Effects of Fertilizers, Captafol, and Atrazine on the Fate and Translocation of [^{14}C]Fonofos and [^{14}C]Parathion in a Soil–Plant Microcosm

E. Paul Lichtenstein,* Tung T. Liang, and Mary K. Koeppe

The effects of fertilizers, captafol, and atrazine on the metabolic fate and translocation of [^{14}C]fonofos and [^{14}C]parathion from soils into oats and corn were studied within open and closed agricultural microcosms. After a 2-week plant growing period in open systems, more radiocarbon was recovered from fonofos-treated soils containing also cow manure, sewage sludge, or atrazine and less from oats or corn grown in these soils. The presence of these amendments in fonofos-treated soil also resulted in an increase of unextractable, bound ^{14}C residues, while ammonium sulfate and captafol had no effect. In parathion-treated soils manure and sludge increased the formation of bound residues, while ammonia sulfate, atrazine, and in particular captafol inhibited their formation. Oat greens from fonofos-treated soils contained primarily insecticidal metabolites. Oats from parathion-treated soil contained less ^{14}C residues, most of them in the form of parathion. Production of $^{14}CO_2$ from the ^{14}C-labeled insecticide treated soils was significantly inhibited by all amendments, in particular by captafol and manure. Results indicate that soil amendments indirectly inhibited the production of $^{14}CO_2$ by directly affecting soil microorganisms, primarily soil fungi, responsible for the degradation of the insecticides. Microbiological degradation of [^{14}C]glucose in soils to $^{14}CO_2$ was inhibited by manure and sludge, was increased in the presence of captafol and atrazine, and was not affected by ammonium sulfate. Results indicate that the fate of pesticide chemicals in the agricultural environment should be studied by taking various ecological factors into consideration.

The environmental behavior of pesticides has in the past been investigated primarily with one specific chemical, usually after its application to soils, water, or plants. Under normal agricultural practices, however, a number of chemicals are often used, resulting in a variety of mixed pesticide residues in soils, water, or crop plants. In addition to pesticide chemicals, organic and inorganic fertilizers are applied that could affect the fate of soil pesticides, possibly via an effect on the soil microflora. Thus Rajaram et al. (1978) reported that a single application of ammonium sulfate or potassium nitrate inhibited the hydrolysis of parathion in flooded soil that had been inoculated with a parathion-hydrolyzing enrichment culture. Doyle et al. (1978), utilizing a closed flow-through incubation unit, reported that "altered rates of pesticide degradation were observed in sludge and/or manure amended soils for a number of structurally unrelated pesticides. ^{14}C-product distribution varied with soil amendments". The pesticides investigated in Doyle's study were primarily herbicides. Studies conducted in our laboratory with [^{14}C]parathion (Ferris and Lichtenstein, 1980) showed that selected fungicides and inorganic nitrogen fertilizers affected the degradation of [^{14}C]parathion in Wisconsin cranberry soils.

For further investigation of interaction phenomena, open and closed agricultural microcosms or systems were used (Figure 1) with or without plants that were grown in insecticide-treated loam soil. With this setup the potential effects of fertilizers, captafol, and atrazine on the fate and translocation of soil-applied insecticides in soils and crop plants were studied.

MATERIALS AND METHODS

Chemicals. [U-*phenyl*-^{14}C]Fonofos (Dyfonate) (sp act. = 17.1 mCi/mM) was obtained through the courtesy of Stauffer Chemical Co., Mountain View, CA, and [2,6-*phenyl*-^{14}C]parathion (sp act. = 2.2 mCi/mM) was purchased from the ICN Corp., Irvine, CA. Specific activities indicated in the different experiments were obtained by dilution with nonradioactive fonofos or parathion. These insecticides were determined to be at least 97% pure by thin-layer chromatography. Nonradioactive fonofos or parathion and some of their potential metabolites were obtained from Stauffer Chemical Co. and from Farbenfabriken Bayer, West Germany, respectively. Analytical-grade captafol (Difolatan) was obtained from Ortho Chevron Chemical Co., Richmond, CA, and atrazine was provided courtesy of Ciba-Geigy Corp., Greensboro, NC. D-[6-^{14}C]Glucose (sp act. = 51.2 mCi/mM) was purchased from New England Nuclear Corp., Boston, MA.

Fertilizers. Cow manure was obtained from the University of Wisconsin dairy barn and sewage sludge from the Madison Metropolitan Sewage District. The sludge was in a liquid, digested form (pH 7.0), as sold to Wisconsin farmers. Both manure and sludge were air-dried at room temperature, ground to a fine powder, and stored at 5 °C. Granular analytical-grade ammonium sulfate was obtained from Mallinkrodt, St. Louis, MO.

Solvents. Acetone, benzene, chloroform, ethyl acetate, dioxane, methyl-Cellosolve, and hexane were redistilled before use. Methanol, toluene, and ethanolamine were of analytical grade.

Soil. A Plano silt loam soil (4.7% organic matter, 5% sand, 71% silt, and 24% clay with a pH of 6.0), free of insecticide residues, was collected at the University of Wisconsin Experimental Farm near Madison and stored in a moist condition at 22 ± 2 °C prior to use.

Soil Treatments. Studies described here deal with the potential effects of cow manure, sewage sludge, ammonium sulfate, captafol, and atrazine on the fate and metabolism of soil-applied [^{14}C]fonofos and [^{14}C]parathion in soils and their translocation into crop plants. Loam soils were first treated at 4 ppm with an acetone solution of one of the insecticides and then thoroughly mixed, while the acetone was removed with a gentle stream of air (Lichtenstein and Schulz, 1959). Immediately following treatment, two 50-g aliquots of each treated soil were removed for extraction and analyses to determine the actual treatment levels. All data are reported as percentages of the initially determined doses. Soil amendments were added to these soils as described later under Experimental Procedures.

So that the effect of these soil amendments on glucose-degrading microorganisms could be studied, soils were

Department of Entomology, University of Wisconsin—Madison, Madison, Wisconsin 53706.

0021-8561/82/1430-0871$01.25/0

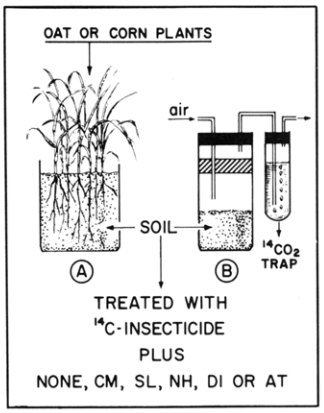

Figure 1. Soils treated with [^{14}C]fonofos or [^{14}C]parathion to which also cow manure (CM), sewage sludge (SL), $(NH_4)_2SO_4$ (NH), Difolatan (captafol) (DI), or atrazine (AT) had been added. (A) depicts the open system with oats or corn and (B) the closed system.

also treated with [^{14}C]glucose followed by the addition of one of the soil amendments.

Extraction. *Soils.* All soils were extracted with a mixture of acetone–methanol–benzene (1:1:1), followed by solvent evaporation and partitioning of the extracted residues between benzene and water as described by Lichtenstein et al. (1973).

Plants. Oats or corn grown in all ^{14}C-labeled insecticide treated soils were extracted with acetone–methanol–benzene as described above. Cleanup of organic extraction phases of oat or corn tops was necessary prior to analysis by thin-layer chromatography. For this purpose benzene extraction phases containing [^{14}C]fonofos or [^{14}C]parathion were purified with charcoal as described by Lichtenstein et al. (1973).

Analyses. Analyses by liquid scintillation counting (LSC), thin-layer chromatography (TLC), and autoradiography as well as gas–liquid chromatography (GLC) were performed as described by Fuhremann and Lichtenstein (1980). Results were expressed in percent of the insecticidal dose as determined by initial soil analyses following soil treatment.

EXPERIMENTAL PROCEDURES

Effects of Soil Amendments on the Fate of ^{14}C-Labeled Insecticides in an Open Soil–Plant Microcosm. Oats or corn were grown in insecticide-treated soils, to which fertilizers, captafol, or atrazine had also been added. Initially two 5000 g (dry weight) each of soil were treated at 4 ppm as described (Lichtenstein and Schulz, 1959) with 125 mL of acetone containing 20 mg of [^{14}C]fonofos (30.2 μCi) or 20 mg of [^{14}C]parathion (53.3 μCi). Each of 14 cartons (9 × 8.5 cm diameter) was then filled with 300 g (dry weight) of [^{14}C]fonofos-treated soil and each of 14 additional cartons with 300 g of [^{14}C]parathion-treated soil. All tests were conducted in duplicate: two cartons containing either [^{14}C]fonofos- or [^{14}C]parathion-treated soil served as controls. Additional soil treatments consisted of mixing 13.5 g of dried cow manure or sewage sludge with insecticide-treated soil, resulting in a dose equivalent to 100 metric tons of manure or sludge per hectare of soil. On the basis of Walsh et al. (1976), the amounts of sludge used in these experiments appear to be on the high side of practical use rates, although Doyle et al. (1978) reported that the rate of 100 metric tons/ha "are in accordance with those rates which have been considered for possible use in Maryland agricultural soils". Ferris and Lichtenstein (1980) showed that in the presence of ammonium sulfate applied at 100 ppm of N equivalent to Wisconsin cranberry soils, parathion was more persistent and amounts of $^{14}CO_2$ produced from [^{14}C]parathion were reduced by 83%. In the experiments reported here, ammonium sulfate was added to the insecticide-treated soils at 106 ppm of N equivalent. Captafol or atrazine was mixed with soils at 100 ppm. These later concentrations, although higher than those used normally, were selected to demonstrate the potential effects of captafol and atrazine (Ferris and Lichtenstein, 1980). Percich and Lockwood (1978) used atrazine at 10, 30, and 100 ppm to study the interaction of atrazine wtih soil microorganisms.

Planting and Growing Oats and Corn. Oats were used as test plants in these experiments with the exception of those where the effects of atrazine were investigated. In these latter tests corn plants were utilized.

Seeds of oats and corn were germinated until the roots reached a length of ca. 1 cm. After soil treatment as described, 25 oat seedlings were planted in the soil of each of 20 cartons (10 with [^{14}C]fonofos-treated soil and 10 with [^{14}C]parathion-treated soil). In addition, eight corn seedlings were planted in each of the soils in eight cartons (four with [^{14}C]fonofos-treated soil and four with [^{14}C]-parathion-treated soil) designated for the study of the effects of atrazine. The soil in each carton was then watered with 40 mL of distilled water and weighed. This initial weight was maintained by addition of water as necessary. The plants were grown for 14 days in a growth chamber at a temperature of 28 °C and on a 14-h light, 10-h dark cycle.

Harvesting Plants and Soils. Tops of plants were cut 1 cm above the soil surface, weighed, rinsed with cold tap water, cut into 1-cm pieces, and immersed in appropriate solvents before extraction as described above. Results obtained after analyses of plant greens were reported as radiocarbon recovered from the leaves in percent of the amount previously applied to the soil and were also calculated on a per gram fresh weight basis (Tables I–III).

After the soils had dried for 2 days, roots were removed but were not analyzed. The soils were sieved through a 2-mm screen and thoroughly mixed. A 100-g aliquot was removed for moisture determination while a second 100-g aliquot was immersed in 200 mL of extraction solvents for analyses.

Effects of Soil Amendments on the Fate and Metabolism of ^{14}C-Labeled Insecticides in a Closed Soil Microcosm. Studies conducted previously in our laboratory with plants grown under bell jars had been designed to obtain a total balance of the soil-applied radiocarbon. Results obtained, however, were quite different from similar studies with open systems (Anderegg and Lichtenstein, 1981). This was due to the different growing conditions and in particular due to reduced rates of leaf transpiration in the closed system. It was for these reasons that the investigations with soil amendments as described above were conducted with plants under the more realistic environmental conditions of open systems. Since the production of $^{14}CO_2$ from ^{14}C-labeled insecticides is a good indicator of the potential effects of soil amendments on the breakdown of ^{14}C-labeled insecticides, additional experiments were performed in duplicate with soils kept in closed systems (Figure 1B) as described by Ferris and Lichtenstein (1980). One hundred grams (dry weight) of [^{14}C]fonofos-treated soil (4 ppm) was placed into each of 12 incubation jars (13 cm × 6 cm diameter). Of these, two served as controls, while cow manure or sewage sludge was applied at 4.5 g (dry weight)/100 g of soil, ammonium sulfate at 106 ppm of N equivalent, and captafol or atrazine at 100 ppm. A second experiment identical with the

Table I. Effects of Fertilizers[a] and Captafol[b] on the Fate and Translocation of [^{14}C]Fonofos in Loam Soils and Oat Plants Incubated for 14 Days in an Open System at 28 ± 1 °C[c]

	^{14}C recovered, % of applied [*ring*-^{14}C]fonofos[d] in soil plus				
	none (control)	cow manure	sewage sludge	ammonium sulfate	captafol (Difolatan)
soil					
benzene:[e] ^{14}C	35.6 ± 0.9	26.8 ± 1.3[i]	40.5 ± 2.2	38.6 ± 1.0	31.5 ± 4.1
FO[f]	36.3 ± 1.0	20.5 ± 0.6[h]	33.8 ± 0.8	38.2 ± 0.4	34.2 ± 5.8
water[e]	1.0 ± 0.1	2.1 ± 0.1[i]	0.7 ± 0.2	0.9 ± 0.1	10.2 ± 0.3[g]
bound[e]	28.3 ± 1.4	43.4 ± 0.8[h]	36.3 ± 0.9[i]	26.0 ± 0.4	29.0 ± 0.1
total (S)	64.9 ± 2.4	72.3 ± 0.3[i]	77.4 ± 1.1[i]	65.6 ± 1.4	70.7 ± 3.9
oat greens					
benzene: ^{14}C[j]	1.0 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	0.2 ± 0.0[h]
water	7.4 ± 0.5	0.7 ± 0.1[h]	0.5 ± 0.1[h]	15.0 ± 1.0[h]	3.7 ± 0.5[i]
bound	0.2 ± 0.0	0.1 ± 0.0[i]	0.1 ± 0.0[i]	0.4 ± 0.0[h]	0.1 ± 0.0[i]
total (O)	8.6 ± 0.5	1.9 ± 0.0[h]	1.6 ± 0.1[h]	16.4 ± 1.0[h]	4.0 ± 0.5[i]
total/g of greens	2.4	0.3	0.2	3.1	1.3
total (S + O)	73.5 ± 1.9	74.2 ± 0.3	79.1 ± 1.0	81.9 ± 0.4[i]	74.7 ± 3.4

[a] Cow manure and sewage sludge were mixed with soil (13.5 g of dry powder/300 g of dry soil) and $(NH_4)_2SO_4$ at 106 ppm of nitrogen equivalent. [b] Captafol was mixed with soil at 100 ppm (10 mg/100 g of dry soil). [c] Results are averages of duplicated tests. [d] [*ring*-^{14}C]Fonofos (1.81 μCi) was mixed with 300 g of soil (dry weight) at 4 ppm. [e] Benzene and water extraction phases of soil. Bound = unextractable ^{14}C. [f] FO = fonofos; the amount was determined by GLC. [g-i] Data are significantly different from controls (none) at the 0.1% (*g*), 1% (*h*), and 5% (*i*) level (Student's *t* test). [j] Over 90% of benzene-soluble ^{14}C compounds were associated with methyl phenyl sulfone (see Figure 2).

Table II. Effects of Fertilizers[a] and Captafol[b] on the Fate and Translocation of [^{14}C]Parathion in Loam Soils and Oat Plants Incubated for 14 Days in an Open System at 28 ± 1 °C[c]

	^{14}C recovered, % of applied [*ring*-^{14}C]parathion[d] in soil plus				
	none (control)	cow manure	sewage sludge	ammonium sulfate	captafol (Difolatan)
soil					
benzene:[e] ^{14}C	42.6 ± 3.7	10.7 ± 2.4[h]	29.5 ± 2.9[i]	28.6 ± 0.2[i]	44.9 ± 0.4
PA[f]	26.4 ± 0.1	7.0 ± 2.1[h]	10.6 ± 1.3[h]	20.7 ± 0.0[g]	39.0 ± 1.7[h]
water[e]	11.3 ± 3.8	24.0 ± 1.4[i]	2.5 ± 0.7	28.0 ± 0.7[i]	41.7 ± 0.5[h]
bound[e]	31.7 ± 0.0	51.0 ± 2.9[h]	60.9 ± 2.7[h]	27.1 ± 0.4[h]	7.3 ± 0.6[g]
total (S)	85.6 ± 0.1	85.7 ± 2.0	92.9 ± 0.6[h]	83.7 ± 0.2[h]	93.9 ± 0.6[h]
oat greens					
benzene: ^{14}C[f]	0.1 ± 0.0	0.1 ± 0.0	trace[j]	0.1 ± 0.0	trace[j]
water	0.3 ± 0.0	0.2 ± 0.0[h]	0.1 ± 0.0[h]	0.3 ± 0.0	0.1 ± 0.0[h]
bound	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0[i]	0.1 ± 0.0
total (O)	0.5 ± 0.0	0.4 ± 0.0[h]	0.2 ± 0.0[g]	0.6 ± 0.0	0.2 ± 0.0[h]
total/g of greens	0.08	0.07	0.05	0.09	0.05
total (S + O)	86.1 ± 0.1	86.1 ± 2.0	93.1 ± 0.5[h]	84.3 ± 0.2[h]	94.1 ± 0.6[h]

[a,b,e,g-i] Footnotes *a*, *b*, *e*, and *g-i* are the same as in Table I. [c] Results are averages of duplicated texts. [d] [*ring*-^{14}C]-Parathion (3.2 μCi) was mixed with 300 g of soil (dry weight) at 4 ppm. [f] PA = parathion; the amounts in soils were determined by GLC. Amounts in greens could not be determined because of impurities. [j] 0.02 or less.

Table III. Effects of Atrazine[a] on the Fate and Translocation of ^{14}C-Labeled Insecticides in Loam Soil and Corn Plants Incubated for 14 Days in an Open System at 28 ± 1 °C[b]

	^{14}C recovered, % of applied[c] to soil			
	[^{14}C]fonofos plus		[^{14}C]parathion plus	
	none	atrazine	none	atrazine
soil				
benzene:[d] ^{14}C	36.6 ± 0.1	36.0 ± 1.6	30.0 ± 6.3	40.4 ± 1.6
IN[e]	30.2 ± 0.1	32.2 ± 0.8[g]	24.9 ± 6.3	33.1 ± 0.8[h]
water[d]	1.2 ± 0.1	2.1 ± 0.1[h]	18.9 ± 3.7	29.9 ± 0.6[h]
bound[d]	23.7 ± 0.4	33.0 ± 1.0[g]	32.0 ± 0.6	20.3 ± 1.0[g]
total (S)	61.5 ± 0.3	71.1 ± 0.7[g]	80.9 ± 2.0	90.6 ± 0.1[h]
corn greens				
benzene: ^{14}C[e]	1.0 ± 0.0	0.2 ± 0.0[f]	0.2 ± 0.0	0.2 ± 0.0
water	14.1 ± 0.4	10.8 ± 0.0[g]	0.2 ± 0.0	0.6 ± 0.1[h]
bound	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
total (C)	15.3 ± 0.4	11.1 ± 0.0[g]	0.6 ± 0.0	1.1 ± 0.1[h]
total/g of greens	1.14	0.87	0.04	0.08
total (S + C)	76.8 ± 0.1	82.2 ± 0.7[g]	81.5 ± 2.0	91.7 ± 0.0[h]

[a] Atrazine was mixed with soil at 100 ppm (10 mg/100 g of dry soil). [b] Results are averages of duplicated tests. [c] Same as footnote *d* in Tables I and II. [d] Benzene and water extraction phases of soil. Bound = unextractable ^{14}C. [e] IN = insecticide (fonofos or parathion, respectively) determined by GLC. Data are not available for greens. [f-h] Data are significantly different from controls (none) at the 0.1% (*f*), 1% (*g*), and 5% (*h*) level (Student's *t* test).

one described was set up, except that [^{14}C]parathion was used as the insecticide. Soils were incubated for 4 weeks at room temperature (23 ± 2 °C) and in the dark, while $^{14}CO_2$ was collected in KOH traps at intervals and quan-

tified by LSC as described by Ferris and Lichtenstein (1980).

At the end of the incubation period, soils from each jar were extracted and analyzed, resulting in data for organic- and water-soluble as well as unextractable, soil-bound radiocarbon. Volatile lipid-soluble compounds retained in the polyurethane trap were extracted twice with 75 mL of hexane.

Effects of Captafol and Atrazine on Populations of Microorganisms in Parathion-Treated Soil. Results obtained in experiments with captafol or atrazine suggested that both the fungicide and the herbicide inhibited soil microorganisms which in turn could have altered the degradation of the insecticides in the soil. For determination of the effects of captafol and atrazine on fungal and bacterial populations in those soils, an experiment complementary to the one described with the closed systems was conducted in duplicate with soils treated with parathion at 4 ppm and with captafol or atrazine at 100 ppm. Soils treated only with parathion served as controls. After a 21-day incubation period, soils were thoroughly mixed, and 15-g samples were removed from each incubation jar and shaken for 20 min with 150 mL of sterilized distilled water to produce a soil inoculum. After that, fungal selective agar media (Martin, 1950) were inoculated with a 1:10 dilution prepared from control or captafol- or atrazine-treated soils. Bacteria selective agar media (Ridge and Rovira, 1971) were inoculated with a 1:1000 dilution prepared from the respective soil samples. After a 3-day incubation period of the inoculated plates at room temperature, they were photographed.

Effects of Soil Amendments on the Activity of [^{14}C]Glucose-Degrading Soil Microorganisms. Studies conducted previously in our laboratory had shown that soil microorganisms in basal salt media utilized parathion as the sole carbon source. Addition of glucose, however, drastically reduced the degradation of the insecticide (Gorder and Lichtenstein, 1980). This phenomenon was also demonstrated with parathion in cranberry soils (Ferris and Lichtenstein, 1980). To test the hypothesis that soil microorganisms are affected by the soil amendments used in the experiments described above, we used [^{14}C]glucose as the substrate instead of ^{14}C-labeled insecticides.

In the first study, the potential degradation of [^{14}C]-glucose by soil microorganisms was investigated. Two jars (10 cm × 5 cm diameter) (Figure 1B) were each filled with 25 g (dry weight) of loam soil that was then sterilized by γ irradiation (45 000 rad for 70 h). The sterility of these soils was confirmed by incubating samples in yeast extract–dextrose medium. Two additional jars were filled with 25 g (dry weight) of nonsterilized soil (controls). Four milliliters of sterilized water containing 50 mg of [^{14}C]-glucose (0.97 μCi) was then pipetted onto each soil surface in the four jars. The soils were then incubated for 28 days at 24 ± 1 °C, and $^{14}CO_2$ produced from [^{14}C]glucose was trapped at intervals during the 4-week period. Sterility tests conducted at the end of the incubation period with the original irradiated soils indicated that these soils were no longer sterile.

The second study was conducted with [^{14}C]glucose-treated soil to which the five amendments had been added as described above. After 100 g (dry weight) of soil in each of two incubation jars had been mixed with either cow manure, sewage sludge, ammonium sulfate, captafol, or atrazine as described, 2 mL of water containing 200 mg of [^{14}C]glucose (1.0 μCi) was added to each of the soils containing amendments and to two control soils. To facilitate a better penetration of [^{14}C]glucose throughout these soils, 10 mL of additional water was added to each soil in the 12 incubation jars. Soils were then incubated as described for 31 days while the released $^{14}CO_2$ was trapped at intervals and quantitated by LSC.

RESULTS AND DISCUSSION

Effects of Soil Amendments on the Fate of ^{14}C-Labeled Insecticides in an Open Soil–Plant Microcosm. The effects of fertilizers and captafol on the fate of soil-applied [^{14}C]fonofos were conducted in open systems as shown in Figure 1A. Due to the presence of the soil amendments, significant changes in the distribution of radiocarbon within the soil–plant system were noticed after the 2-week incubation period (Table I). While the total recoveries of ^{14}C-labeled compounds from soils plus oats (Table I, total, S + O) were similar under all conditions (except for ammonium sulfate), significant differences were noticed when results from soils or oats were compared with those of controls. Thus, more total radiocarbon was recovered from soils containing cow manure or sewage sludge and considerably less from oats that had grown in these soils.

Effects of soil amendments on the distribution of radiocarbon in the extraction phases of [^{14}C]fonofos-treated soils were primarily noticed with cow manure. In comparison to controls, manure-treated soils contained less benzene-soluble radiocarbon and less fonofos (21% of applied as opposed to 36% in controls) but showed a significant increase in bound ^{14}C residues (43% of applied as opposed to 28% in controls). In the presence of sewage sludge, an increase in bound soils residues had occurred, while in the presence of captafol a 10-fold increase in water-soluble radiocarbon was noticed. The pH of control soils and of those containing also cow manure, sewage sludge, or ammonium sulfate were initally 5.9, 6.4, 6.5, and 5.9, respectively. Fourteen days later, these figures were 4.9 (controls), 6.0, 6.5, and 5.0, respectively. Determinations of the pH in captafol-treated soils are not available.

Differences observed with oats due to manure or sludge were reflected by a 90% reduction in the formation of water-soluble radiocarbon amounting to only 0.7% or 0.5% of applied ^{14}C, respectively, as compared to 7.4% in controls. It appears that in the presence of manure and sludge—where an increase in binding of radiocarbon to soil had occurred—less water-soluble ^{14}C had been translocated from soils into the leaves or the metabolism within leaves of benzene-soluble ^{14}C into water-soluble radiocarbon had been drastically reduced. The presence of ammonium sulfate in soils, however, resulted in an increase in water-soluble ^{14}C within oat greens (15% of applied as opposed to 7.4% in controls).

For identification of organic-soluble compounds, benzene extraction phases of both soils and oats were also analyzed by TLC, autoradiography, and GLC (soils only). Results are shown in Figure 2, where the right-hand column presents in figures the total amounts of benzene-soluble radiocarbon recovered from soils and oats (expressed in percent of soil-applied ^{14}C). The horizontal bar graphs, however, depict the percent distribution of the various compounds in these benzene extraction phases. It is evident, that fonofos was the major compound recovered from soils, amounting from 49% to 72% of all benzene-soluble compounds. In oat greens, however, most of the ^{14}C recovered (1% or less of the soil-applied radiocarbon) was associated with the fonofos breakdown product methyl phenyl sulfone ($MPSO_2$). Small amounts of the oxygen analogue of fonofos (F=oxon in Figure 2) were found in both soils and oats. Compounds recovered in trace quantities ("others" in Figure 2) were thiophenol, diphenyl

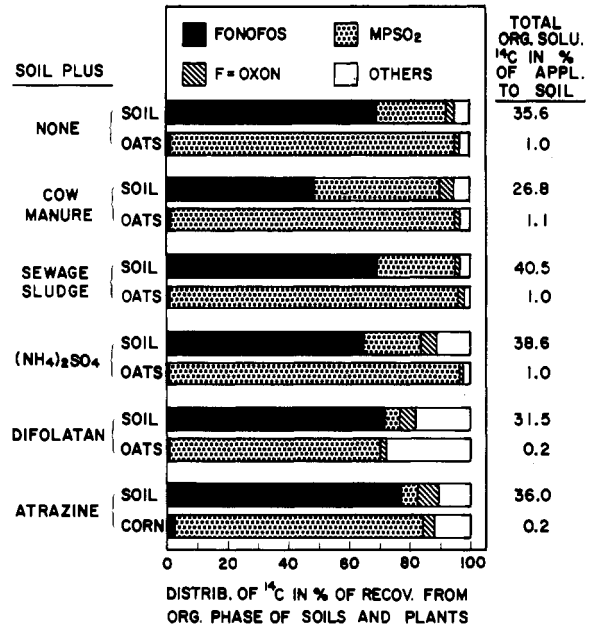

Figure 2. Metabolism of [*ring*-^{14}C]fonofos in soils and plants growing in an open system. Data are based on TLC of the organic extraction phase, followed by autoradiography, LSC, and GLC (soils). $MPSO_2$ = methyl phenyl sulfone; F=oxon = oxygen analogue of fonofos; others = thiophenol, diphenyl disulfide, and 2-, 3-, or 4-hydroxymethyl phenyl sulfone.

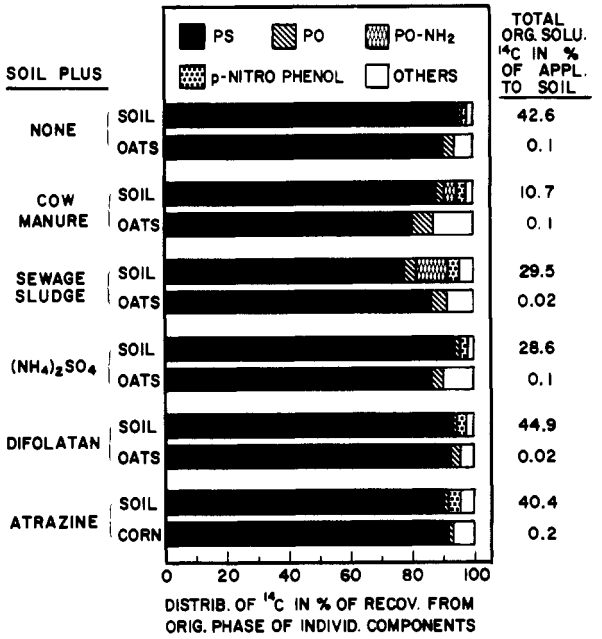

Figure 3. Metabolism of [*ring*-^{14}C]parathion in soils and plants grown in an open system. Data are based on TLC of the organic extraction phase, followed by autoradiography, LSC, and GLC (soils). PS = parathion; PO = paraoxon; $PO\text{-}NH_2$ = amino-paraoxon; others = aminoparathion and *p*-aminophenol.

disulfide, and 2-, 3-, or 4-hydroxymethyl phenyl sulfone. As demonstrated by Liang and Lichtenstein (1980) with houseflies, only fonofos (0.1 μg/fly) and its oxygen analogue (0.1 μg/fly) are insecticidal and "no mortalities were observed after flies had been treated with 1 μg of methyl phenyl sulfone, thiophenol, diphenyldisulfide, or one of the hydroxy methyl phenyl sulfone compounds". It is evident, therefore, that after 2 weeks of incubation, soils still contained 20% to 38% of the applied fonofos (Table I), while nearly all of the radiocarbon recovered from oat greens was associated with detoxified, noninsecticidal metabolites of fonofos.

As shown in Figure 2, relatively more $MPSO_2$ was recovered from soils amended with cow manure and less from soils amended with ammonium sulfate. The addition of captafol to soils, however, suppressed the production of $MPSO_2$, which amounted to only 5% of all benzene-soluble ^{14}C in the soil.

Experiments identical with those described for [^{14}C]fonofos in open systems were also conducted with [^{14}C]-parathion. As shown in Table II, the effects of soil amendments on the fate of [^{14}C]parathion were quite pronounced, although they were different in nature from those observed with [^{14}C]fonofos. The overall recoveries (total, S + O in Table II) of [^{14}C]parathion-derived radiocarbon were larger in the presence of sewage sludge and captafol, due to larger recoveries from soils. In the presence of ammonium sulfate, however, the total recovery of radiocarbon was slightly small than in controls. Due to the presence of manure, recoveries of benzene-soluble ^{14}C and of parathion (determined by GLC) from soils were drastically reduced and to a lesser extent in the presence of sludge and ammonium sulfate. Conversely, the presence of captafol resulted in an increased persistence of parathion as determined by GLC.

Most noticeable were the effects of these soil amendments on the production of unextractable, bound ^{14}C residues in soils. While in control soils 31.7% of the applied radiocarbon was associated with bound soil residues, the presence of manure and sludge increased these bound residues to 51% and 61% of applied, respectively, while ammonium sulfate and captafol drastically decreased the formation of bound residues. It appears that in particular captafol inhibited soil microorganisms responsible for the reduction of parathion to amino compounds, which in turn are bound to soils (Katan and Lichtenstein, 1977). This might explain one of the reasons for the increased persistence of the originally applied parathion and the decreased binding of [^{14}C]parathion derivatives in captafol-treated soils. Results relative to the binding of [^{14}C]-parathion-derived radiocarbon are different from those observed with [^{14}C]fonofos. This was not surprising, since previous investigations with [^{14}C]fonofos showed that the mechanism of binding to soil is different than that observed with [^{14}C]parathion (Lichtenstein et al., 1977).

The amounts of ^{14}C water-soluble compounds produced in [^{14}C]parathion-treated soils increased from 11% of applied in controls to 24%, 28%, and 42% of applied in the presence of manure, ammonium sulfate, and captafol, respectively, and decreased to 2.5% of applied when sewage sludge had been added.

Oat leaves grown in these soils contained small amounts (0.2–0.5% of applied or 0.05–0.09%/g of fresh greens) of radiocarbon. On the basis of TLC, autoradiography, and GLC (soils only), parathion was the major compound recovered from the benzene extraction phases of both soils and oat greens (Figure 3), although in greens the actual amounts were minimal. Paraoxon, aminoparaoxon, and *p*-nitrophenol were the major metabolites detected.

All data, therefore, indicate that the effects of soil amendments on the fate of [^{14}C]parathion in these soil–plant systems were primarily noticeable with soils, where they especially affected the production of bound and water-soluble [^{14}C]parathion derivatives. In plants, however, effects were not noticeable.

So that potential insecticidal activity of oat greens grown in fonofos or parathion-treated soils could be tested organic-soluble compounds extracted from leaves were also bioassayed with fruit flies (*Drosophila melanogaster* Meig.) as described by Lichtenstein (1960). In a separate experiment, two containers as shown in Figure 1A were each

Table IV. Effects of Fertilizers,[a] Difolatan, and Atrazine[b] on the Fate of Insecticides in a Loam Soil Incubated for 28 Days in a Closed System at 24 ± 2 °C[c]

	^{14}C recovered from soil treated[d] with			
	[^{14}C]fonofos		[^{14}C]parathion	
soil plus	$^{14}CO_2$[e]	bound[f]	$^{14}CO_2$	bound
	Percent of Applied[d]			
none (control)	16.7 ± 0.5	30.8 ± 0.4	23.2 ± 1.1	48.5 ± 0.1
	Percent of Control (=100)			
cow manure	7.8[g]	99.4	4.3[h]	178.6[g]
sewage sludge	21.6[g]	78.3[h]	4.3[h]	172.8[g]
ammonium sulfate	61.7[h]	97.1	85.3[i]	84.1[g]
captafol	2.4[g]	89.0[i]	4.3[h]	46.0[g]
atrazine	26.4[h]	115.9[i]	26.7[h]	79.4[h]

[a] As in Table I, except that 4.5 g dry powder of manure or sludge per 100 g of soil was added. [b] Difolatan or atrazine was mixed with soil at 100 ppm (10 mg/100 g of dry soil). [c] Results are averages of duplicated tests. [d] [*ring*-^{14}C]Fonofos (0.69 μCi) or [*ring*-^{14}C]parathion (1.1 μCi) was mixed with 100 g of soil (dry weight) at 4 ppm. [e] Cumulative $^{14}CO_2$ trapped over a 28-day incubation period in KOH within the closed system. [f] Bound = unextractable ^{14}C soil residues. [g-i] Data are significantly different from controls (none) at the 0.1% (*g*), 1% (*h*), and 5% (*i*) level (Student's *t* test).

filled with soil treated with fonofos at 4 ppm, two with parathion treated at 4 ppm, and two with untreated soil (control). Oat greens were harvested after a 2-week growing time in these soils and were extracted as described. Duplicate organic solvent extracts, representing 5.8, 5.6, and 5.7 g (fresh weight) of oat greens from fonofos-treated soils, from parathion-treated soils, and from control soils, respectively, were transferred into bioassay jars. As an additional control, only solvents were pipetted into two bioassay jars. After evaporation of these solvents, fruit flies were exposed to the dry residues in the eight jars and held for 48 h. At the end of the exposure period to solvent controls, oat extracts from nontreated soils, fonofos-treated soils, and parathion-treated soils insect mortalities of 4%, 6%, 4%, and 33%, respectively, were observed. Data, therefore, indicate, that only oat greens from parathion-treated soils exhibited some toxicity to fruit flies.

Effects of Soil-Applied Atrazine on the Fate of Insecticides in a Soil–Corn System. Since corn plants were used in experiments with atrazine, results are reported separately in Table III. On the basis of analyses by GLC of the benzene extraction phase of soils (IN in Table III), both fonofos and parathion were more persistent in the presence of atrazine. Binding of radiocarbon to soils was increased with [^{14}C]fonofos but decreased with [^{14}C]parathion. In addition, more water-soluble ^{14}C compounds had been produced from both insecticides in the presence of the herbicide.

Corn greens grown in [^{14}C]fonofos-treated soils (controls) contained a total of 15% of the applied radiocarbon but only 11% when atrazine was also present. As shown in Table III and Figure 2, respectively, most of these residues in corn were in the form of water-soluble fonofos metabolites and 81% of the benzene-soluble radiocarbon was associated with the noninsecticidal methyl phenyl sulfone.

Corn greens grown in [^{14}C]parathion or [^{14}C]parathion plus atrazine treated soils contained only 0.6% and 1.1%, respectively, of the originally soil-applied radiocarbon. Of these residues, over one-third were water-soluble metabolites (Table III) and most of the benzene-soluble radiocarbon was in the form of parathion (Figure 3).

Effects of Soil Amendments on the Fate of ^{14}C-Labeled Insecticides in a Closed Soil Microcosm. Since these experiments were conducted in closed systems (Figure 1B) without plants, results obtained cannot be strictly compared with those obtained with open systems. Furthermore, soils in closed systems were incubated for 28 days as opposed to 14 days with open systems. However, soil amendments did alter the fact of both [^{14}C]fonofos and [^{14}C]parathion in soils incubated in closed systems. Although analytical data for benzene-soluble and water-soluble radiocarbon are available, the most significant effects of the soil amendments were noticed in the production of $^{14}CO_2$ and of bound ^{14}C residues and are, therefore, reported here. Data presented in Table IV show the recoveries of $^{14}CO_2$ and of bound ^{14}C compounds for controls in percent of the amounts of ^{14}C-labeled insecticides applied to soil. However, data pertaining to the effects of the various soil amendments are expressed relative to controls and were calculated as ^{14}C recoveries in percent of those determined for controls (=100%). Production of $^{14}CO_2$ from the ^{14}C-labeled insecticide treated soils was significantly inhibited by all amendments. With [^{14}C]fonofos this inhibition was most pronounced in the presence of captafol (2.4% of control or 97.6% inhibition) and cow manure (7.8% of control or 92.2% inhibition). With [^{14}C]parathion, 95.7% of inhibition was noticed with cow manure, sewage sludge, or captafol. Since microorganisms affect the persistence of insecticides in soils (Lichtenstein and Schulz, 1964) and also the formation of unextractable soil-bound insecticide derivatives (Katan and Lichtenstein, 1977), it appears that the soil amendments indirectly inhibited the production of CO_2 by directly affecting soil microorganisms. No lipid-soluble volatile ^{14}C was found in the polyurethane traps.

The production of soil-bound ^{14}C residues was also affected by the amendments (Table IV). With [^{14}C]fonofos, less binding occurred in the presence of sewage sludge and captafol and more with atrazine. With [^{14}C]parathion, however, increased binding was noticed with cow manure and sewage sludge and decreased binding with the remaining amendments.

Data, therefore, indicate that the two insecticides were affected in different ways. This makes it difficult or impossible to draw general conclusions from results obtained with one particular chemical.

Effects of Captafol and Atrazine on Populations of Microorganisms in [^{14}C]Parathion-Treated Soil. Populations of fungi or bacteria in parathion-treated soils (controls) and in soils containing also captafol or atrazine were estimated by using selective agar media. After a 3-day incubation period, no fungal growth had occurred on plates inoculated with microorganisms from soils that had also been treated with captafol or atrazine, but bacterial colonies had appeared (Figure 4). The fact that atrazine also inhibited fungal growth was somewhat surprising, although this phenomenon has been reported. As stated by Kaiser et al. (1970) "triazines have highly variable effects on the soil mycoflora. They have no action at times (four authors), they stimulate the mycoflora of certain

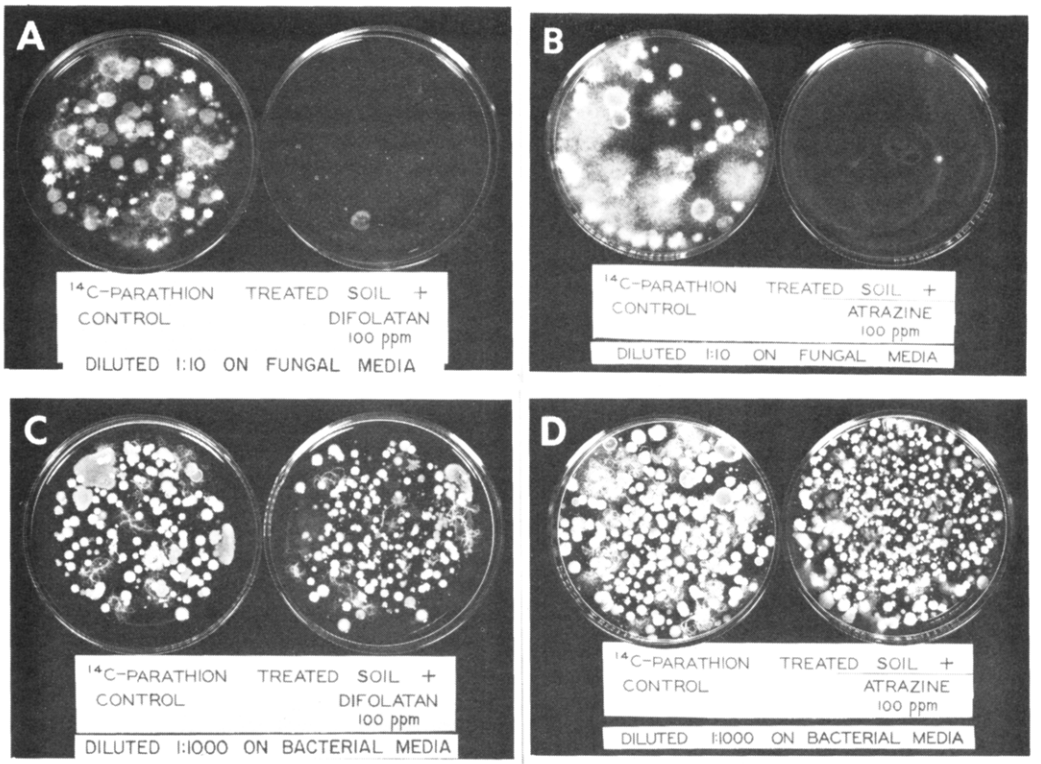

Figure 4. Fungal (A and B) and bacterial (C and D) growth on nutrient media inoculated with microorganisms from soils treated with parathion (controls) or parathion plus Difolatan (captafol) or atrazine.

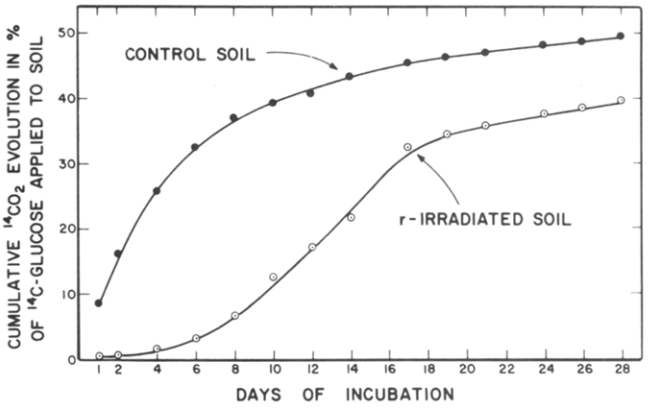

Figure 5. Evolution of $^{14}CO_2$ from soil-applied [^{14}C]glucose in soils sterilized by γ irradiation and in nonirradiated soils (controls).

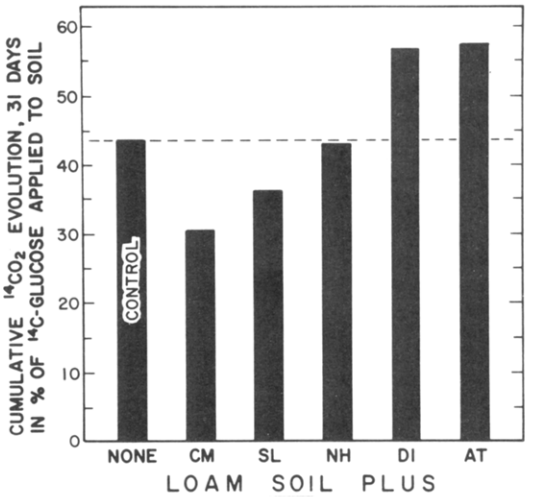

Figure 6. Effects of fertilizers (CM = cow manure; SL = sewage sludge; NH = ammonium sulfate), Difolatan (captafol) (DI), and atrazine (AT) on the evolution of $^{14}CO_2$ from soil-applied [^{14}C]-glucose during a 31-day incubation period. None = controls (soil treated only with [^{14}C]glucose). Differences observed from controls are significant at the 0.02% level (Student's t test) except for ammonium sulfate.

species (six authors), or inhibit fungi (eight authors)". Results seem to indicate that soil fungi were involved in the effects exhibited by captafol and atrazine on the fate of parathion in the soils. No attempt was made to identify fungal or bacterial species.

Effects of Soil Amendments on the Activity of [^{14}C]Glucose-Degrading Soil Microorganisms. [^{14}C]-Glucose was rapidly degraded to $^{14}CO_2$ in nonsterilized soils but not in irradiated soils (Figure 5). Two days after soil treatment 16.12 ± 0.3% of the radiocarbon applied to control soils was released as $^{14}CO_2$ but only 0.48 ± 0.1% from irradiated soils. Four days later (day 6), these figures were 32.4 ± 1.2% and 3.0 ± 0.03%, respectively. After that, a rapid increase in $^{14}CO_2$ production took place in the irradiated soils, similar to the end of a lag phase and the beginning of a logarithmic growth phase of soil microorganisms. After 18 and 28 days of incubation the amounts of $^{14}CO_2$ released from control soils were 45.4 ± 0.9% and 49.6 ± 1.3% of the applied [^{14}C]glucose and from irradiated soils 32.8 ± 0.1% and 39.8 ± 0.1%, respectively. Tests conducted at that time with the irradiated soils showed that they were no longer sterile. The production of $^{14}CO_2$ in these irradiated soils indicates that microorganisms developed and grew along lines of a typical microorganism population growth curve. It appears, therefore, that the degradation of [^{14}C]glucose to $^{14}CO_2$ was related to the activity of soil microorganisms.

The effect of soil amendments on the degradation of [^{14}C]glucose in soils was studied with closed systems (Figure 1B), and the production of $^{14}CO_2$ was used as the criterion to apraise these effects. Results (Figure 6) indicated that cow manure and sewage sludge significantly (at the 0.1% level) decreased the production of $^{14}CO_2$ from 43.4 ± 0.6% in controls to 30.5 ± 1.4% and 36.1% ± 0.5%

of the applied radiocarbon, respectively. Conversely, captafol and atrazine significantly increased the production of $^{14}CO_2$ to 56.9 ± 0.4% and 57.3 ± 3.3%, respectively. Ammonium sulfate in [^{14}C]glucose-treated soils, however, had no effect. Soil amendments apparently affected certain soil microorganisms that were responsible for the breakdown of [^{14}C]glucose to $^{14}CO_2$. It is conceivable, therefore, that the effects of soil amendments on the fate of fonofos or parathion in soils also took place via an effect on soil microorganisms, although different groups might have been involved.

Results obtained with the insecticides under the different environmental conditions do show that soil amendments affect the persistence, metabolism, and translocation of both fonofos and parathion in soil and plants but the nature of these effects was different for each insecticide and no general conclusions can be drawn. It appears that the effects of some amendments occurred via a direct effect on soil microorganism populations that in turn are responsible for the degradation of the insecticides. In addition, organic fertilizers such as cow manure and sewage sludge are not of a specific, fixed chemical and microbiological composition. Their qualitative and quantitative makeup could be dependent on the source of the fertilizer and possibly on the food of the producing organism at a particular time. The major fact, however, is that the soil amendments did in one way or another change the fate and metabolism of the insecticides under the experimental conditions employed. It is for these reasons that the behavior and fate of pesticide chemicals in the agricultural environment should be studied by taking various ecological factors into consideration.

LITERATURE CITED

Anderegg, B. N.; Lichtenstein, E. P. *J. Agric. Food Chem.* **1981**, *29*, 733.
Doyle, R. C.; Kaufman, D. D.; Burt, J. W. *J. Agric. Food Chem.* **1978**, *26*, 987.
Ferris, I. G.; Lichtenstein, E. P. *J. Agric. Food Chem.* **1980**, *28*, 1011.
Fuhremann, T. W.; Lichtenstein, E. P. *J. Agric. Food Chem.* **1980**, *28*, 446.
Gorder, G. W.; Lichtenstein, E. P. *Can. J. Microbiol.* **1980**, *26*, 475.
Kaiser, P.; Pochon, J. J.; Cassini, R. *Residue Rev.* **1970**, *32*, 211.
Katan, J.; Lichtenstein, E. P. *J. Agric. Food Chem.* **1977**, *25*, 1404.
Liang, T. T.; Lichtenstein, E. P. *J. Econ. Entomol.* **1980**, *73*, 204.
Lichtenstein, E. P. *J. Agric. Food Chem.* **1960**, *8*, 448.
Lichtenstein, E. P.; Fuhremann, T. W.; Schultz, K. R.; Liang, T. T. *J. Econ. Entomol.* **1973**, *66*, 863.
Lichtenstein, E. P.; Katan, J.; Anderegg, B. N. *J. Agric. Food Chem.* **1977**, *25*, 43.
Lichtenstein, E. P.; Schulz, K. R. *J. Econ. Entomol.* **1959**, *52*, 118.
Lichtenstein, E. P.; Schulz, K. R. *J. Econ. Entomol.* **1964**, *57*, 618.
Martin, J. P. *Soil. Sci.* **1950**, *69*, 215.
Percich, J. A.; Lockwood, J. L. *Can. J. Microbiol.* **1978**, *24*, 1145.
Rajaram, K. P.; Rao, Y. R.; Sethunathan, N. *Pestic. Sci.* **1978**, *9*, 155.
Ridge, E. H.; Rovira, A. D. *New Phytol.* **1971**, *70*, 1017.
Walsh, L. A.; Peterson, A.; Keeney, D. *Res. Rep.—Univ. Wis., Coll. Agric. Life Sci., Res. Div.* **1976**, *R2779*.

Received for review February 25, 1982. Accepted May 19, 1982. This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, WI, and by a grant from the Agricultural Research and Development Fund-BARD (I-282-80). Contribution by project 1387, "Environmental Implications and Interactions of Pesticide Usage", from the Wisconsin Agricultural Experiment Station as a collaborator under North Central Regional Cooperative Research Project NCR 124, entitled "Fate and Interactions of Soil Pesticides under Conservation Tillage".

Short-Term Fate of Mirex and 2,8-Dihydromirex in Rats

Janice E. Chambers,* Roger S. Case, Earl G. Alley, and James D. Yarbrough

Adult female and male rats were given a single oral dose of 38 μg of either [^{14}C]mirex or 2,8-dihydro-[^{14}C]mirex. Less than 0.6% of the mirex and 0.1% of the 2,8-dihydromirex doses were excreted in the urine during the 2-week period in either sex. During the first 2 days, fecal elimination of greater amounts of mirex in both sexes indicated that less mirex than 2,8-dihydromirex was absorbed. Fecal elimination between 3 and 14 days was about 3% and 1–2% of the mirex and 2,8-dihydromirex doses, respectively. Both compounds accumulated in the highest concentrations in fat. Concentrations of radioactivity in a number of tissues declined between 1 and 2 weeks. The patterns of distribution and excretion of mirex and 2,8-dihydromirex were very similar, indicating that the latter, a dechlorinated derivative of mirex, is no more readily eliminated than is the parent compound.

Mirex is a chemically stable compound composed of a polycyclic carbon skeleton with all available valences occupied by chlorine atoms. It is extremely refractory to biodegradation but is degraded slowly photochemically to hydrogen and/or oxygen-substituted derivatives (Ivie et al., 1974a). Some of these photodecomposition products are more polar and more water soluble than mirex and are potentially more susceptible to biotransformation and excretion (Ivie et al., 1974c).

Mirex is extremely lipophilic and resistant to metabolism; therefore, it partitions readily into animal fat and has a high potential for bioaccumulation. The disposition and excretion of mirex have been studied in the laboratory rat, Japanese quail, mosquitofish, rhesus monkey, goat, and cow (Gibson et al., 1972; Mehendale et al., 1972; Dorough and Ivie, 1974; Ivie et al., 1974b,c; Pittman et al., 1976; Smrek et al., 1978). In these studies, mirex was retained for long periods in body fat, and it was excreted at low rates in the feces and in only trace amounts in the urine.

Department of Biological Sciences and Mississippi State Chemical Laboratory, Mississippi State University, Mississippi State, Mississippi 39762.

0021-8561/82/1430-0878$01.25/0