Characterization of a Mirex Metabolite from Monkeys

by V. B. Stein, K. A. Pittman, and M. W. Kennedy
Institute of Comparative and Human Toxicology
Albany Medical College
Albany, N.Y. 12208

Mirex (Figure 1) is a chlorinated insecticide used in the Southeastern United States for control of the imported fire ant (*Solenopsis richteri* and *Solenopsis invicta*). The systematic name for this compound is dodecachloropentacyclo [5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$] decane while Chemical Abstracts lists it under the name (1,3,4) methano-(2H)cyclobuta (CD) pentalene dodecachlorooctahydro. The structure of Mirex has been established by X-ray diffraction (7,14) infrared spectroscopy (17,18) and mass spectroscopy (4,5).

Previous studies of Mirex metabolism in animals have not produced evidence for its biotransformation (8,11,13,15). Feces, urine and tissue from rats given a single oral dose of ^{14}C-Mirex as well as *in vitro* liver preparations from the rat, mouse and rabbit incubated with ^{14}C-Mirex were found to be free of any metabolite of Mirex (15). Rats receiving a single oral dose of ^{14}C-Mirex ex-

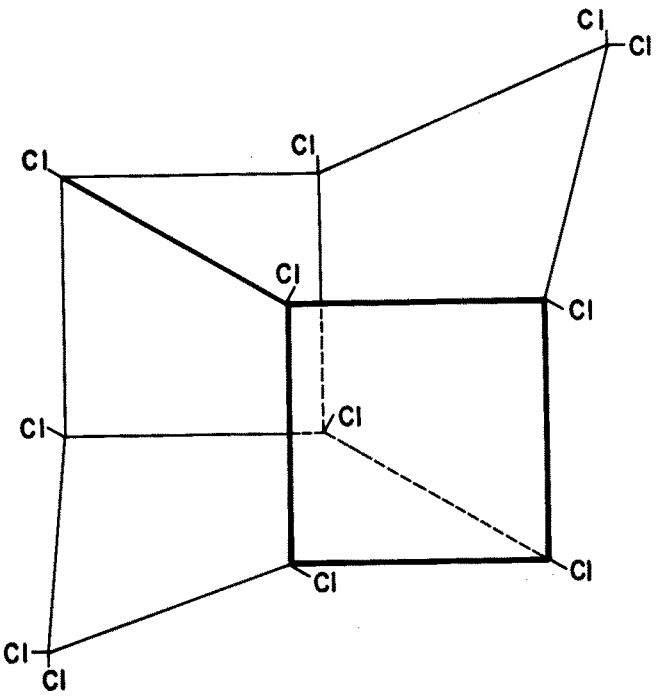

Figure 1. Mirex - dodecachloropentacyclo [5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane

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creted essentially all the radioactivity as unmetabolized Mirex (8), as did rats given repeated doses of ^{14}C-Mirex (10). Japanese Quail treated orally with ^{14}C-Mirex were found to contain undegraded Mirex in tissues, feces and egg yolk (11). No metabolites of Mirex were found in dairy cattle (6), or houseflies (16).

Mirex was found not to be degraded by soil microorganisms (12), but a suspected biodegradation product of Mirex has been reported to be formed under anaerobic conditions in sewage sludge. Identification and structure determination of this compound are presently incomplete (3).

Photodegradation products of Mirex in solution in hydrocarbons and in the presence or absence of aliphatic amines have been characterized and reported (1,2,8). The major photoproducts were identified as monohydro- and dihydro-derivatives of Mirex.

A recent study of the uptake and accumulation of Mirex in the rhesus monkey was undertaken in our laboratories and small quantities of a suspected metabolite were detected in the feces (13). The present communication reports the characterization by high-pressure liquid chromatography (HPLC) of the suspected metabolite of Mirex found in extracts of the feces of rhesus monkeys (*Macaca mulatta*) given i.v. doses of ^{14}C-Mirex.

Materials and Methods

Chemicals: ^{14}C-Mirex, 5.76 Ci/mole (obtained from Mallinckrodt) was checked for purity by thin-layer (TLC), gas-liquid (GC) and HPLC. Only radioactivity could be monitored when using the HPLC, since Mirex could not be detected with the UV detector attached to the instrument. The TLC systems were run on 0.25 mm silica gel plates (Merck, Darmstadt) and the ^{14}C located by use of a Tracerlab 4π scanner with a thin layer attachment. The four TLC systems used are given with Rf values for Mirex in parenthesis; 1) Heptane (0.65), 2) hexane:acetone, 4:1 v/v (0.86). 3) cyclohexane (0.77), 4) cyclohexane:benzene, 4:1 v/v (1.0). The GC and HPLC systems used in this study are described below.

The Mirex photodegradation products 1,2,3,4,5,6,7,8,9,10-decachloropentacyclo [5.3.0.0^{2,6}.0^{3,9}.0^{4,8}] decane (I), 1,3,4,5,5,6,7,9,10,10-decachloropentacyclo [5.3.0.0^{2,6}.0^{3,9}.0^{4,8}] decane (II), 1,2,3,4,5,5,6,7,8,9,10-undecachloropentacyclo [5.3.0.0^{2,6}.0^{3,9}.0^{4,8}] decane (III), and 1,2,3,4,5,5,6,7,9,10,10-undecachloropentacyclo [5.3.0.0^{2,6}.0^{3,9}.0^{4,8}] decane (IV) (2) and Kepone (1,2,3,4,6,7,8,9,10,10-decachloropentacyclo [5.3.0.0^{2,6}.0^{3,9}.0^{4,8}] decane-5-one) and reduced Kepone (1,2,3,4,6,7,8,9,10,10-decachloropentacyclo [5.3.0.0^{2,6}.0^{3,9}.0^{4,8}] decane 5-ol) were obtained from Dr. B.R. Layton of the Mississippi State Chemical Laboratory. Standard solutions for HPLC analysis were prepared by diluting stock solutions of the Mirex derivatives in hexane with acetonitrile to give a concentration of about 50 µg/ml. Standard solutions of kepone and reduced kepone were prepared by diluting stock solutions in aceton-

itrile to give concentrations of 70 µg/ml and 98 µg/ml respectively. Standards for GC analysis were prepared by diluting the stock solutions of Mirex and of its photodegradation products with hexane to give concentrations of about 5 µg/ml and by diluting stock solutions of kepone and reduced kepone with acetonitrile to give concentrations of about 3.5 µg/ml for kepone and 5 µg/ml for reduced kepone.

Sample Collection: Fecal samples were collected separately from the urine of female rhesus monkeys which had been given an i.v. dose of about 1 mg/kg of ^{14}C-Mirex. The feces were found to contain measurable quantities of ^{14}C for at least 13 months (13). Samples were collected daily at first; as the ^{14}C content decreased, collections were made every two to three days. All samples were kept frozen until they were analyzed.

Extraction of Fecal Samples: Fecal samples were homogenized in a Waring Blender with enough water to make a pipettable slurry. The slurry was extracted by adding to it two volumes of acetonitrile, stirring the mixture for one hour and allowing it to settle. The supernatant liquid was decanted through a Buchner funnel and the residue extracted again with another two volumes of acetonitrile. The combined filtrates were placed under a hood on a hot plate at about 45°C, the organic solvent was allowed to evaporate and the remaining aqueous mixture was extracted three times with equal volumes of hexane. The combined hexane extracts were dried over anhydrous sodium sulfate, filtered and taken to dryness in a rotary evaporator. The residue was then extracted exhaustively with acetonitrile. The combined acetonitrile extracts were partitioned into hexane until no more radioactivity could be extracted into the hexane. The hexane extract was evaporated to dryness at 35°C in a rotary evaporator, brought to 5 ml with hexane, and quantitatively transferred. The extract was then passed through a chromatographic column (Chromaflex column, Kontes Glass Co., size 22) fitted with a glass wool plug and containing 16 grams of sodium sulfate above 16 grams of PR grade florisil (Sigma Chemical Company, 100/120 mesh); the column was pre-wetted with 40 ml of hexane and eluted with 20 ml of hexane. Collection of the eluate was made in a 100 ml round bottom flask, evaporated to dryness using a rotary evaporator and finally was brought to an appropriate volume with acetonitrile for analysis by HPLC.

HPLC Analysis: Ten microliters of each sample of fecal extract were injected into a DuPont model 830 Liquid Chromatograph fitted with a reverse phase column (Permaphase ODS) and eluted, using 50% acetonitrile as the mobile phase at 1000 psi. The flow rate was approximately 0.75 ml/min.

Fractions were collected at three minute intervals until the elution volume of Mirex was reached; thereafter, one minute collections were made. The ^{14}C in the fractions was then determined by liquid scintillation spectrometry using a Packard Tri-Carb Model 3380. Fecal extracts, a Mirex standard, and a co-chromatographed fecal extract plus standard Mirex were run on the same day.

Twenty-five microliters of standard solutions of Mirex photodegradation products (Figure 3) were co-chromatographed with ten microliters of a fecal extract using the conditions described above. Fractions were collected at two minute intervals and chromatographed on GC to determine the elution volume of the Mirex photoproducts. The ^{14}C content of each fraction was measured to determine the elution volume of the suspected metabolite; Mirex photodegradation products III and IV were also co-chromatographed with fecal extracts using one minute collection times. All runs were performed in duplicate.

Fifteen microliters of kepone or of reduced kepone were co-chromatographed with twenty microliters of fecal extract. Fractions were collected at one minute intervals during elution of kepone and reduced kepone and at two minute intervals during elution of the suspected metabolite.

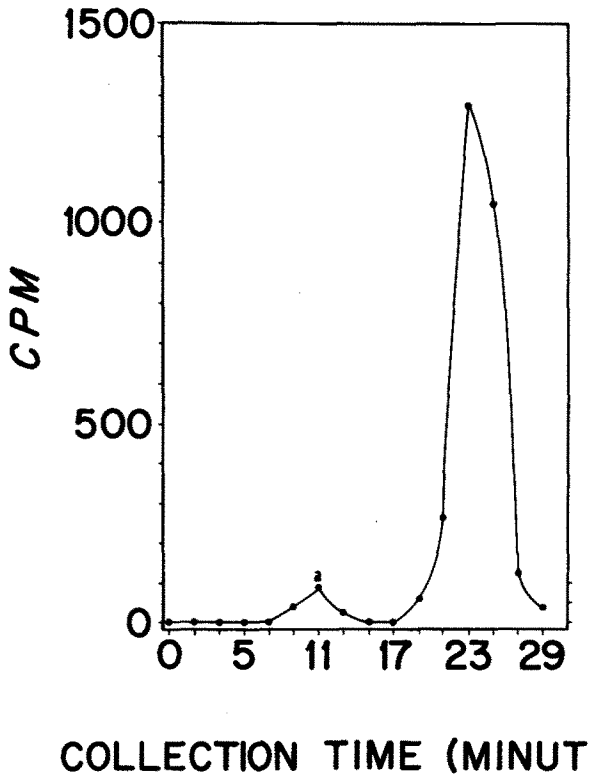

Figure 2. High pressure liquid chromatographic analysis of an extract of a typical fecal sample from a rhesus monkey given ^{14}C-Mirex intravenously. The large peak is Mirex.

Gas Chromatographic Analysis: A Hewlett Packard Model 5713A gas chromatograph, equipped with a ^{63}Ni electron capture detector held at 300°C and containing a 6' x ¼" (0.25mm I.D.) glass column filled with 3% OV-1 on Chromsorb Q, 100/120 mesh, was maintained at an oven temperature of 245°C for analysis of Mirex and its photodegradation products and was maintained at 230° for analysis of kepone and reduced kepone. A carrier gas of 5% methane in argon was used at a flow rate of 50 ml/min.

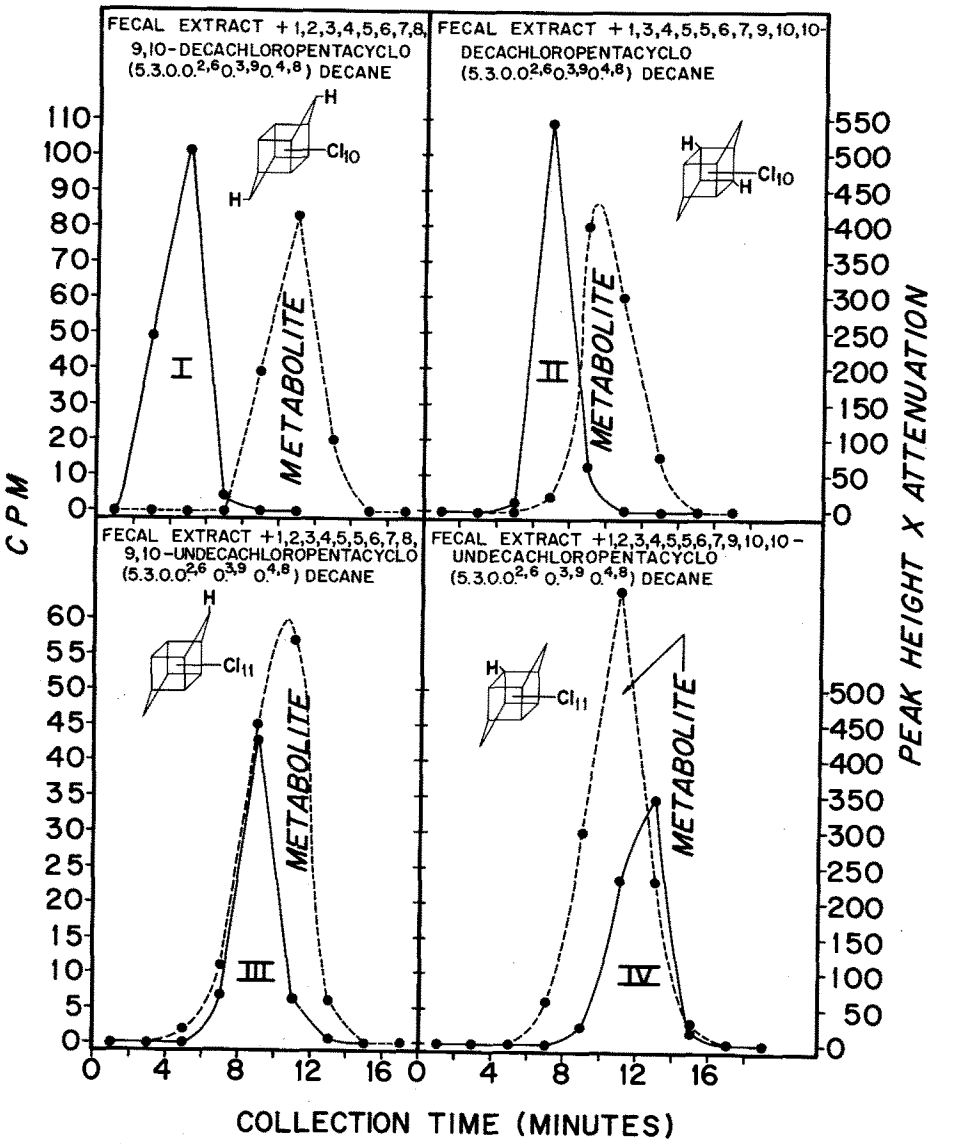

Figure 3. Co-chromatography of Mirex photodegradation products with monkey fecal extracts.

Fractions from each HPLC analysis of fecal extracts co-chromatographed with Mirex derivatives were analyzed by GC to determine which fractions contained the Mirex derivatives. Peak heights were measured to obtain a quantitative estimate. Identification of the Mirex derivatives was made by a comparison of the retention times of the standards to samples.

Results and Discussion

Thin-layer, gas and high pressure liquid chromatographic analysis of ^{14}C-Mirex failed to show the presence of detectable quantities of any chemical or radiochemical impurity.

HPLC analysis of extracts of feces collected individually from two rhesus monkeys at about 2 and 5 weeks after ^{14}C administration, showed a peak with a smaller elution volume than Mirex (Figure 2). This peak was absent in fecal extracts collected a few days after ^{14}C administration.

The results of the co-chromatography of Mirex photodegradation products on HPLC with monkey fecal extracts (Fig. 3) show that the chromatographic system employed clearly separated the suspected metabolite from the $C_{10}H_2Cl_{10}$ Mirex derivatives, both of which had smaller elution volumes. Of the $C_{10}HCl_{11}$ derivatives, one had an elution volume slightly smaller and the other had an elution volume slightly larger than the suspected metabolite. Though the metabolite and both of the $C_{10}HCl_{11}$ photodegradation products of Mirex behaved quite similarly, the differences in elution

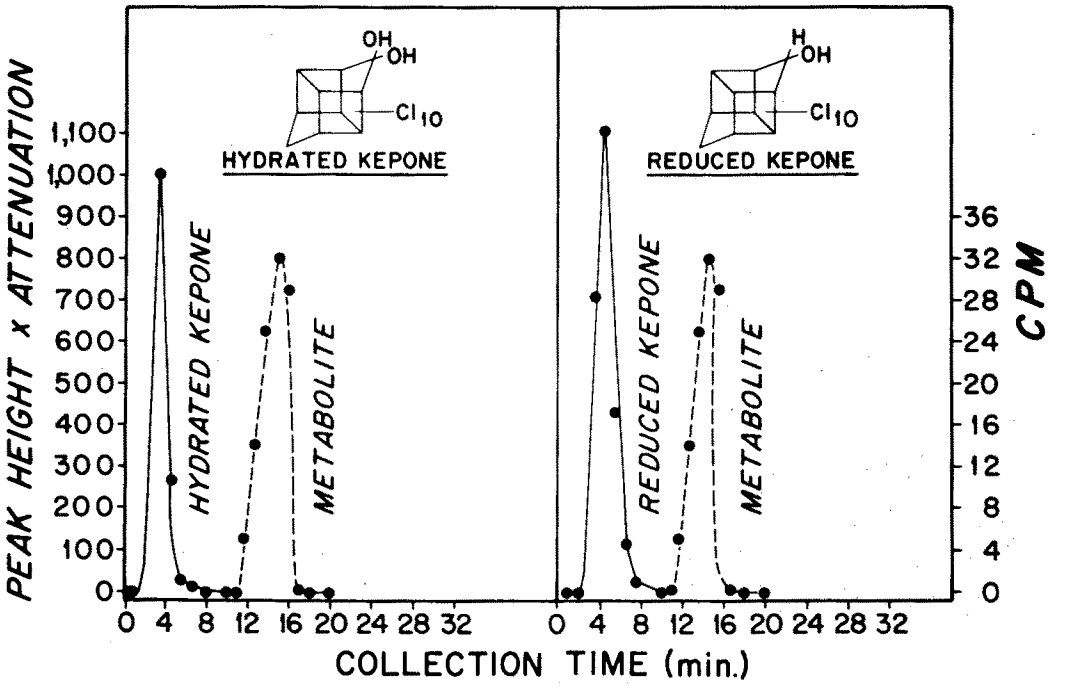

Figure 4. Co-chromatography of kepone and reduced kepone with monkey fecal extracts.

volumes demonstrated in Figure 3 were invariably maintained when the analysis was repeated. The possibility that the suspected metabolite might be an oxidation product of Mirex, which was retarded in the reverse-phase HPLC system due to interaction with the solid support or column tubing, was eliminated by co-chromatography with kepone and reduced kepone (Fig. 4). The elution volumes of both of these compounds were substantially less than that of the suspected metabolite, making it unlikely that the latter was an oxidation product of Mirex. The amount of the compound obtained by combining all fecal extracts was insufficient for location on GC or for purification for mass spectrometry.

It is believed that the suspected metabolite arose as a result of bacterial action in the lower gut or in the feces, since no evidence of a metabolite was found in extracts of fat, where the concentration of radioactivity was always several orders of magnitude higher than in the feces (13). The lipophilic nature of the suspected metabolite, as demonstrated on HPLC, should have caused it to be taken up in the fat, had it arisen as the result of hepatic or other tissue metabolism. The results of this experiment lead to the conclusion that the behavior of the suspected metabolite is consistent with that expected from a non-polar derivative of Mirex, perhaps 1,2,3,4,5,5,6,7,8,10,10-undecachloropentacyclo [5.2.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$] decane, the only monohydro photodegradation product which we were not able to obtain.

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