ANALYSIS OF ANIMAL FOOD PRODUCTS FOR
CHLORINATED INSECTICIDE RESIDUES.

II. SOME FACTORS INVOLVED IN USING ELECTRON CAPTURE GAS CHROMATOGRAPHY

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Summary

The use of ECGC along with proper sample clean-up provides an accurate analytical method for the detection and measurement of trace amounts of chlorinated insecticide residues. Levels of 0.01 ppm of several insecticides may be determined, providing a degree of accuracy comparable to other analytical procedures, if the following precautions are observed: (a) a representative sample is available; (b) the linearity of the instrument is determined for each compound; (c) all reagents are redistilled; (d) only high purity nitrogen is used as the carrier gas; (e) the chromatographic clean-up column is not overloaded with fat; (f) proper elution techniques are followed; (g) the best column packing is used for the insecticides being analyzed; (h) an all glass system is used including a borosilicate glass injector tube and a borosilicate glass column; (i) careful control of temperature and rate of gas flow is performed; and (j) analytical instrument is carefully calibrated with standard insecticide solutions at specified times.

This paper contains more details and problems encountered in developing the technique reported in the first paper of the series (8). Some of these problems can only be avoided by the analyst if he is aware of certain details about reagents and procedures.

The use of microcoulometric (4) and electron capture (9) gas chromatography for the trace analysis of chlorinated insecticides in animal products has certain advantages over chemical methods including the Mills procedures (10) and Schechter, et al. (13). Instrumental methods are more sensitive and in most cases require less sample clean-up.

There are a number of factors which must be considered in using gas chromatography for insecticide residue analysis. Cassil, et al. (3) attempted to inject solvent extracts of vegetable samples directly into a microcoulometric gas chromatograph for analysis. However, failure to separate some residues from impurities resulted in instrument contamination, reduced sensitivity and inaccurate analysis. Coulson, et al. (4) and Morley (11) reported that absorbents used for sample clean-up occasionally contained impurities which made it difficult to achieve the desired sensitivity and accurate results. Beckman and Bevenne (1) and Phillips, et al. (12) indicated that certain insecticides were decomposed when metal analytical columns were used for gas chromatographic analysis.

Langlois, et al. (8) in the first paper in this series outlined a rapid one-step sample clean-up procedure for animal products prior to chlorinated insecticide residue analysis with electron capture gas chromatography (ECGC). However factors other than sample clean-up also must be considered in order to obtain accurate analyses. This paper includes more information on several factors which are involved in the analysis of samples for chlorinated insecticide residues using the one-step sample clean-up method in combination with ECGC.

Methods and Procedures

Clean-up of samples.

The sample clean-up procedure previously described (8) was used with some modifications. All solvents and chemicals used in the procedure were checked for possible contaminants by gas chromatography. Florisil and Florex absorbents were used with and without partial deactivation with water. Two methods of solvent evaporation from samples were examined. The effect of type of sample on accuracy achieved was investigated.

Analytical conditions.

Analytical columns for the Wilkins Aerograph Hi-Fi Model 600 gas chromatograph were prepared from % OD stainless steel, monel metal and borosilicate glass tubing. The columns were packed with acid washed Chromosorb "W" or HMDS treated Chromosorb "W" coated with different amounts of Silicone Dow 11, QF-1 and Epon 1001 as the liquid phase. The columns were conditioned by baking out at 225 C for at least 24 hr with nitrogen gas flow before being placed in a gas chromatograph equipped with an electron capture detector. Standard in-

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secticide solutions in hexane were injected into the gas chromatograph. Peak areas were measured with a Leeds and Northup Model H 1 mv recorder equipped with a disc integrator unit.

Factors which were studied included column length, column material, rate of gas flow, type of packing material and column temperature. Retention times and breakdown of insecticides on columns made from various column tubing materials were compared. Studies were made on the effects of a completely borosilicate glass system including a glass injector port liner on insecticide analysis.

The linear range of the analytical instrument for each insecticide was determined. Factors which might affect the linear range such as impurities in the samples, were considered. A calibration curve for each insecticide was constructed by plotting quantity of insecticide versus area response on log-log paper. Factors affecting the necessity for frequent injection of standard solutions to insure quantitative results were studied. This included the effect of impurities in reagents and samples.

**Results and Discussion**

During development of the one-step column clean-up technique it was found that acetonitrile and sodium sulfate could not be used due to contaminants which each contained. These contaminants interfered in the analysis for nanogram quantities of insecticides. This may have contributed to the artifact as reported by Klein, et al. (7). No artifact problem was encountered in this work using the confirmation test for DDT described by Klein, et al. (7). Florex absorbent was used in preliminary work but considerable fatty material was eluted from florex columns and work with it was discontinued.

Two methods of solvent evaporation were considered, the use of a steam bath and a 50-60 C water bath. Early in the study, recovery experiments indicated that the steam bath method caused too much bumping of samples with occasional loss of solvent and reduction in recovery of insecticide residues.

Variations may be obtained between duplicate samples analyzed by this method depending on the type of sample being analyzed. Fluid milk samples and other dairy products are no problem to sample and appear to have an even distribution of the insecticide residues on a fat basis. Coulson, et al. (4) reported large variations in results on a series of samples from a human liver. The same problem was encountered with poultry and other meat tissue samples in this study. It is desirable to grind a chicken thigh or breast and sample the homogenous mixture when possible. Sampling procedures from non-homogenous materials are of great importance as only 1 or 2 g of tissue are used for an analysis.

Attempts to reduce the volume of eluant needed for sample clean-up were not successful. If florisil was partially deactivated with over 5% water or the concentration of methylene chloride in the eluant exceeded 20%, impurities which interfered with the analysis were eluted from the clean-up column. All samples were carefully checked by visual observation for fatty residue on the bottom of the beakers following the evaporation of the eluant. Any fatty residues indicated a need for repetition of the particular sample to prevent contamination of the analytical column.

Gunther, et al. (6) reported that DDT breakdown on analytical columns could be prevented by addition of tris (2-biphenyl) phosphate to the sample.

**Figure 1. Comparison of DDT analysis on all glass analytical column system versus all metal analytical column system.**

![Figure 1](http://meridian.allenpress.com/jfp/article-pdf/27/8/231/2396612/0022-2747-27_8_231.pdf)
However, Phillips, et al. (12) found no noticeable improvement in elimination of DDT breakdown on metal columns upon use of this compound. Beckman and Bevenne (1) noted that the greatest amount of breakdown occurred on copper columns, less on stainless steel or aluminum columns, and the least on quartz columns. A comparison of results with DDT on borosilicate glass and stainless steel columns is presented in Figure 1. The results presented here agree with results reported by Beckmann and Bevenne (1). The same type of breakdown occurs when a glass injector insert is not used with a glass column.

Breakdown of endrin into two products on metal and borosilicate glass columns as reported by Phillips, et al. (12) was also noted. Temperature and gas flow rate studies were not performed on these breakdown products.

A series of column packings including QF-1, SE 30, Epon 1001, and Dow 11 were used with metal and borosilicate glass columns. Most of these were discarded for a variety of reasons: tailing of peaks, inability to separate combinations of insecticides, long retention times, instability at high column temperatures, and breakdown of insecticides.

Increasing the amount of liquid resulted in longer retention times and more diffuse peak areas. Attempts to use a smaller mesh chromosorb to improve separation led to greatly increased retention times.

The effects of a borosilicate glass column and liner in the injector port of the instrument on the retention time of the five insecticides studied are presented in Table 1. The use of the injector liner resulted in decreased retention times and more sharply defined peaks. An increase in temperature or rate of gas flow also resulted in decreased retention times. Poor peak resolution was obtained at temperatures below 180°C and a nitrogen flow rate of 40 ml per minute.

The optimum resolution of the five insecticides studied was obtained using a 1/8-inch by 5 ft coiled borosilicate glass column packed with 5.0% Dow 11 on 60/80 mesh hexamethyldisilazane (HMDS) treated Chromosorb "W". Separation of the insecticides was achieved within 10 min at the temperature of 191°C and a nitrogen flow rate of 60 ml per minute. DDE and dieldrin were separated as one peak under these conditions.

Work performed by Bonelli, et al. (2) has shown that a polar QF-1 analytical column can separate insecticides which are not separated by a non-polar Dow 11 Column. By this means, compounds such as dieldrin and pp’ DDE, which are not separated by a Dow 11 column can be readily identified. Goodman, et al. (5) have also studied ways by which identification of peaks can be achieved. The use of various detection systems, changing of column parameters, and use of different stationary phases were studied. By noting peak retention times on various analytical columns, the identity of individual peaks may be more positively determined.

To obtain quantitative results, it is important to determine the linear range of the analytical instrument with electron capture detector. The linear ranges for selected insecticides are presented in Table 2.

During the study, results indicated that the injection of standard solutions at the start of analysis, after each series of ten samples and at the end of the analysis, was sufficient providing no contaminated samples were encountered. When a contaminated
Table 2. Linearity of Gas Chromatograph for Selected Chlorinated Insecticides

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Linear range (nanograms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT</td>
<td>0.5 to 100</td>
</tr>
<tr>
<td>Lindane</td>
<td>0.1 to 10</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>0.1 to 10</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>0.1 to 50</td>
</tr>
<tr>
<td>Endrin</td>
<td>0.1 to 50</td>
</tr>
</tbody>
</table>

sample was injected, it was necessary to check the accuracy of the instrument with an injection of a standard solution before proceeding further. Contamination shows up as large peaks on the chromatograph with retention times of 16 to 25 min under the column conditions used in this study.

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References


