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# ANALYSIS OF ANIMAL FOOD PRODUCTS FOR CHLORINATED INSECTICIDE RESIDUES

## I. COLUMN CLEAN-UP OF SAMPLES FOR ELECTRON CAPTURE GAS CHROMATOGRAPHIC ANALYSIS.[1, 2, 3]

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SUMMARY

A one step method for sample clean up prior to analysis for chlorinated insecticides by electron capture gas chromatography is outlined. The method has been applied to a variety of animal product samples with excellent success. Sufficient sensitivity is attained so the procedure can be used as a screening procedure or a quantitative research tool. Using the procedure a technician can analyze 25 to 35 samples in an 8 hour day and detect nanogram quantities of chlorinated insecticides in a variety of samples.

Several methods have been used for the analysis of insecticide residues in animal products. Two methods used extensively for analysis of insecticide residues in animal products are the Mills paper chromatagraphic procedure and the Schechter-Haller colorimetric method for DDT (5, 7). These methods require time consuming sample preparations for accurate, sensitive results.

Recently, two special detection systems for gas chromatography, the microcoulometric cell (1), and the electron capture detector (3), have turned the analysts' attention to instrumental analysis of insecticide residues.

Watts and Klein (8) reported on the use of electron capture gas chromatography for detection of residues in food products. These authors found it necessary to use a modified Mills clean up procedure to prevent contamination of the detector system.

Moats (4) reported the use of a single column clean up procedure for butterfat which was later adapted by Langlois, et al. (2) for clean up of all dairy products. Since the procedure was a simple, rapid means of sample preparation for dairy products, attempts to expand its use for clean up of other fat containing animal products were made. This paper presents results with the method on a variety of animal food products and tissue samples.

### METHODS

Reagent grade methylene chloride and technical grade petroleum ether, B.P. 30-60 C, were redistilled before use. The Entomological Society of America insecticides reference standards used were obtained from Nutritional Biochemical Corporation. The standards and unkown samples were prepared in hexane, B.P. 65-67 C, and stored at 2-5 C. Florisil, 60/100 mesh activated by the supplier at 650 C, was obtained from the Floridin Company, Tallahassee, Florida. The florisil was re-activated by heating at 140 C for 12-14 hr. Five per cent water was uniformly mixed with the florisil and the mixture was held in an air tight container for 48 hr before use. This time was necessary for the water-florisil mixture to reach equilibrium. The eluant was a mixture of 20% methylene chloride in petroleum ether.

Chromatographic columns were 20 mm OD x 600 mm pyrex tubes plugged at one end with glass wool. Analytical columns were ¼-inch OD x 4 ft pyrex glass, packed with 2.5 or 5.0% Dow 11 Silicone on 60/80 mesh hexamethyldisilazane (HMDS) treated Chromosorb W. The Chromosorb W was obtained in the HMDS treated form from the supplier. The analytical instrument was either a Wilkens Aerograph Hi-Fi Model 600 gas chromatograph with an electron capture detector containing a 250 mc tritium ionization source, or the Wilkens Pestilyzer Model 650 with the same size tritium source. Both instruments were operated with a 90V potential across the detector. The recorder was a 1 mv Leeds and Northrup Model H with a disc integrator unit.

Fluid samples were weighed directly. Solid samples were cut as fine as possible prior to weighing. Weighed product samples were ground with 25 to 30 g of florisil in a glass mortar to form a free flowing powder. Twenty-five g of florisil were poured into a chromatographic column to form the bottom layer. This was prewashed with 50 ml of an equal mixture of methylene chloride and petroleum ether to remove some impurities from the florisil. The

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washings were discarded. Next the sample-florisil mixture was poured into the chromatographic column to form the top layer. From 150 to 650 ml of the eluant mixture was used, depending on which insecticide residues were being eluted. The eluant was evaporated to dryness from a beaker in a water bath at 50-60 C. The residues were transferred from the beaker to a calibrated test tube and made up to a 5 or 10 ml standard volume with hexane.

The analytical column was operated at temperatures of 185 to 195 C with a nitrogen (high purity) carrier gas flow of 60 to 80 ml per minute. Standard insecticide solutions were analyzed before and after each series of unknown samples. Depending upon the quantity of insecticide present, from 5 to 25 $\mu$l of unknown samples in hexane were used for analysis.

## RESULTS AND DISCUSSION

The sample size is limited by its fat content and the size of florisil clean up column used. Overloading the florisil column results in elution of fat and other impurities. If traces of fat are injected into the analytical instrument, they will cause poor stability of the analytical column and loss of instrument sensitivity. Typical sample sizes used for various products are presented in Table 1. By adhering to these sample sizes, proper clean up is achieved as indicated by the use of one analytical column for 400 to 500 analyses before replacement becomes necessary.

This one step extraction and clean up technique has been used for the detection of selected chlorinated insecticides in numerous types of samples as listed in Table 1. Recovery data for added insecticides to samples are presented in Table 2. This is a summary for all types of samples which explains somewhat the standard deviation of 3%. Duplicate samples of milk, for example, agree more closely.

Table 3 contains information on the amounts of eluant needed to eluate the various insecticides studied. Use of less eluant results in a reduction in per cent recovery of residues.

The sensitivity of the combination clean up procedures and electron capture gas chromatographic analysis for five chlorinated insecticides is presented in Table 4. A range is given for each insecticide to allow for various sample sizes as listed in Table 1. The effectiveness of the clean up procedure on a particular sample also effects sensitivity of the analytical procedure. Other factors which enter into the analysis will be discussed in a later paper.

Using analytical column conditions as outlined, an analysis on the instrument for the five insecticides was completed in less than 10 min. DDE and dieldrin were difficult to separate on this particular analytical column. Heptachlor and heptachlor epoxide were

TABLE 1. SAMPLE SIZE USED FOR ANALYSIS

| Sample | g |
|---|---|
| Whole milk | 10.0 |
| Cream | 2.0 to 5.0 |
| Butter | 1.0 |
| Cheese | 2.0 |
| Dried whole milk | 2.0 |
| Evaporated milk | 5.0 |
| Egg yolk | 2.0 |
| Chicken fat | 1.0 |
| Chicken tissue | 1.0 |
| Lard | 1.0 |
| Blood | 2.0 |
| Soil | 1.0 |
| Ground animal tissue | 1.0 to 2.0 |

TABLE 2. RECOVERY OF INSECTICIDES ADDED TO VARIOUS SAMPLES

| Insecticide | Percent recovery* 0.1 ppm | 1.0 ppm |
|---|---|---|
| DDT | 94.0 ± 3% | 95.0 ± 3% |
| Lindane | 91.0 ± 3% | 91.5 ± 3% |
| Heptachlor | 91.5 ± 3% | 92.0 ± 3% |
| Dieldrin | 92.0 ± 3% | 92.5 ± 3% |
| Endrin | 88.0 ± 3% | 88.5 ± 3% |

*Average of 6 samples.

TABLE 3. AMOUNT OF ELUANT REQUIRED TO ELUTE SELECTED INSECTICIDES FROM FLORISIL

| Insecticide | Ml of eluant |
|---|---|
| DDT | 200 |
| DDE | 200 |
| Lindane | 200 |
| Heptachlor | 300* |
| Heptachlor epoxide | 300* |
| Dieldrin | 600* |
| Endrin | 700* |

*Eluted only from partially deactivated florisil.

TABLE 4. MINIMUM SENSITIVITY OF THIS METHOD FOR SELECTED INSECTICIDES IN VARIOUS SAMPLES

| Insecticide | Minimum levels detectable (ppm) |
|---|---|
| DDT | 0.05 to 0.20 |
| Lindane | 0.01 to 0.05 |
| Heptachlor or heptachlor epoxide | 0.01 to 0.05 |
| DDE | 0.01 to 0.05 |
| Dieldrin | 0.01 to 0.05 |
| Endrin | 0.05 to 0.20 |

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easily separated. Endrin was eluted as described by Phillips, et al. (6).

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