Fate of Mirex and Its Major Photodecomposition Product in Rats

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Rats receiving single oral doses of radiolabeled mirex (0.5 μCi, 0.2 mg/kg) eliminated about 18% of the administered radiocarbon during a 7-day period. Of the total quantity eliminated, 85% was excreted in the feces within 48 hr after administration. Only trace amounts of radioactivity were detected in the urine. Essentially all of the excreted radioactivity was as unmetabolized mirex. Prefeeding rats with unlabeled mirex resulted in a slight decrease in intestinal absorption of a single oral dose of the ^{14}C material. A major mirex photoproduct was prepared, and its chemical nature and fate in rats were evaluated. This product exhibited almost identical behavior to mirex after oral administration to rats, in that it was not metabolized by the animal to any appreciable extent. Both mirex and its photoproduct showed strong lipophilic tendencies, with concentrations of mirex equivalents in fat 7 days after treatment being 0.9 and 1.1 ppm, respectively. Mirex levels in fat did not decline below the 7-day level when the experimental period was extended to 28 days.

The introduction of persistent pesticides into the environment without extensive study could result in needless environmental hazards and possibly serve to enhance any existing crises. To minimize such occurrences it is essential that the toxicity, tissue accumulation, metabolism, and excretion of both the parent compound and its environmental derivatives by nontarget species be investigated.

Mirex (1,3,4-methenododecachlorooctahydro-2*H*-cyclobuta[*c*,*d*]pentalene) is widely used for control of the imported fire ant (*Solenopsis richteri* Forel, *S. invicta* Buren) in the Southeastern United States. However, only limited data are available relative to the accumulation, tissue distribution, metabolism, and excretion of mirex in nontarget species.

Mirex is relatively nontoxic to vertebrates (Dewitt *et al.*, 1964; Kenaga and Allison, 1969), but crustaceans are particularly susceptible to this compound. Ludke *et al.* (1971) have shown mirex to be toxic to crayfish at concentrations in water as low as 0.1 ppb. Several other crustaceans, including juvenile blue crabs, juvenile pink shrimp, grass shrimp, fiddler crabs, and penaeid shrimp also are susceptible to mirex (Lowe *et al.*, 1971). Van Valin *et al.* (1968) found that while mirex was essentially nontoxic to bluegill sunfish and goldfish, it did cause gill and kidney lesions at high concentrations. They also reported that absorbed mirex was highly resistant to removal from or degradation by the tissues, the level of residues remaining relatively constant for as long as 300 days after the fish were removed from the pesticide source. Pinfish were reported to accumulate considerable body burdens when fed mirex treated food (Lowe *et al.*, 1971).

Mirex in the diet of laying hens at 600 ppm did not reduce egg production, but hatchability and survival rate of chicks were significantly lowered (Naber and Ware, 1965). Swiss mice of the BALB/c strain fed 5 ppm of mirex in their diets for 120 days experienced high mortality rates and low fertility and fecundity (Ware and Good, 1967). An adverse effect on reproduction was also noted under similar conditions for the CFW strain of Swiss mice, but with no increase in parent mortality. Sherman rats fed diets containing 25 ppm of mirex for as little as 45 days had fewer offspring born alive, fewer offspring survived to weaning, and many pups developed cataracts (Gaines and Kimbrough, 1970). Analyses of milk and fetuses showed excretion of mirex in the milk and passage through the placental barrier. The livers of mirex-treated rats weighed more than those of controls, and electron microscopic examination of the liver cells showed a proliferation of smooth endoplasmic reticulum.

This study was undertaken to examine the absorption, tissue distribution, and excretion of radiocarbon after oral administration of mirex-^{14}C to rats. Studies involving the preparation, chemical nature, and fate in rats of a major mirex photodecomposition product also are described.

MATERIALS AND METHODS

Chemicals. Technical and analytical samples of mirex were supplied by Allied Chemical Corp., Baltimore, Md. Mirex-^{14}C (6.34 mCi/mmol) was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. The unlabeled and labeled samples of mirex were chemically and radiochemically pure, as determined by tlc and glc analysis.

Analytical Procedures. Infrared (ir) spectra were recorded as potassium bromide pellets with a Beckman IR5A spectrophotometer. Mass spectra (ms) were determined on a Hitachi RMU-7 instrument, and proton magnetic resonance (pmr) spectra were taken in deuterated chloroform on a Varian T-60 spectrometer, using tetramethylsilane as an internal reference. The ms and pmr studies were conducted in the Department of Chemistry, University of Kentucky. Melting points were determined in sealed capillary tubes with an Electrothermal Melting Point Apparatus.

Tlc was accomplished on silica gel F_{254} precoated chromatoplates (0.25 mm gel thickness, Merck AG, Darmstadt, Germany), using the following four solvent systems: heptane; hexane–acetone, 4:1; cyclohexane; and cyclohexane–benzene, 4:1. Solvent system using heptane was generally employed for routine analyses. After development of the plates, visualization was afforded either by radioautography for radioactive products or by spraying the plates with diphenylamine reagent and exposing them to ultraviolet light for the unlabeled compounds (Ivie and Casida, 1971). Glc samples were analyzed on a Varian Aerograph Model 1400 equipped with an electron capture detector and 6 ft × 1/8 in. pyrex column. The column and operating parameters were either: 5% OV-210 on 100–120 mesh Chromosorb W-HP, injector temperature 205°, column 180°, detector 205°; or 4% SE-30 + 6% QF-1 on 100–120 mesh Chromosorb W-HP, in-

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Table I. Elimination of Radiocarbon in Urine and Feces of Rats Receiving Single Oral Doses of Mirex-^{14}C and Its Major Photodecomposition Product[a]

Days after administration	Percent elimination of administered radiocarbon			
	Mirex		Photoproduct	
	Urine	Feces	Urine	Feces
1	0.04	6.74	0.04	14.70
2	0.05	8.52	0.04	1.75
3	0.05	1.28	0.02	0.70
4	0.04	0.82	0.01	0.36
5–7	0.08	0.67	0.02	0.89
Total	0.26	18.03	0.13	18.40

[a] Doses = 0.5 μCi; ^{14}C = 0.2 mg/kg.

jector 210°, column 200°, detector 210°. In each case, the nitrogen flow rate was maintained at 60 ml/min.

Treatment of Animals. Radioactive compounds (0.5 μCi, 0.2 mg/kg dose) were administered to mature rats (Sprague–Dawley female) *via* a stomach tube in 0.5 ml of corn oil. The animals were held in stainless steel–glass metabolism cages for separation and collection of urine and feces. Food and water were provided *ad libitum*.

Extraction and Quantitation of Radiocarbon Residues. Urine was assayed directly by liquid scintillation counting; feces samples were mixed, and portions were air dried and then combusted in a Parr oxygen bomb. Aliquots of the carbon dioxide trap solution (2:1 2-methoxyethanol–2-aminoethanol) were analyzed by liquid scintillation counting. Remaining portions of urine and feces samples were frozen for later analysis. At appropriate time intervals after treatment the animals were sacrificed and tissue samples were collected and air dried for combustion.

Feces samples, up to 20 g, were homogenized for 10 min in four parts (w/v) of a 1:1 acetonitrile–water mixture, and the homogenate was then filtered under vacuum. The residue was extracted two additional times with acetonitrile, and then once with hexane. The extracts were combined in a separatory funnel, and sufficient sodium chloride was added (with shaking) to cause separation of the acetonitrile–water mixture into two phases. The funnel was then shaken vigorously, and the hexane layer was removed. The water–acetonitrile was partitioned three more times with 100-ml volumes of hexane, and the hexane extracts were combined. Aliquots of each of the three phases were radioassayed by liquid scintillation counting. The hexane extract, after concentration, was cleaned-up for glc and tlc analysis by eluting the compounds with hexane through a column containing 30 g of aluminum oxide (5% deactivated) above 30 g of PR grade Florisil. Portions of the extracted feces residue were combusted to quantitate the unextractable radiocarbon.

Fat samples were solubilized in hexane and cleaned-up using the Florisil–aluminum oxide column. The solutions were analyzed by glc and tlc as described above.

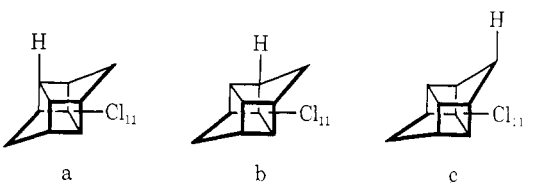
Preparation of a Major Mirex Photoproduct. Mirex-^{14}C was exposed in the environment for periods up to 3 months as deposits on thin layers of silica gel (4 μg, 0.7–0.9 cm diameter spots, Eastman Chromagram Sheet No. 6061). After exposure, the gel was extracted with methanol, and the extracts were analyzed by tlc and glc. Under these exposure conditions, mirex was converted to a product having a shorter glc retention time (column a: mirex 18.4 min; photoproduct 13.9 min), and lower R_f on tlc (solvent system using heptane: mirex 0.43; photoproduct 0.35). However, after 3 months exposure of mirex, this compound comprised only approximately 5% of the total persisting residue. Although both tlc and glc indicated that one or more other very minor mirex derivatives were produced, the remaining residue consisted almost exclusively of unchanged mirex.

The major mirex photoproduct was prepared in radioactive form by irradiating silica gel deposits of mirex-^{14}C with artificial light (0.05 μCi/cm^2, Westinghouse Fluorescent Sunlamp, double tubes, 7 cm lamp to subject, 48 hr). After exposure, the gel was extracted with hexane and the photoproduct-^{14}C (22%) was isolated by tlc. The compound prepared by artificial light exposure exhibited identical behavior on tlc and glc as the product obtained with environmental exposure of mirex. The derivative was prepared in milligram quantities by exposing unlabeled mirex to artificial light as described above (60 μg/cm^2, 7 day exposure). Isolation of the material on tlc and recrystallization twice from methanol gave white crystals, tlc and glc pure, mp >360°.

RESULTS AND DISCUSSION

Chemical Nature of Mirex Photoproduct. The ir spectrum of the photoproduct indicated the absence of unsaturation and of any apparent functional groups. Pmr showed a singlet centered at 3.79 ppm. The ms exhibited a molecular ion at *m/e* 506 (Cl = 35), indicating the compound to be a monohydro derivative, $C_{10}Cl_{11}H$. Earlier studies have shown that the predominant mode of fragmentation in the mirex series is cleavage of the pentacyclodecane nucleus in half, with subsequent loss of a chlorine atom (Dilling and Dilling, 1967). Thus, both mirex and its photoproduct give abundant ions at *m/e* 270 ($C_5Cl_6^+$) and *m/e* 235 ($C_5Cl_5^+$), but the product shows an additional fragment at *m/e* 236 ($C_5Cl_5H^+$).

The symmetry of the carbon nucleus of mirex permits only three isomeric monohydro derivatives:



a b c

The known **c** was prepared by lithiumaluminum hydride reduction of Kepone and subsequent chlorination of the alcohol with phosphorus pentachloride (Dilling *et al.*, 1967; Gilbert *et al.*, 1966), and found to differ from the photoproduct on the basis of tlc, glc, ir, and pmr. Thus, the mirex photoproduct is either **a** or **b**, although the data obtained in the current studies do not permit distinction between these two isomers. Dilling and Dilling (1967) have prepared a monohydro derivative from mirex (either **a** or **b**) by reaction with lithium, water, and dry ice. Dilling's compound probably differs from the mirex photoproduct, as evidenced by comparison of the reported ir absorption maxima of the lithium derivative with those of the photoproduct. The mirex photoproduct isolated in the current studies is probably the same compound observed by other workers after exposure of mirex to ultraviolet light in hydrocarbon solvents (Alley, 1972).

Fate of Mirex in Rats. Rats given a single oral dose of mirex-^{14}C excreted 18% of the total administered radioactivity during a 7-day period after treatment (Table I). Fecal elimination during the initial 48 hr accounted for about 85% of the total radiocarbon excreted and probably represents unabsorbed material. A virtual lack of urinary elimination of ^{14}C (0.3% of total dose) during the 7-day period, and fecal elimination of only 2% of the administered dose after the

Table II. Elimination of Radiocarbon in Urine and Feces of Rats Receiving a Single Oral Dose of Mirex-^{14}C[a] after the Animals were Fed 250 ppm of Unlabeled Mirex in the Food for 7 Days

Days after administration	Percent elimination of administered radiocarbon	
	Urine	Feces
1	0.03	12.84
2	0.01	7.18
3–7	<0.02	4.94
Total	~0.06	24.96

[a] Dose = 0.5 μCi of ^{14}C = 0.2 mg/kg.

Table III. Partitioning Characteristics and Chemical Nature of Radiocarbon Excreted in Feces of Rats Following Oral Treatment with Radiolabeled Mirex and Its Major Photodecomposition Product[a]

Days after treatment	Percent radiocarbon in indicated fraction			
	Water	Acetonitrile	Hexane[b]	Residue
Mirex				
1	<0.1	<0.1	98.4	1.6
2	<0.1	<0.1	98.1	1.9
3	<0.1	<0.1	95.1	4.9
4	<0.1	<0.1	95.2	4.8
5–7	<0.1	<0.1	94.7	5.3
Photoproduct				
1	0.6	<0.1	98.1	1.3
2	7.5	<0.1	83.6	8.9
3	4.5	<0.1	88.1	7.4
4	2.3	<0.1	95.2	3.5
5–7	14.9	<0.1	85.1	<0.1

[a] Dose = 0.5 μCi of ^{14}C = 0.2 mg/kg. [b] Tlc and glc analysis revealed that all hexane fractions contained only unaltered parent material.

initial 48 hr suggested that there was relatively little metabolic turnover of mirex in the rat.

Intestinal absorption of mirex was slightly decreased by the presence of an existing body burden (Table II). Rats fed 250 ppm of unlabeled mirex in food for 7 days prior to administration of a single dose of mirex-^{14}C eliminated 25% of administered radiocarbon as opposed to 18% for control animals given only a single radioactive dose (Table II).

Radiocarbon voided in the feces after treatment of rats with mirex consisted primarily of unaltered parent compound (Table III). The low levels of radioactivity remaining in the feces residue after extraction could consist of metabolic products; however, the unextractable radiocarbon may be comprised entirely or in part of bound mirex. Attempted extraction of the low levels of radiocarbon from the urine with a variety of organic solvents was unsuccessful.

Distribution and concentrations of radiocarbon in rat tissues resulting from a single oral dose of mirex-^{14}C substantiated previous indications that there was very little turnover of mirex in rats (Table IV). As with many other organochlorine pesticides, mirex demonstrated an affinity for lipids; and once absorbed into fatty tissue, the residue levels remained essentially unchanged for periods up to 28 days. Other tissue concentrations maximized within a few days and declined thereafter. Tlc and glc analysis revealed that the radiocarbon residues in the fat consisted entirely of unaltered mirex.

Table IV. Radiocarbon Residues in Tissues of Rats Receiving Single Oral Doses of Mirex-^{14}C and Its Major Photodecomposition Product[a]

Days after administration	Parts per million mirex equivalents				
	Brain	Fat	Kidney	Liver	Muscle
Mirex					
1	0.08	1.02	0.19	0.40	0.10
2	0.07	1.08	0.14	0.28	0.19
7	0.04	0.85	0.09	0.11	0.10
14	0.01	0.92	0.03	0.06	0.04
28	0.01	1.24	0.03	0.04	0.02
Photoproduct					
7	0.03	1.14	0.06	0.06	0.02

[a] Dose = 0.5 μCi of ^{14}C = 0.2 mg/kg.

Fate of Mirex Photoproduct in Rats. The mirex photoproduct-^{14}C behaved very much like mirex after oral administration to rats. Essentially no radiocarbon was excreted in the urine, while only a small portion of the dose was eliminated through the feces (Table I). Tlc and glc analysis indicated that the radiocarbon eliminated in the feces was primarily unaltered material (Table III). As with mirex, the absorbed photoproduct was highly lipophilic in nature (Table IV), and chromatographic analysis revealed that radiocarbon stored in the fat 7 days after treatment was entirely unmetabolized material. However, there was a somewhat greater amount of unextractable and water-soluble radiocarbon in the feces of rats treated with the photoproduct (Table III). This may indicate that the photoproduct is slightly susceptible to metabolic attack by the rats. Because of the slow conversion, however, it is unlikely that environmental conversion of mirex to this product would yield a more metabolically labile compound or act to lessen accumulation of residues in laboratory rats. Whether the same is true of other species, or of other photoproducts of mirex, must be determined by further experimentation.

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