Fate of Mirex-^{14}C in the Rat and Plants

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Due to its proposed use over a large portion of the Southeastern United States, the insecticide mirex has become the subject of much recent controversy (1). The application of the insecticide by airplane over such a large land area, and the fact that mirex has a completely, chlorinated cage-like carbon skeletal structure which is quite resistant to biological, chemical, photochemical and even pyrolytic degradation have been matters of concern. However, on the positive side, the incorporation of mirex into a bait which is uniquely specific for its target organism is an excellent example of using a pesticide in the most efficient manner.

Mirex has a low acute oral toxicity compared to other pesticides (2). However, chronic low doses of the pesticide resulted in parent mortality and reduced litter size in mice (3), decreased litter size and produced cataracts and proliferation of the liver endoplasmic reticulum in rats (4). The carcinogenicity of mirex in two strains of mice has been reported (5).

Mirex was reported to be a persistant residue in fish (2,6); however, goldfish were sensitive only to high levels, and bluegills were not affected by the pesticide (7). Among other aquatic organisms tested, the crayfish proved particularly sensitive to mirex and accumulated high levels (6). Residues in these studies were analyzed by GLC and no metabolites of mirex were detected.

Although present evidence points out that mirex is resistant to biological degradation, the evidence is only incidental, and a study of its metabolic fate in animals and plants has not been reported. This study was undertaken to determine the fate of a single dose of mirex such as might be encountered by an animal in the environment. This paper reports the routes and rates of excretion and amount and loci of storage in the bodies of a group of male rats fed a single dose of 6.0 mg/kg of mirex-^{14}C. The first half-life is reported and the second half-life is projected from the excretory pattern. In vitro studies with various liver and plant root preparations are also included in this report, as well as preliminary experiments on plant uptake of mirex at environmental levels.

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EXPERIMENTAL

Uniformly labeled mirex-^{14}C (sp. Act. 1.74 mCi/mM was obtained from Mallinckrodt Nuclear, St. Louis, Mo. Unlabeled technical mirex was obtained from City Chemical Corporation, New York, N. Y. Nictinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), NADPH, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), uridine diphosphoglucuronic acid (UDPGA), uridine diphosphoglucose (DUPG), adenosine triphosphate (ATP), uridine triphosphate (UTP) and adenosine phosphosulfate (APS) were obtained from Sigma Chemical Co., St. Louis, Mo.

In Vivo Metabolism: White rats (CD-1) obtained from Charles River Breeding Labs, Inc., Wilmington, Massachusetts were maintained in the animal rearing facilities until used. Five male rats weighing approximately 200 g each were administered mirex-^{14}C (6.0 mg/kg) in corn oil by oral intubation. Treated animals were maintained in metabolism cages (model E 110, Maryland Plastics, Inc.) and fed Wayne Lab Blox and water ad libitum for seven days. Feces and urine were collected daily and at the end of seven days the animals were sacrificed and the major organs and samples of various tissues were removed. Aliquots of urine (0.2 ml) were radio-assayed directly in a liquid scintillation counter (Model 3384, Packard Tri-Carb liquid scintillation spectrometer) using Bray's Cocktail. The daily samples of feces were dried, powdered, and 50 mg samples were oxidized using a Tri-Carb sample oxidizer (Packard Instrument Co., Downers Grove, Illinois) and the radioactivity counted. Samples (200 mg) of various organs and tissues were dried and oxidized and radioassayed similarly.

Extraction and Analysis: Pooled urine was extracted twice using a 3:2 mixture of hexane and isopropanol and the extracts were pooled, concentrated, and analyzed by thin layer chromatography using precoated Silica Gel G plates from Anal Tech Inc., Newark, Delaware. Feces and tissues were extracted thrice and the extracts were analyzed as described for urine. Tissues were homogenized using Virtis 45 homogenizer using the same solvent prior to extraction. One and two dimentional TLC was used for analyses of the extracts. Solvents used were: hexane:acetone (9:1); benzene and benzene:hexane (1:9). Radioactive spots were detected by exposing the developed plates to no-screen medical X-ray films (Eastman Kodak Co.).

In Vitro Metabolism: Mirex-^{14}C was incubated with rat, mouse and rabbit liver preparations with the following cofactors individually and in various combinations; NADP, NADPH, FAD, FMN, UDPGA, UDPG, ATP, UTP, and APS. Incubations were in Tris-KCl

(0.1 m, 7.4 pH) buffer at 37°C in 25 ml Erlenmeyer flasks in a metabolic shaker. Reactions were terminated after 1, 2, 8, 24 and 36 hours of incubation by adding 4.0 ml of the hexane-isopropanol extraction mixture. Extraction of the reactions, and analyses were carried out as described above for the urine.

Mirex-^{14}C was also incubated with bean (Phaseolus vulgaris, L., Dwarf Horticulture variety) and pea (Pisum sativum, L., Alaska Wilt Resistant variety) root preparations known to be biochemically active (8). These reactions were extracted and analyzed as described above.

Uptake in Plants: Mirex-^{14}C (5 x 10^5dpm) was dissolved in methylcellosolve. Additional unlabeled mirex was added to make a final concentration of 1, 5 and 10 ppm and 1% methylcellosolve in water. Two week old pea and bean plants were allowed to grow for 48 hours in water containing the indicated amounts of mirex-^{14}C. At the end of two days plants were removed from the water and the roots were rinsed well and stored (-20°C) until extracted. Roots and shoots were separated, weighed, homogenized and extracted with hexane-isopropanol as described above. Remaining water along with the rinse was extracted, radioassayed, and analyzed by TLC and radioautography.

RESULTS AND DISCUSSION

Excretion: Mirex was found to be excreted primarily in the feces (Fig. 1). Fifty-five percent of the administered dose of mirex was excreted in the feces within the first 48 hours after administration. This first rapid excretion in the feces most probably represents that portion of the insecticide dose which passed directly through the animal without being absorbed from the gut. The fact that total excretion levels off rapidly after 48 hours and that very little mirex is ever excreted in the urine indicate that once absorbed, the insecticide is readily stored in the body and slowly excreted. Thus, the first half-life for a small dose of mirex is approximately 38 hours whereas the second half-life for the same dose of mirex is in excess of 100 days and the third half-life is probably even longer. The results shown in Fig. 1 were obtained by administering a dose of 6 mg/kg to five animals. A similar study using 1.5 mg/kg yielded a similar excretory pattern. Other chlorinated pesticide compounds such as aldrin and its metabolite dieldrin are also excreted primarily in the feces; however, these compounds are excreted primarily as their metabolites (9,10). Extraction and the analysis of the radioactivity excreted in any of the daily samples of feces failed to yield a radioactive compound other than mirex.

Extraction of mirex in the urine accounted for less than one percent of the total dose in seven days. This slow rate of

excretion probably reflects the very low water solubility of mirex and the rate at which the absorbed portion of the insecticide dose was released. Extraction and analysis of the radioactivity excreted in the urine also failed to reveal the presence of any radioactive compound other than mirex.

Storage: Seven days after the administration of a single dose of mirex the tissues and organs retained approximately 34% of the total dose (Table 1). On a per gram wet weight basis, fat had the maximum (1.54%) followed by kidney, liver and small and large intestines. Estimated on the basis of 9% body fat (11) and 40% muscular tissue (12) these rats had 27.8% of the total dose of mirex stored in fat followed by 3.2% in the muscle. Each of the tissues was extracted and the extracts analyzed as described for the feces. No radioactive compound other than mirex was detected.

In spite of the relatively poor absorption of mirex from the gut, its extraordinarily long half-life in the tissues indicates that animals environmentally exposed, even at low doses, can be expected to concentrate high levels of this insecticide in their adipose tissue. Furthermore, since mirex is not degraded by either plants or animals, accumulated mirex can be expected to be recycled upon the death of the organism.

In Vitro Studies: Liver preparations known to dechlorinate chloroform (13), chloroethanes and chloropropanes (14) were incubated with mirex for up to 36 hours. Extraction and analysis of these incubations gave good recoveries of mirex but failed to indicate the presence of any dechlorinated metabolite of mirex. Mirex-^{14}C was also incubated with rat, mouse and rabbit liver preparations and various cofactors and combinations of cofactors for varying durations of time. These in vitro studies also failed to yield metabolites of mirex. Dechlorination of chloroalkanes (14) was dependent on the extent of chlorination and with a molecule such as mirex ($C_{10}Cl_{12}$) high resistance to enzymatic dechlorination might be expected.

Plant root preparations known to metabolize other chlorinated hydrocarbons (8) were incubated with mirex, but failed to yield a metabolite. These incubations were made, with and without added NADPH, and for periods up to 24 hours. All of the mirex-^{14}C was recovered in the organic layer of the extractions.

Uptake of Mirex by Bean and Pea Plants from Water: Due to solubility problems, mirex was introduced into 250 ml of water as a methylcellosolve solution and two week old pea and bean plants were allowed to grow in this water for 48 hours. Mirex was taken up by both bean and pea roots in proportion to the concentration provided (Table 2), the concentrations factors, roots vs. water, were 9.2 ± 1.35 and 7.2 ± 1.27 respectively. Translocation to the aerial parts was proportional to the mirex in the roots averaging

TABLE 1. PERCENT OF TOTAL MIREX-^{14}C ADMINISTERED ORALLY.*

Tissues or Organs	Total	Per g of Tissue
Fat	27.8	1.54
Liver	1.75	0.15
Small Intestine	0.76	0.10
Muscle	3.20	0.04
Large Intestine	0.23	0.10
Stomach	0.06	0.05
Heart	0.03	0.05
Kidney	0.09	0.25
Brain	0.07	0.04
Testes	0.12	0.06
Lung	0.08	0.07
Subtotal	34.19	
Excretion		
Feces	58.5	
Urine	0.69	
Total recovery	93.38	

*6.0 mg/kg mirex-u-^{14}C administered by oral intubation

TABLE 2. UPTAKE OF MIREX BY PEA AND BEAN PLANTS*

MIREX-^{14}C in Water	PEAS (PPM)		BEANS (PPM)	
ppm	Roots	Shoots	Roots	Shoots
1	6.487	0.586	8.478	0.496
5	40.696	1.521	45.364	3.867
10	71.285	8.408	101.604	6.834

*Mirex-u-^{14}C (5 x 10^6 dpm) along with desired amount of unlabeled mirex was introduced in water as methylcellosolve (1%) solution.

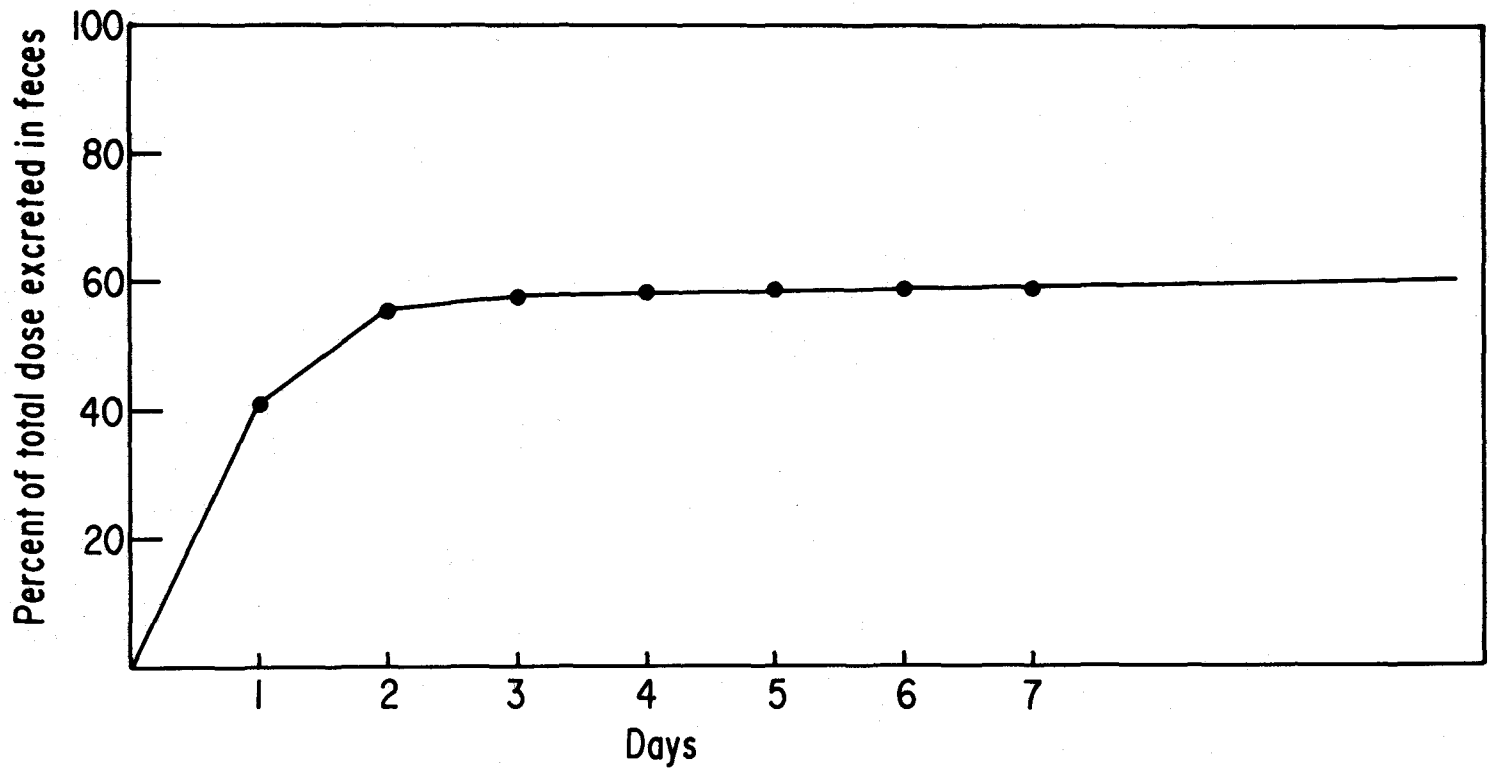

Fig. 1. Percent of cumulative excretion of radioactivity in rat feces for seven days after administration of a single dose of mirex-u-^{14}C (6 mg/kg) by oral intubation.

7.0 and 8.2% of the root content for bean and pea plants, respectively. Although these experiments were not done under field conditions, the results might indicate that plants do take up mirex inspite of its low water solubility. Under field conditions, the soil pH, weather conditions and infinitely longer exposure will undoubtedly affect the amount of uptake by various plants. Root crops grown in the area of mirex application might be expected to pick up residues of mirex and constitute an as yet undetected source of contamination. Vegetation in ponds treated with 1 ppm mirex concentrated maximum mirex residues for 7 to 14 days after treatment and although the residue levels dropped after that, significant residues were found even after 168 days (3). A careful examination of mirex residues in root and vegetable crops may be warranted.

SUMMARY

About 58.5 percent of the uniformly labeled mirex-^{14}C administered to rats as a single oral dose was excreted in feces and 0.69% in urine after 7 days. Considerable tissue storage of mirex was observed; fat, muscle, liver,kidneys and intestines contained 27.8, 3.20, 1.75, 0.76 and 0.23 percent of the total dose, respectively 7 days after treatment. While the first half-life of mirex was 38 hours, the projected second half-life was in excess of 100 days indicating a very slow rate of elimination from the body. No metabolite of mirex was detected in the feces, urine or any of the tissues. Nor was any mirex metabolite detected on incubation with rat, mouse, and rabbit liver preparations or plant root preparations. Mirex was concentrated by pea and bean roots and smaller amounts were translocated to the aerial parts when the plants were allowed to grow in water containing 1, 5 and 10 ppm mirex for 2 days. The resistance of mirex to biodegradation and its long biological half-life indicate that this insecticide may have an environmental half-life which far surpasses that of any previously studied insecticide.

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