Persistence of Mirex and Its Effects on Soil Microorganisms

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Up to 1 g of mirex per 50 g of soil had no demonstrable effects on total populations of soil fungi or bacteria. The 0.5- and 1.0-g concentrations significantly reduced populations of actinomycetes in one of three soils. A variety of soil fungi and a few bacteria colonized mirex bait but did not degrade the mirex. No degradation of mirex occurred in nine aerobic soils or four anaerobic lake sediments after 6-months incubation, or in liquid cultures of bacteria after 1 month. ^{14}C-Labeled mirex in bait placed outdoors for 6 months remained primarily in the bait particles; only 6.6% was found in the top 15 mm of soil. No mirex leached through the 100 × 110 × 45 mm soil layer with approximately 3 l. of collected rainfall.

Although mirex [dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta(*cd*)pentalene] has been the standard insecticide for control of the imported fire ant (*Solenopsis richteri* Forel, *S. invicta* Buren) since 1963, relatively little is known about the effects of this insecticide on soil microorganisms or about the effects of the microorganisms on it. Mehendale *et al.* (1972) reported that orally administered mirex was found in organs and tissues of white mice, but that it was not metabolized. They also reported that pea and bean plants took up mirex in their roots and translocated it to aerial parts, but again the insecticide did not appear to be metabolized. Gibson *et al.* (1972) reported photodecomposition of mirex after outdoor exposure for up to 3 months on thin layers of silica gel. The photoproduct comprised only 5% of the total persisting residue and behaved similarly to mirex in rats.

The study reported here was initiated to determine: (1) the effect of mirex on soil microbial populations, (2) the ability of microorganisms to colonize mirex bait, (3) possible microbial degradation of mirex in bait preparations, (4) the ability of microorganisms in soils and lake bottom sediments to degrade mirex under aerobic and anaerobic conditions, and (5) the movement of mirex from the bait into soil under natural conditions.

EXPERIMENTAL SECTION

Chemicals. Uniformly labeled mirex-^{14}C (sp act. 1.74 and 5.76 mCi/mmol) was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Unlabeled mirex (analytical and 98% technical), prepared bait, and the materials necessary for preparing bait were supplied by Allied Chemical Corp., Baltimore, Md.

Mirex-^{14}C labeled bait was prepared by mixing 12.75 g of corncob grits with 2.25 g of soybean oil containing 0.045 g of unlabeled mirex and 0.15 mg of mirex-^{14}C.

Soils. Study soils (Table I) were selected to provide a wide range of textures, organic matter contents, and vegetative covers, and, thus, a wide variation in microorganisms. Sediment was collected from the beds of four lakes in the Research Triangle Park, N. C., area.

The water-holding capacity of each soil was determined on a pressure plate at 0.2 atm (approximately field capacity).

Influence of Mirex on Soil Microorganisms. Flasks were prepared from soils 2, 3, and 5 by mixing 50 g of air-dried soil with 0.0, 0.01, 0.1, 0.5, or 1.0 g of technical mirex, and adding distilled water to bring the soils to approximately field moisture capacity. Each soil–mirex combination was replicated three times.

After incubation for 2 months at 25°, 200 ml of 0.1% water agar was added to each flask and the soil suspension stirred for 15 min with a magnetic stirrer. Duplicate series of dilutions from each soil sample were made in 0.1% water agar. Final dilutions of 1:5000 were made for fungi and 1:50,000 for bacteria and actinomycetes from each of the two subsamples. For enumeration of fungi, a 5-ml aliquot of 1:5000 dilution was placed in 100 ml of cooled but still liquid Martin's Rose Bengal–streptomycin medium (Martin, 1950), mixed, and poured in approximately equal quantities into five sterile petri dishes. A 1-ml aliquot from the 1:50,000 dilution was spread over the surface of hardened Thornton's medium (Thornton, 1922) for enumeration of bacteria and actinomycetes. Five replicate plates were made for each sample.

Total colony counts were made after 7 days incubation at room temperature. No attempt was made to identify all species of the microorganisms, but frequently occurring species of fungi were identified. Effects of mirex concentration on fungi, bacteria, and actinomycetes were determined by linear regression analysis.

Colonization of Mirex Bait by Soil Microorganisms. Duplicate petri plates were filled with soil (Table I) and moistened to about field capacity. Approximately 100 pieces of mirex bait sterilized with ethylene oxide were placed on each soil surface. After 3 days, 1 month, and 2 months, 25 pieces of bait were removed from each plate, surface sterilized in 5% hydrogen peroxide, and placed on malt extract agar. After incubation for 3–7 days, organisms growing from the bait were transferred for identification. The plates were also examined at least once a week at 40× magnification. Fungi sporulating on the individual bait pieces were noted and, when possible, isolated in pure culture.

Effect of Microorganisms on Mirex. *Soils and Sediments.* Flasks were prepared for each soil by mixing 0.5 g of mirex (98% technical) with 100 g of air-dried soil. Distilled water was added initially and after 3 months to bring each soil to approximately field capacity. Control flasks were autoclaved at 121° for 30 min, and all flasks were held at room temperature. Autoclaved soils were analyzed for mirex after 3 months, and nonsterile soils were analyzed after 3 and 6 months. Each 100-g soil sample was mechanically shaken for 2 hr with 300 ml of benzene–isopropyl alcohol (2:1). The extract was filtered and concentrated under an air stream.

To study degradation of mirex in lake sediments, 100 g of each sediment was air-dried, mixed with 0.5 g of technical mirex, and placed into 8-oz French square bottles. Lake water was added to cover the top of the sediment 125 mm. Nitrogen was bubbled into the water for 1 min to remove most of the oxygen. Then the bottles were tightly capped and held at room temperature. Duplicate samples from each lake bed were analyzed for mirex after 4 and 6 months. The aqueous phase was decanted from each 100-g sediment sample and extracted twice with an equal volume of hexane. The sediments were then extracted as de-

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Table I. Descriptions of Soils and Sediments Used in Various Experiments

Soil no.	Mechanical anal., % Sand	Silt	Clay	Org matter, %	Location	Vegetative cover
1	69	22	9	2.61	RTP, N. C.[a]	Soybeans
2	82	15	3	0.98	Conway, S. C.	Bermuda grass
3	92	7	1	4.94	Conway, S. C.	Pine[b]
4	59	25	16	0.75	RTP, N. C.	Millet
5	87	10	3	0.63	RTP, N. C.	Fescue grass
6	87	8	5	1.32	Conway, S. C.	Bermuda grass
7	93	6	1	3.65	Conway, S. C.	Pine
8	91	8	1	0.97	RTP, N. C.	Tobacco
9	66	29	5		RTP, N. C.	Mixed grasses
Sediment no.						
1	87	10	3	0.55	RTP, N. C.	
2	83	12	5	2.12	RTP, N. C.	
3	79	16	5	2.60	RTP, N. C.	
4	38	33	29	1.20	RTP, N. C.	

[a] Research Triangle Park, N. C. [b] Soil collected from within abandoned fire ant nest.

scribed for soils, and the organic solvent fractions combined and concentrated.

Mirex Bait. Fifteen grams of unlabeled mirex bait or bait containing labeled mirex-^{14}C was placed in 125-ml flasks. Ten milliliters of malt extract was added to moisten the bait and to enhance the growth of the microorganisms. The bait was then autoclaved for 15 min at 121° and inoculated with microorganisms which were either isolated directly from soil or trapped from soil using standard mirex bait. The unlabeled bait was inoculated with *Absidia orchidis, Aspergillus cervinus, Chaetomium fusisporus, Cunninghamella elegans, Gliocladium catenulatum, G. virens, Penicillium* sp., *Rhizopus arrhizus, Streptomyces* sp., and *Trichoderma harzianum.* ^{14}C-Labeled bait was inoculated with *Bacillus* sp., *Cunninghamella elegans, Fusarium oxysporum, Gliocladium virens, Gongronella butleri, Minimedusa polyspora, Mucor* spp. (2), *Rhizoctonia* sp., *Trichoderma hammatum, T. harzianum,* and a reproductively sterile fungus.

The unlabeled bait was incubated 1 month and the ^{14}C-labeled bait was incubated for 6 months at room temperature. For analysis each 15-g bait sample was mixed with 150 ml of benzene–isopropyl alcohol (2:1) and allowed to stand for 1 hr with occasional stirring. The solvent was decanted and an additional 150 ml was added to the bait and allowed to stand overnight. The samples were filtered and the combined solvent fractions concentrated.

Liquid Cultures. Flasks were prepared by adding approximately 1×10^6 cpm of mirex-^{14}C to 100 ml of the following medium: 0.5 g of K_2HPO_4, 0.5 g of KNO_3, 0.2 g of $FeCl_3$, and 0.5 g of yeast extract per liter of H_2O (pH 6.5). The flasks were autoclaved and inoculated with 13 bacterial isolates (mostly *Bacillus* spp.) obtained from mirex bait incubated for 2 months on moist soil. After incubation for 2 and 4 weeks at 25°, the cultures were analyzed for mirex. Each 100-ml sample was extracted twice with an equal volume of hexane.

Leaching Studies with Mirex Bait. Fifteen grams of ^{14}C-labeled mirex bait was placed on the surface of a soil column 45 mm deep in a plastic box 100 × 110 mm containing a drainage tube connected to a 1-l. reservoir. Three boxes were prepared and placed outside during January and February. The rainwater which leached through the bait and soil was collected, measured, concentrated, and checked for radioactivity. After 2 months, the bait was removed from the surface of the soil and extracted twice with benzene–isopropyl alcohol (2:1), and the mirex content was determined. The soil column was carefully removed from the boxes and the edges trimmed away with a spatula. The remaining column was then divided into three 15-mm layers. Each layer and the edges were then extracted twice with benzene–isopropyl alcohol (1:3 w/v) and the amount of mirex determined.

Three additional soil columns were similarly prepared except that only 8 g of ^{14}C-labeled bait was placed on the surface. These columns were placed outside during May and remained until November. The soil columns and bait were analyzed for mirex as above.

Analytical Procedure. Gas–liquid chromatography (glc) was performed on the crude extracts described above, utilizing a Hewlett-Packard Model 7620A gas chromatograph equipped with a flame ionization detector and $\frac{1}{8}$ in. × 6 ft stainless steel columns packed with 2% OV-17 on Gas Chrom Q (80–100 mesh), obtained from Applied Science Laboratories, State College, Pa., and 2% stabilized DEGS on Anakrom ABS (90–100 mesh), obtained from Analabs, Inc., New Haven, Conn. With the OV-17 columns, the operating conditions were: injector and detector temperature of 300° and oven temperature programmed from 170 to 270° at 6°/min. For the DEGS columns, the injector temperature was 230°, the detector temperature was 240°, and the oven temperature was programmed from 120 to 230° at 6°/min. The carrier gas flow rate was 30 ml/min in both systems.

Thin-layer chromatography (tlc) plates with 0.25-mm layers of silica gel GF_{254} (Brinkmann Instrument Co., Westbury, N. Y.) were prepared and conditioned at 110° for 1 hr prior to use. The chromatograms were developed in heptane and visualized with Rhodamine B and 10% Na_2CO_3 (Stahl, 1969). The identity of mirex was confirmed by cochromatography with analytical mirex.

^{14}C-Labeled spots on chromatograms of bait extracts were located by autoradiography with Kodak No-Screen X-ray film.

Quantitation and Ir Spectra. The crude extracts were cleaned-up by elution through a 2 × 10 cm silicic acid column with hexane. Recovery of unlabeled mirex from the soil samples was determined by weight. Recovery of mirex-^{14}C was quantitated in a Beckman LS-100 liquid scintillation counter (lsc), using a toluene–Triton X-100 cocktail with PBD and PBBO as fluors.

Ir spectra were obtained with a Beckman IR 8 spectrophotometer. Samples were analyzed as 10% solutions in CS_2, following silicic acid column clean-up of the crude extracts.

RESULTS AND DISCUSSION

Influence of Mirex on Soil Microorganisms. None of the concentrations of mirex added to the three soils significantly affected the total populations of fungi or bacteria (Table II). The only evidence that high concentrations of mirex might cause some shift in fungal populations was a large increase of *Gliocladium catenulatum* with a concom-

Table II. Influence of Mirex on Soil Microbial Populations

Soil no.	Concentration of mirex[a] 0.0	0.01	0.1	0.5	1.0	p[b]
			Fungi[c]			
2	495 ± 48	645 ± 104	485 ± 46	553 ± 64	692 ± 110	0.0590
5	408 ± 26	430 ± 49	428 ± 60	422 ± 35	433 ± 84	0.7412
3	632 ± 74	913 ± 85	885 ± 148	972 ± 64	535 ± 13	0.1199
			Bacteria[c]			
2	6800 ± 1658	7216 ± 486	6867 ± 1025	6950 ± 377	7917 ± 1901	0.2733
5	6533 ± 1229	7400 ± 477	7333 ± 840	5500 ± 265	7583 ± 1666	0.8929
3	4883 ± 1235	5933 ± 633	5450 ± 132	6267 ± 2894	5950 ± 1203	0.5183
			Actinomycetes[c]			
2	1117 ± 29	1150 ± 229	1250 ± 529	883 ± 236	617 ± 333	0.0108[d]
5	1317 ± 375	2017 ± 473	1767 ± 275	1267 ± 202	1117 ± 597	0.0523
3	4867 ± 351	6867 ± 465	6700 ± 709	6450 ± 563	6667 ± 2801	0.5139

[a] In grams per 50 g of soil. [b] p values from linear regression analysis of concentration of mirex *vs.* numbers of fungi, bacteria, and actinomycetes in each soil type. [c] In thousands per gram of soil. Mean ± standard deviation for three replicates. [d] Significant only at 5% level.

itant decrease in other species of fungi in two of the three replications of the 1.0-g mirex level in soil no. 2. No attempt was made to determine the effect of mirex on species diversity in the bacterial or actinomycetes populations. Mirex did affect total actinomycete populations in soil no. 2, however. Applications of 0.5 and 1.0 g of mirex per 50 g of soil decreased numbers of actinomycete colonies, while applications of 0.01 g appeared to increase actinomycete numbers.

It is improbable that mirex concentrations applied to the soil to control fire ants would influence total populations of fungi, bacteria, and actinomycetes. The bait formulation currently being used contains 0.3% mirex and is applied at the rate of 1.7 g of mirex per acre. However, the foraging activity of the fire ant which concentrates the mirex bait in the mound areas might result in localized levels of mirex high enough to influence actinomycete populations in pasture soils.

The two pasture soils were similar in total populations of the three groups of microorganisms as well as in fungal species present. The forest soil had much higher populations of actinomycetes, slightly higher populations of fungi, and slightly lower populations of bacteria than the pasture soils.

Colonization of Mirex Bait by Soil Microorganisms. When mirex bait was applied to the soil surface, the individual pieces of bait were rapidly colonized by a wide variety of soil-inhabiting microorganisms (Table III).

Table III. Fungi Isolated from Mirex Bait Incubated on Moist Soil

Aspergillus niger
Aspergillus ustus
Chaetoceratostoma longicolleum
Chaetomidium sp.
Chaetomium fusisporus
Chaetomium globosum
Chaetomium spp. (4)
Chloridium chlamydosporis
Cochliobolus spicifer (conidial)
Cunninghamella echinulata
Cunninghamella elegans
Curvularia protuberata
Fusarium sp.
Gelasinospora cerealis
Gliocephalotrichum simplex
Gliocladium catenulatum
Gliocladium roseum
Gliocladium virens
Melanospora ornata
Minimedusa polyspora
Minimedusa sp.
Myrothecium roridum
Myrothecium verrucaria
Myrothecium spp. (2)
Mucor spp. (2)
Paecilomyces elegans
Penicillium funiculosum
Penicillium multicolor
Phialocephala sp.
Phialomyces macrosporus
Rhinocladiella sp.
Rhizoctonia sp.
Rhizopus arrhizus
Stachybotrys atra
Staphylotrichum coccosporum
Talaromyces luteum
Thozetella cristata
Trichoderma hammatum
Trichoderma harzianum
Trichoderma koningii
Verticillium cinnarbarinus
Zygorhynchus macrocarpa
Zygorhynchus moelleri

Three days after it was placed on the soil, the bait was colonized by a small but similar group of fungi on all nine soils tested. The most prevalent were *Cunninghamella elegans* and *C. echinulata*, which in addition to being isolated from surface-sterilized bait were sporulating profusely on individual bait pieces. *Trichoderma harzianum*, *T. hammatum*, and *T. koningii* were also commonly isolated from baits on all soils. *Rhizopus arrhizus*, *Zygorhynchus moelleri*, and *Mucor* spp. were isolated less commonly but were found on bait from most soils. No bacteria or actinomycetes were isolated from bait after 3 days of incubation.

The fungi listed above were also commonly isolated from surface-sterilized bait after 1 and 2 months incubation, but with the exception of *Trichoderma* spp. they were no longer sporulating on the surface of the bait. The only other fungus isolated from bait on all soils was *Gliocladium virens*. *Minimedusa polyspora* (=*Papulospora polyspora*) was commonly isolated from three soils. *Penicillium funiculosum*, *P. multicolor*, *Gliocephalotrichum simplex*, *Paecilomyces elegans*, and *Chaetomium globosum* were occasionally isolated from bait placed on one or more soils.

Bacteria (*Bacillus* sp.) were isolated from only two pieces of bait from two soils after 1 month. At 2 months, bacteria were occasionally isolated from five of the nine soils. At least four species, three of which were species of *Bacillus*, were present. No actinomycetes were isolated at any time, nor were they observed sporulating on the bait.

A large number of fungi, many of which were not isolated from surface-sterilized bait, sporulated on the surface of the bait during the study. *Cunninghamella* spp. sporulated first. *Chaetomium globosum*, *C. fusiforme*, four unidentified species of *Chaetomium*, *Paecilomyces elegans*, *Minimedusa polyspora*, *Chloridium chlamydosporis*, *Penicillium funiculosum*, *Gliocladium roseum*, *Chrysosporium pannacum*, *Melanospora ornata*, and several species of *Myrothecium* sporulated, sometimes profusely, on bait pieces on most of the soils. Many other fungi found less frequently are listed in Table III.

With the exception of *Trichoderma* spp., most of the early colonizers of the bait are Phycomycetes. Many members of this group of fungi readily utilize oils as carbon sources, and they are probably responsible for early breakdown of oil which renders the bait nonattractive to the ants after a few days. Many of the other fungi are common inhabitants of organic debris and are probably responsible for the eventual decomposition of the cracked corncobs.

The low numbers of bacteria found on bait could be due to competition with the fungi, or the unavailability of adequate nutrients from the oil or corncob, or a combination of both. The fact that nine bacteria isolated from the soil

Table IV. Recovery of Mirex from Soils and Sediments (%)[a]

	Months	Soil no. 1	2	3	4	5	6	7	8	9
Autoclaved (control)	3	98	98	98	95	96	98	98	96	92
Nonsterile	3	95	99	96	94	96	97	95	99	88
	6	98	93	95	95	95	93	93	95	99

	Sediment no. 1	2	3	4
4	98	99	96	96
6	97	98	97	92

[a] Per cent of original weight. No correction was made for extractable residues from untreated soils.

Table V. Recovery of Mirex-^{14}C from Bait Cultures (%)[a]

Microorganism	Extract	Bait residue
Bacillus sp.[b]	97.6	2.4
Cunninghamella elegans	99.5	0.5
Fusarium oxysporum	99.9	0.1
Gliocladium virens	99.7	0.3
Gongronella butleri	99.6	0.4
Minimedusa polyspora	99.9	0.1
Mucor sp.	99.4	0.6
Mucor sp.	99.3	0.7
Rhizoctonia sp.	99.2	0.8
Trichoderma hammatum	99.8	0.2
Trichoderma harzianum	99.7	0.3
Sterile fungus	99.9	0.1

[a] Per cent of recovered radioactivity after 6-months incubation. [b] One-month incubation.

Table VI. Recovery of Mirex-^{14}C from Bacterial Cultures (%)[a]

Culture no.	2 weeks Hexane	2 weeks H_2O	4 weeks Hexane	4 weeks H_2O
1	99.3	0.7	99.5	0.5
2	99.6	0.4	99.3	0.7
3	98.3	1.7	99.3	0.7
4	98.7	1.3		
5	97.6	2.4	99.3	0.7
6	97.9	2.1	99.7	0.3
7	99.0	1.0	99.7	0.3
8	98.6	1.4	98.9	1.1
9	98.9	1.1	97.1	2.9
10	98.9	1.1	98.6	1.4
11	98.7	1.3	98.4	1.6
12	99.2	0.8		
13	98.4	1.6	98.4	1.6
Control	99.2	0.8	99.8	0.2

[a] Per cent of recovered radioactivity.

Table VII. Recovery of ^{14}C-Labeled Mirex from Leached Bait after 2 and 6 Months (%)[a]

Sample	2 months	6 months
Bait	93.20	88.48
Upper 15 mm	0.85	6.56
Middle 15 mm	0.01	Negl.
Lower 15 mm	Negl.	Negl.
Edge	0.20	2.37
Total recovery	94.26	97.41

[a] Per cent of applied radioactivity. Average of three replicates.

grew poorly or not at all when inoculated on sterile bait tends to reinforce the possibility that the bait was not an adequate growth medium. Because high concentrations of mirex in soil had no adverse effect on bacterial populations, it is unlikely that the mirex in the bait inhibited bacterial growth.

The absence of actinomycetes supports the previous finding that mirex in high concentrations may adversely affect actinomycetes.

Effect of Microorganisms on Mirex. *Soils and Sediments.* No degradation of mirex had occurred in the soils or sediments after 6-months incubation. Gas chromatographic analysis of the crude extracts showed a single, symmetrical peak, with the same retention time as mirex on two different columns. Tlc gave a single spot which had the same R_f as mirex, and ir spectra were identical with analytical mirex. With the soils, average recovery from the autoclaved controls was 96.6%, and for the nonsterile flasks 95.4 and 95.1% for 3- and 6-months incubation, respectively. Recovery from sediments averaged 97% after 4 months and 96% after 6 months (Table IV).

Mirex Bait. Chromatographic analysis of the unlabeled bait cultures after 1 month incubation indicated that no degradation of mirex had occurred, although the soybean oil triglycerides had been almost completely hydrolyzed. Identity of mirex in these samples was confirmed by ir spectroscopy.

After 6-months incubation, the cultures on mirex-^{14}C labeled bait showed no degradation of mirex. Autoradiography of tlc plates showed a single ^{14}C-labeled spot corresponding to mirex. Ir spectroscopy of the material from silicic acid column-cleanup confirmed the identity.

Extraction efficiency from the bait immediately after preparation was approximately 90% of the theoretical applied dose. Quantities of mirex were calculated on the basis of recovered rather than applied radioactivity. Aliquots of the extracts and 1-g samples of the bait residue were counted, and with the exception of the *Bacillus* culture, more than 99% of the radioactivity was present as mirex (Table V). The residue from the *Bacillus* culture contained 2.4% radioactivity, but reextraction yielded only mirex.

Liquid Cultures. No degradation of mirex occurred during 2 or 4 weeks of incubation with bacteria. About 99% of the radioactivity was in the organic phase (Table VI). Subsequent autoradiography of both aqueous and organic phases indicated the presence of only mirex. Identity was confirmed by glc and ir.

These studies constituted a survey of soil microbial populations which might come into contact with mirex. Although a microorganism capable of degrading mirex may be isolated or developed by adaptation, it is unlikely that microbial action will be important in the removal of mirex from the environment. The fact that mirex appeared to be innocuous to microorganisms also suggests that it will probably not exert enough pressure on microbial populations to enhance development of the capability to degrade it.

Leaching Studies with Mirex Bait. The recovery of mirex in bait and individual soil layers is shown in Table VII. Even after 6 months, most of the mirex was still present in the bait. The individual bait particles were partially decomposed; however, most still held their structural integrity. It is likely that much of the activity measured in the upper soil layer after 6 months came from decomposed bait that could not be separated from soil since only negligible activity was detected in the middle and lower soil layers. That the mirex is tightly adsorbed to the bait is shown by the fact that almost 3 l. of rainwater leached

through the bait and soil during the 6-month period and no trace of radioactivity could be detected in the leachate. Thin-layer chromatography and autoradiography of the extracts from weathered bait and soil showed all radioactivity to be in a single spot corresponding to mirex. There was no evidence of mirex degradation.

These field studies support the conclusion drawn in laboratory tests that mirex is not degraded by soil microorganisms. Under field conditions, mirex probably remains in unconsumed bait on the soil surface until the bait is completely decomposed. Decomposed bait and mirex are probably incorporated into the soil slowly. Because of the small amount of mirex applied to the soil and its low solubility in water, it is doubtful that significant amounts of mirex leach into ground water. Transportation of bait particles by surface runoff is a more likely source of stream and lake contamination.

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Kinetics of Azinphosmethyl Losses in the Soil Environment

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The kinetics of Azinphosmethyl losses in sterile and nonsterile soil as affected by temperature and moisture content was studied. The fate of Azinphosmethyl in soil involved two steps: one after application when the initial concentration remained constant and the second when the disappearance starts and the Azinphosmethyl losses follow first-order kinetics. It was found that the "lag period" before Azinphosmethyl losses begin is more than a biological effect. The water and temperature affect both the lag period and the rate of disappearance.

One of the pesticides commonly used in irrigated agriculture of arid and semiarid regions is the Azinphosmethyl (*O,O*-dimethyl *S*-[4-oxo-1,2,3-benzotriazin-3(4*H*)-yl methyl] phosphorodithioate) also called Guthion or Gusathion. Studies on the persistence and degradation of Azinphosmethyl *in vitro* dealt with chemical decomposition of this pesticide in an organic solvent system (Harvey *et al.*, 1969) and in an aqueous solvent system (Liang and Lichtenstein, 1972). In soils the persistence of Azinphosmethyl was studied only with respect to the formulation and mode of application (Schultz *et al.*, 1970).

The aim of the present research was to isolate three environmental factors (biological, temperature, and moisture) important in the irrigated field and to establish their contribution to the disappearance of Azinphosmethyl in the soil.

EXPERIMENTAL SECTION

Soil. The soil used in the experiment was a silty loamy loessial sierozem (Haplargid) (from the Gilat Regional Experiment Station) with an organic matter content of less than 1%, pH 8.4, and a cation exchange capacity of 13.4 mequiv/100 g.

Sterile soil was obtained by irradiating subsequent samples of the initial soil in a JS-6000 irradiator provided with a ^{60}Co radiation source. The radiation dose was 3 Mrads.

Chemicals. Pure Azinphosmethyl has been synthesized in our laboratory from the commercially available methyl anthranilate in an overall yield of 35%. The intermediate and final products were identified by nmr spectroscopy. In the nmr spectrum (recorded with a Varian A-60 spectrometer in deuteriochloroform solution with tetramethylsilane as internal standard), the crystallized product displayed three sets of signals: doublet at τ 6.20 (J_{PCH3} = 15.5 Hz) for the methoxy group protons, doublet at τ 4.18 (J_{PCH} = 16 Hz) for the methylene protons, and multiplet at τ 1.9 for the aromatic protons.

Analytical Methods. Azinphosmethyl in soil was extracted by mechanically shaking with a 1:2 mixture of chloroform–methanol (9:1) and water in a soil:solvent ratio of 1.1. The soil was separated from the solvents by centrifugation (4000 rpm) and the aqueous phase was separated from the organic solvent in a separatory funnel. Preliminary tests showed that by this procedure a 100% recovery is obtained. A Packard Model 873 gas chromatograph with a flame ionization detector was used. The conditions for glc were: glass column, 180 cm × 3 mm i.d. filled with 10% SE-30 on Gas Chromosorb Q, 80–100 mesh; inlet and column temperature, 245°; detector temperature, 200°; nitrogen carrier gas flow, 60–70 ml/min. Azinphosmethyl solution (2 μl) at a concentration of 50 ppm in chloroform was injected as a standard after each two injections of sample. Peak height was used for quantitation.

Procedure. Two grams of soil (air dried and passed through a 60-mesh sieve), sterile and natural, respectively, were shaken with 2 cm^3 of an Azinphosmethyl solution of 50 ppm in chloroform for 30 min. Preliminary tests showed that the soil population was not affected by the treatment with chloroform. The solvent was then evaporated and 1 cm^3 of water (50% moisture content equivalent to the soil-saturated paste value) was added to half of the samples. Samples were incubated at three temperatures, 6, 25, and 40°, and analyzed at different intervals for up to 2 months. The entire experiment was conducted in duplicate. The bottles were sealed with paraffin in

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