposit was unaccounted for owing to volatilization and probably some hydrolysis of the absorbed parathion by plant enzymes. The diminishing rate of surface residue loss on the leaves indicates persistence of the insecticide in the cuticular lipids in a solubilized or bound state. The relative percentage of the cuticular residues would increase with time with a corresponding decrease in evaporation and absorption losses. Surface degradation products of parathion on leaves appeared to be identical with those on glass surfaces. Traces of paraoxon first appeared at 2 days followed by *p*-nitrophenol, *S*-ethyl parathion, and two unidentified substances, indicating that photochemical reactions occurring on leaves include oxidation, hydrolysis, and isomerization of parathion. Benjamini, Metcalf, and Fukuto (*1, 2*) reported formation of the *S*-ethyl isomer of *O,O*-diethyl *O*-*p*-methyl sulfinylphenyl phosphorothionate on leaf and glass surfaces and as a principal metabolite within the plant. The degradation product tentatively identified as *S*-ethyl parathion here had chromatographic mobilities identical with the major heat isomerization product of parathion on thin-layer plates.

Paraoxon was the principal anticholinesterase degradation product of parathion both on the leaf surface and within the plant tissues. Total paraoxon residues remained relatively constant at approximately 1% of original parathion deposit from 4 to 10 days. Paraoxon of the leaf surface initially accumulated faster and then diminished more rapidly than internal paraoxon, the latter showing a slower but steady increase throughout the first 8 days. This would indicate that paraoxon, initially formed from parathion on the leaf surface through the participation of radiant energy and atmospheric oxygen, is absorbed into the

plant system. When paraoxon residues were calculated as percentages of total residue remaining at each posttreatment interval, as determined by the PNBP method, they steadily increased with time and represented about 25% of the remaining residue on glass surfaces and 10% of the total plant residue at 10 days. Other anticholinesterase degradation products were detected in trace amounts in the internal extracts, and evidence suggests that they accumulate at least partially through cuticular absorption of surface degradation products. Relative amounts of paraoxon arising from photochemical oxidation processes on the leaves and from enzymatic oxidation within the plant system are not known and, indeed, would be difficult to determine. Parathion carefully injected into the vascular bundle region of the stem gave rise to paraoxon in the plant, but that does not eliminate the possibility of photochemical oxidation within the cells of the leaves following translocation.

The root absorption studies demonstrated that parathion was absorbed readily into roots of bean plants from nutrient solution, but very little was translocated to aerial parts. The rate of parathion absorbed by roots of bean plants in this study would be expected to be much higher than by plants grown in soil, since several workers have demonstrated such a relationship (*3*). Water absorption by the roots may be enhanced further by action of the insecticide. Tietz (*16*) found that demeton stimulated water uptake by *Phaseolus vulgaris* roots during the first few hours of treatment.

Paraoxon accumulated in the roots and, to a much lesser extent, in the aerial plant parts. However, the ratio of paraoxon to parathion in aerial parts was lower, suggesting that paraoxon is formed more rapidly in aerial parts of the plant or is translocated to a larger extent than parathion because of its greater water solubility.

Acknowledgment

The technical assistance of Magdalena L. Briones, Emelita G. Demeterio, and Phyllis A. Lofgren in various phases of the research is sincerely appreciated.

Literature Cited

(1) Benjamini, E., Metcalf, R. L., Fukuto, T. R., *J. Econ. Entomol.* **52,** 94 (1959).
(2) *Ibid.*, p. 99.
(3) Bennett, S. H., *Ann. Rev. Entomol.* **2,** 279 (1957).
(4) Casida, J. E., "Radioisotopes and Radiation in Entomology," pp. 49–64, International Atomic Energy Agency, Vienna, 1962.
(5) Casida, J. E., Allen, T. C., *Agr. Chem.* **7,** 41 (1952).
(6) Coffin, D. E., *Residue Rev.* **7,** 61 (1964).
(7) David, W. A. L., Aldridge, W. N., *Ann. Appl. Biol.* **45,** 332 (1957).
(8) El-Refai, A., Hopkins, T. L., J. Agr. Food Chem. **13,** 477 (1965).
(9) Getz, M. E., Watts, R. R., *J. Assoc. Offic. Agr. Chemists* **47,** 1094 (1964).
(10) Giuffrida, L., *Ibid.*, **47,** 293 (1964).
(11) Gunther, F. A., Blinn, R. C., "Analysis of Insecticides and Acaricides," Interscience, New York, 1955.
(12) Heath, D. F., "Organophosphorus Poisons, Anti-Choline Esterases and Related Compounds," Pergamon Press, New York, 1961.
(13) Knaak, J. B., Stahmann, M. A., Casida, J. E., J. Agr. Food Chem. **10,** 154 (1962).
(14) Metcalf, R. L., March, R. B., *J. Econ. Entomol.* **46,** 288 (1953).
(15) Storherr, R. W., Getz, M. E., Watts, R. R., Friedman, S. J., Erwin, F., Giuffrida, L., Ives, F., *J. Assoc. Offic. Agr. Chemists* **47,** 1087 (1964).
(16) Tietz, H., *Hoefchen Briefe* **7,** 1–55 (1954).
(17) Van Middelem, C. H., *Residue Rev.* **2,** 77 (1963).
(18) Zeid, M. M. I., Cutkomp, L. K., *J. Econ. Entomol.* **44,** 898 (1951).

Received for review May 8, 1966. Accepted August 8, 1966. Contribution No. 894, Department of Entomology, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, Kan. Research supported in part by the North Central Regional Project 33, "Pesticide Residues on or in Food, Feed, and Forage Crops."

HERBICIDE METABOLISM

The Metabolism of Carbon-14 Diphenamid in Strawberry Plants

TOMASZ GOLAB, R. J. HERBERG, S. J. PARKA, and J. B. TEPE

Greenfield Research Laboratories, Eli Lilly and Co., Greenfield, Ind.

Diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide) is a selective pre-emergence herbicide, which is registered for use on many horticultural and agronomic crops. A residue of diphenamid was detected in strawberry fruit harvested from plants grown in diphenamid-treated soil. To determine the nature of the total residue concentration and the metabolic products of the herbicide, bearing strawberry plants were grown in greenhouse soil treated with 3.83 pounds per acre of diphenamid labeled with carbon-14 in the carbonyl position. Unaltered diphenamid was found as the major product incorporated in strawberry fruit and plants. *N*-methyl-2,2-diphenylacetamide, present in much lower concentration, was identified as the major diphenamid metabolite.

Diphenamid (Dymid, Elanco Products Co., a division of Eli Lilly and Co.) is a selective, pre-emergence herbicide whose properties were discovered by Eli Lilly and Co. Research Laboratories (*8*). This compound is useful in the pre-emergence control of a wide variety of annual grasses and broadleaf weeds. Tolerant crops include direct-seeded and transplanted tomatoes and peppers, peanuts, Irish potatoes, sweet potatoes, and nonbearing strawberry plants.

When diphenamid applications are made to established strawberry plants from 1 to 5 months prior to producing strawberry fruit, residues of diphenamid in the range of 0.05 to 0.5 p.p.m. were found in the strawberry fruit. The presence of a residue indicated that diphenamid was absorbed by the strawberry plants from treated soil, suggesting that the strawberry plant would be useful in studying the metabolism of diphenamid. The purpose of this investigation was to detect and identify the metabolites of diphenamid in the strawberry fruit and the strawberry plant.

Material and Methods

Labeled Diphenamid. Diphenamid labeled with carbon-14 in the carbonyl group was prepared in the Lilly Research Laboratories (*6*). The radiochemical