ANALYTICAL PROCEDURE

Extraction and Gas Chromatographic Analysis of Chlorinated Insecticides from Animal Tissues

PETER W. SASCHENBRECKER AND DONALD J. ECOBICHON

An extraction procedure for chlorinated hydrocarbon insecticides gives high quantitative yields from several distinctly different tissues (plasma, liver, breast muscle, and brain). Recoveries of added insecticides ranged from 84.1 to 100.3%. Recoveries of *p,p'*-DDD were somewhat lower, ranging from 78.7 to 83.2%. With the exception of neutral fat and glandular oils, this method is applicable to a variety of tissues and to a number of insecticides.

Many of the cleanup procedures developed for the extraction of chlorinated hydrogen insecticides are excellent for separation from one particular tissue. Poor recoveries are often obtained when the methods are applied to a variety of different tissues such as blood, liver, kidney, skeletal and smooth muscle, and brain (*1*, *4*, *5*). A recovery procedure is not ideal unless it is broad enough in scope to be applicable to a wide range of biological materials and to a number of insecticides and their degradation products and metabolites. The present report describes an extraction procedure which gives quantitatively high yields of seven commonly used insecticides from several tissues of adult roosters.

Experimental

Apparatus. CHROMATOGRAPH. An Aerograph HyFI Model 600-D equipped with an electron-capture detector containing a 250-mc. tritium foil source (Varian Aerograph, 2700 Mitchell Drive, Walnut Creek, Calif.).

RECORDER. An L & N Speedomax H, Model S, 0 to 1 mv. (Leeds and Northrup Co., Toronto, Canada).

COLUMN. Coiled borosilicate glass tubing, 1/8-inch o.d. × 4-feet packed with acid-washed Chromosorb W 60/80-mesh coated with 4% G.E. SE-30 methyl silicone and 6% D.C. QF-1 fluorosilicone. The column was prepared according to McCully and McKinley (*5*) and was preconditioned at 225°C. for 24 hours before use.

FILTERING FUNNELS, 150-ml. capacity, with medium porosity fritted disks; fitted with ⅌ vacuum adapters (Emerald Glass Co., Toronto, Canada).

Reagents. Nitrogen, prepurified (Linde of Canada, Ltd., Toronto, Canada).

Sodium sulfate, anhydrous, Analar grade (British Drug Houses, Ltd., Toronto, Canada).

Polyethylene-coated alumina (Kensington Scientific Corp., Oakland, Calif., Catalog No. K-3209).

All the solvents employed in this study were redistilled in glass at least once. *n*-Heptane was redistilled twice. Each redistilled solvent was checked for impurities prior to use by the gas-liquid chromatographic analysis of a 10-μl. sample.

Division of Pharmacology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

A standard solution of insecticides in acetone was prepared from analytical grade chlorinated hydrocarbon standards so that each microliter contained 2×10^{-4} μg. of heptachlor, aldrin, *p,p'*-DDE, and dieldrin, 2×10^{-3} μg. of *o,p'*-DDT, and *pp'*-DDT, and 1×10^{-3} μg. of *p,p'*-DDD.

Sample Preparation. Five grams of tissue were homogenized with known amounts of the above standard solution in an explosion-proof Waring Blendor with 45 ml. of acetonitrile, 15 ml. of acetone, and 5 grams of anhydrous sodium sulfate for 4 minutes at low speed and, subsequently, 2 minutes at high speed. The homogenate was then filtered under suction through a fritted funnel and the residue washed with two 10-ml. portions of acetonitrile. The filtrate was adjusted with sufficient distilled water to produce an acetonitrile-water ratio of 2.5 to 1 (see results).

A slurry of polyethylene-coated alumina in acetonitrile–water (ratio 2.5 to 1) was prepared and poured into a glass chromatographic column (20 × 400 mm.) to produce a packed column at least 8 cm. in height. The acetonitrile–water extract of tissue was passed through the column and the column was subsequently washed twice with 35-ml. portions of a 2.5 to 1 acetonitrile–water mixture, and finally with 25 ml. of acetonitrile. The combined column eluates were transferred into a 250-ml. separatory funnel, 20 ml. of distilled water and 45 ml. *n*-heptane were added, and the funnel was shaken manually for 2 minutes. After separation of the layers, the lower (aqueous) layer was drained into another separatory funnel and re-extracted with 45 ml. of *n*-heptane. The upper (heptane) layers from these two steps were combined and filtered under suction through a fritted funnel containing 30 grams of anhydrous sodium sulfate.

The water-free *n*-heptane extract was concentrated to near-dryness by rotary flash evaporation at 38° C. The evaporation flask was rinsed with several small portions of acetone, these being transferred into a glass-stoppered graduated test tube. The solution was then either concentrated under a stream of nitrogen or suitably diluted for analysis.

The operating conditions of the gas chromatograph were as follows:

Column temperature	178° C.
Injector temperature	200° C.

Range	EC 1
Attenuation	8.0
Carrier gas	Prepurified nitrogen
Flow rate	80 to 85 ml. per minute
Chart speed	0.5 inch per minute

Chromatogram Analysis. The most common procedure for the quantitative evaluation of peaks in a gas chromatogram is the measurement of the area by triangulation. Under isothermic and isobaric conditions, it is acceptable to take the peak height alone into consideration in the quantitation of a compound, provided the response of the detector is linearly related to the quantity of compound, or, in the case of the Aerograph Model 600-D, the peak height does not exceed $^1/_8$ of the total standing current (*2*). For comparison, the chromatograms of 1 μl. of the standard insecticide solution both before and after the injection of 1 μl. of sample were compared.

Insecticides were qualitatively identified by direct comparison of the retention times of the unknown insecticides with those of the different standard compounds relative to that of aldrin.

Results and Discussion

Separation on the gas chromatographic column and retention times of various chlorinated hydrocarbons were comparable to the results reported by McCully and McKinley (*5*).

A problem encountered with the organic solvents employed in cleanup procedures is the retention of small amounts of lipids in the final extract which appear on the chromatogram as "dirt" peaks and which readily contaminate the column and the electron-capture detector. The extraction of tissues with acetonitrile proved to be no exception. A subsequent step in the cleanup procedure was required.

Polyethylene-coated alumina is known to adsorb most fats and waxes, while insecticides can be eluted easily. Insecticide recovery from a column of this material was thoroughly studied. Acetonitrile extracts of chicken liver treated with known amounts of insecticide were diluted with varying proportions of water and applied to the column. To assess the amount of background interference due to contamination, untreated liver samples were processed in the same manner and the background attenuation was measured. Figure 1 shows the per cent recovery of *p,p'*-DDE and contaminants as a function of the acetonitrile–water ratio. The best results were obtained using an acetonitrile–water ratio of 2.5 to 1. While ratios higher than 2.5 to 1 resulted in essentially 100% elution of the insecticide, a higher background level, indicative of interfering material, was observed. For optimal results, different insecticides required slightly different solvent ratios; however, the ratio adopted here represented a good compromise for all the chlorinated hydrocarbons studied with little background interference.

Low yields of insecticides were obtained when the acetonitrile–water extracts were just filtered through the polyethylene-coated alumina columns. Quantitative

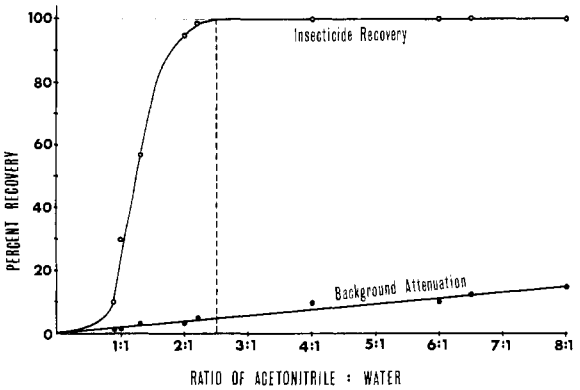

Figure 1. Per cent recovery of *p,p'*-DDE and interfering material from chicken liver as a function of acetonitrile-water ratio as determined by gas-liquid chromatography

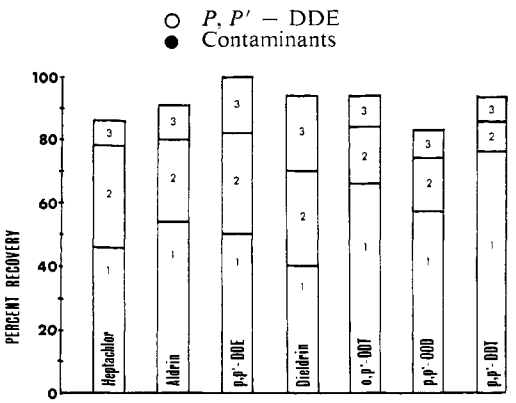

Figure 2. Recovery of chlorinated hydrocarbon insecticides from a column of polyethylene-coated alumina by consecutive elution

1. 35 ml. of acetonitrile-water, 2.5 to 1
2. 35 ml. of acetonitrile-water, 2.5 to 1
3. 25 ml. of acetonitrile

yields were obtained by subsequent elution of the column with two 35-ml. portions of a 2.5 to 1 acetonitrile–water mixture. Figure 2 illustrates the per cent recovery with each washing of seven chlorinated hydrocarbons. The first two washings of the column yielded a recovery ranging from 70% for dieldrin to 86% for *p,p'*-DDT. A third washing with a small amount (25 ml.) of pure acetonitrile improved the quantitative elution of the insecticides without a considerable increase in interfering material. Excessive washing with acetonitrile alone resulted in the removal of contaminating lipids from the column.

A series of controlled experiments was carried out on plasma, liver, breast muscle, and brain of adult roosters. Known amounts of the standard solution of seven chlorinated hydrocarbons were added to the sample in the Waring Blendor before homogenization. The extraction procedure described above was carried out and the final extracts were quantitatively analyzed by gas-liquid chromatography. The averaged results for each insecticide with each tissue, expressed as per cent recovery, are summarized in Table I. High quantitative yields were obtained for all the hydro-

Table I. Average Recovery of Chlorinated Hydrocarbon Insecticides from Control Studies of Tissues from Adult Roosters

Insecticide	Per Cent Recovery ± Standard Error			
	Plasma	Liver	Breast muscle	Brain
Heptachlor	86.7 ± 2.28	84.1 ± 1.79	93.4 ± 1.45	93.8 ± 1.32
Aldrin	87.0 ± 1.70	93.6 ± 1.47	90.3 ± 1.85	92.4 ± 1.60
p,p'-DDE	100.3 ± 0.41	98.8 ± 0.32	99.5 ± 0.32	98.8 ± 0.43
Dieldrin	93.7 ± 1.51	93.4 ± 1.44	95.0 ± 0.91	94.1 ± 1.88
o,p'-DDT	94.1 ± 1.72	93.5 ± 1.31	95.0 ± 1.13	91.6 ± 1.45
p,p'-DDD	83.2 ± 1.46	80.9 ± 1.10	83.2 ± 1.29	78.7 ± 1.17
p,p'-DDT	94.3 ± 1.68	92.8 ± 1.27	95.5 ± 0.84	90.9 ± 1.49
No. of individual tissue analyses	14	17	12	12

Table II. Minimum Detectable Levels for Insecticides Investigated

Insecticide	Minimum Detectable Level, P.P.M.
Heptachlor	0.002
Aldrin	0.002
p,p'-DDE	0.005
Dieldrin	0.005
o,p'-DDT	0.006
p,p'-DDD	0.005
p,p'-DDT	0.006

carbons. The lowest recovery from the tissues was observed for *p,p'*-DDD. The yields reported in Table I compare favorably with those reported in the literature (*3*, *6*, *7*). This extraction procedure has the advantage of being applicable to a variety of different tissues and to a number of commonly used chlorinated hydrocarbons. In addition to the tissues reported above, quantitatively similar yields have been obtained for hydrocarbon residues in whole blood, kidney, gastrointestinal smooth muscle, and cardiac muscle of a variety of avian specimens (unpublished results).

Using the extraction technique, the instrument, and the operating conditions described, the lowest levels of the various insecticides which could be detected were determined and are summarized in Table II. These values are based on the extraction of insecticides from sample sizes in the order of 5 grams of tissue or 5 ml. of plasma or blood resulting in a 2-ml. final volume of extract and injecting 1.0-μl. quantities into the gas chromatograph. The detection of smaller amounts is speculative, as the peaks become obscured by background noise.

This method is not recommended for samples of neutral fat or glandular oils. The recovery of chlorinated hydrocarbon residues from the uropygial or "preen" gland of birds was far from satisfactory because of the high content of low molecular weight lipids (unpublished results). For body fats and oils, the fat-precipitation method developed by McCully and McKinley (*5*) has proved ideal.

Acknowledgment

The authors acknowledge the kind cooperation of the Velsicol Chemical Corp., Chicago, Ill., for reference grade heptachlor; Geigy Agricultural Chemicals, Ardsley, N. Y., for standard *p,p'*-DDT, *o,p'*-DDT, and *p,p'*-DDE; and K. A. McCully, Food and Drug Directorate, Ottawa, Canada, for the purified *p,p'*-DDD. The technical assistance of H. E. Tel is gratefully acknowledged.

Literature Cited

(1) De Faubert Maunder, M. J., Egan, H., Godly, E. W., Hammond, E. W., Roburn, J., Thomson, J., *Analyst* **89,** 168 (1964).
(2) Dimick, K. P., Hartmann, H., Abstracts of Papers, p. 44A, 143rd Meeting, ACS, Cincinnati, Ohio, January 1963.
(3) Jain, N. C., Fontan, C. R., Kirk, P. L., *J. Pharm. Pharmacol.* **17,** 362 (1965).
(4) Kadis, V. W., Jonasson, O. J., *Can. J. Pub. Health* **56,** 433 (1965).
(5) McCully, K. A., McKinley, W. P., *J. Assoc. Offic. Agr. Chemists* **47,** 653 (1964).
(6) Stanley, R. L., Le Favoure, H. T., *Ibid.*, **48,** 666 (1965).
(7) Taylor, A., Rea, R. E., Kirby, D. R., *Analyst* **89,** 497 (1964).

Received for review June 6, 1966. Accepted October 10, 1966. Work supported by a grant from the Canadian Wildlife Services, Department of Northern Affairs and National Resources of Canada.