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Gas-Liquid Chromatographic Determination of Mirex in Plasma, Liver and Fat from Mice and Monkeys

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Mirex is used in the southeastern United States for control of the imported fire ant (Solenopsis richteri and S. invicta). VAN VALIN et al. (1977) found Mirex to be very resistant to degradation in soil. Mirex has been detected in milk (LOFGREN et al. (1964), beef (BAETCKE et al. 1972) fish and birds (BORTHWICK et al. 1973). From recent findings of Mirex in fish caught from Lake Ontario (KAISER 1974) Mirex may be more widely distributed in parts of the country other than the southern United States. WATERS et al. (1977) have published an informative review summarizing these findings.

Several methods have been employed for the determination of Mirex in biological samples. The distribution of Mirex in rat tissues was studied and recoveries greater than 90% were reported by KHERA et al. (1976) However, the method required large amounts of sample and reagents, and no minimum detectable levels were reported. BONG (1975) described a procedure for the determination of hexachlorobenzene and Mirex in fatty materials, utilizing an acetonitrile-petroleum ether partition and final measurements by gas chromatography. Recoveries of greater than 90% were again obtained. HAWTHORNE et al. (1974) and GIBSON et al. (1972) used an acetonitrile-hexane partitioning method for the analysis of Mirex following its extraction from commercially raised catfish and from rat feces but did not report % recoveries or minimum detectable levels.

Although numerous papers have been written describing the determination of Mirex in biological samples, most of these methods are tedious, time consuming and require large volumes of solvent and bulky glassware. Minimum detectable levels and recovery data for the particular analysis were overlooked in most of these studies.

The purpose of our study was to develop a rapid and sensitive method using small quantities of sample for the analysis of Mirex in tissues and plasma.

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METHODS

Reagents

Solvents used were those suitable for pesticide residue analysis, Florisil (60-100 mesh, PR grade activated at 677°C) and sodium sulfate were stored in an oven held at 120°C. Mirex standard solutions were prepared containing 10-1000 µg Mirex/mL and these were diluted with hexane to obtain solutions containing 0.001-10 µg Mirex/mL.

Apparatus

(a) Chromatographic column - Chromaflex size 22, 7 mm (Kontes Glass Co.)

(b) Graduated centrifuge tubes - 15 mL.

(c) Micro centrifuge tube - Graduated, 2.5 mL capacity, for mice plasma extraction.

(d) Test tube - Culture, round bottom.

(e) Centrifuge tube - 50 mL capacity for extraction.

(f) Centrifuge - Model EXD, size 2 (No. 8196, obtained from International Equipment Co. (IEC), Needham, MA).

(g) Gas chromatograph - Hewlett-Packard Model 5713A fitted with ^{63}Ni electron capture detector. Operating conditions: temperature (°C) - detector 300, oven 240, 1.8 m x 4 mm id (0.25 mm id) glass column (Hewlett-Packard) packed with 3% OV-1 (on 100-200 mesh Gas Chrom Q; carrier gas 5% methane in argon at ca 50 mL/minute flow rate. Under these conditions retention time of Mirex is about 2.6 min.

(h) Homogenizer - Super Dispax Model SDT 182 Tissuemizer.

Extraction

(a) Liver - Measure up to 0.3 g into glass scintillation vials and fortify each sample with 10 µL of hexane (blank) or 10 µL standard solutions containing 1.0-100 µg Mirex/mL. Do the same for b, c, and d below. Homogenize samples in 2.5 mL acetonitrile, centrifuge for 5 min in glass vials at ca 1500

rpm and draw off the organic extract using a Pasteur pipet. Repeat twice more, transferring the extracts to a 50 mL centrifuge tube. After the addition of 7.5 mL of 2% sodium sulfate, partition the acetonitrile extracts into hexane with one 5 mL and two 2.5 mL portions and combine the extracts in glass test tubes. If necessary centrifuge to eliminate emulsions that may form. After extraction concentrate the hexane extracts to about 5 mL and proceed to the cleanup procedure described below.

(b) Fat - Measure up to 0.3 g samples into glass scintillation vials and fortify each sample with 10 µL of standard solutions containing 1.0-100 µg Mirex/mL. Homogenize in 2.5 mL acetonitrile and extract with one 5 mL and two 2.5 mL portions of hexane as above. Draw off the hexane layer with a Pasteur pipet and combine the extracts in glass test tubes. Concentrate the combined extracts to about 5 mL and proceed to the cleanup procedure described below.

(c) Mouse plasma - Measure up to 0.5 mL samples in 2.5 mL-graduated centrifuge tubes, add 10 µL standard solutions containing 0.2-100 µg Mirex/mL, and extract three times with 1 mL hexane. Centrifuge to eliminate emulsions, combine the extracts in 15-mL graduated centrifuge tubes, and concentrate to an appropriate volume by drying under a stream of nitrogen. Proceed to gas-liquid chromatographic analysis.

(d) Monkey plasma - Measure up to 1.0 mL samples in glass test tubes, fortify with 10 µL standard solutions containing 1-100 µg Mirex/mL, add 2.5 mL acetonitrile to precipitate the proteins and centrifuge at ca 1500 rpm for 5 min. Draw off the acetonitrile extract using a Pasteur pipet, repeat twice more and combine the extracts in a 50-mL centrifuge tube. The remaining procedure is identical to that described above for liver.

Florisil Column Cleanup

Prepare the chromatographic columns over glass wool plugs by adding 1.6 g Florisil and 1.6 g sodium sulfate, in that order; tamp to ensure uniform packing. Pre-wet each packed column with 10 mL hexane, pass each biological extract through a separate column, and elute with 5 mL of hexane. Collect the eluates in separate 25-mL Kuderna-Danish concentrator tubes and evaporate to an appropriate volume by drying under a stream of nitrogen.

Gas-Liquid Chromatography

Inject 2 µL standards containing 0.0001-1.0 µg Mirex/mL (direct standards).

Inject 2 µL sample and measure height of symmetrical Mirex peak (extracted standards). Obtain regression analysis for peak height x attenuation vs. concentration, for direct and extracted standards. Use slope and intercepts obtained for direct standards to calculate concentration of Mirex in sample.

RESULTS AND DISCUSSION

Samples of liver and fat were homogenized in scintillation vials because these containers are of convenient size, and the caps are lined with aluminum foil; this prevents extraction of organic material into low-boiling solvents such as hexane. Acetonitrile-hexane partition is preferred over direct hexane extraction because the acetonitrile removes some of the more polar interferring materials. Direct hexane extraction was attempted for monkey plasma, but recoveries of less than 60% were obtained.

The proposed procedure for the extraction of Mirex from plasma, liver and fat can be applied to other tissues such as kidney, thyroid gland, gonads, adrenals, intestines, sciatic and peripheral nerves and also from feces, with recoveries exceeding 90%.

Plots of GLC response vs. concentration of both extracted and direct standards were linear over the range indicated in Table 1, with correlation coefficients greater than 0.99. Recoveries of Mirex from mouse tissue and plasma ranged from 90 to 107% (see Table 1) and from monkeys, 93 to 102% (see Table 2). Average recoveries ($\pm$ standard deviations) from plasma were 97.8 $\pm$ 6.1% for the mouse and 95.7 $\pm$ 3.5% for the monkey. Average recoveries for mouse liver and fat were 98.1 $\pm$ 6.5% and 98.6 $\pm$ 4.9% respectively, while those for monkey, liver and fat were 96.5 $\pm$ 5.0% and 95.3 $\pm$ 3.2%, respectively. The method is sensitive to 0.01 ppm for both mouse and monkey plasma, and to 0.03 ppm for mouse and monkey liver. The sensitivities for mouse and monkey fat were determined to be 0.017 ppm.

Using the same definition of detection limit described in an article by HARTMAN (1971), as the amount of sample which gives a response equal to twice the background level (5), the detection limit

of Mirex by GLC was determined to be 0.2 pg. The same criteria was used for the determination of sensitivities.

TABLE 1
Recovery (%) of Mirex from Mouse Plasma, Liver and Fat

Added, µg	Sample		
	Plasma	Liver	Fat
0.005	98	---	97
0.01	93	102	97
0.025	98	94	92
0.05	100	101	90
0.10	90	94	101
0.25	101	101	99
0.50	107	99	102
1.0	95	95	101
Mean	97.8	98.1	98.6
Std. dev.	6.1	6.5	4.9

Values are averages of duplicate determinations.

TABLE 2
Recovery (%) of Mirex From Monkey Plasma, Liver and Fat

Added, µg	Sample		
	Plasma	Liver	Fat
0.01	101	---	---
0.025	99	---	---
0.05	94	---	97
0.10	94	104	92
0.25	94	94	99
0.50	96	93	94
1.0	93	95	94
Mean	95.7	96.5	95.3
Std. dev.	3.5	5.0	3.2

Values are averages of duplicate determinations.

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