treatment. In the length of day experiment, the initial deposits varied between 1467 and 876 p.p.m. on snap beans and between 547 and 301 p.p.m. on potatoes. Here the deposit was in the 70 to 300 p.p.m. range at 11 days after treatment.

These data have a very direct application to the use of phosphamidon under field conditions and support the previous observations made on spinach and broccoli. In practice, one will have to consider the conditions of temperature under which phosphamidon is used. More time will have to be allowed between last application and harvest when colder temperatures are expected. However, the effects observed herein are greatly magnified because of the high concentrations of phosphamidon used. At dosages used in the field, one would not expect to find such pronounced differences because the deposits of phosphamidon are considerably less.

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INSECTICIDE ANALYSIS

The Quantitative Determination of Heptachlor in Pesticide Formulations by Gas Chromatography

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By using lindane as an internal standard, heptachlor can be determined quickly and accurately with an argon ionization detector. A method for determining heptachlor by gas chromatography has been developed and applied to several commercial products. The calibration curve can be prepared in 1 hour when four points are plotted. Additional samples can then be run in 15 minutes. The method is easy, fast, and accurate.

SPECIFIC CHEMICAL ANALYSES of pesticides are often difficult, time consuming, and cumbersome. Gas chromatography has made it possible to determine many pesticides accurately and rapidly. Previous work by Coulson, Cavanagh, and Stuart (*1*); Zweig and Archer (*5*); and Hughes and Freed (*2*) has shown the advantages of this method. More recently, the availability of stationary phases capable of withstanding high temperatures and the sensitive argon ionization detector have made the technique more useful.

Repetitive injections of a given volume of a sample by means of a syringe give variable peak heights unless extreme care is exercised. This variation is undesirable in routine quantitative work. Experience has proved that different analysts employ different injection techniques, and that even when the techniques were standardized, reproducibility was poor. Therefore, an internal standard or marker was employed. In the present work, an unsuccessful attempt was made to use a nonhalogenated internal standard; however, when a halogenated compound was employed, the results became very useful.

Apparatus and Reagents

Gas Chromatograph. Barber-Colman Model 10 equipped with a radium sulfate ionization detector and 5-mv. recorder.

Stationary Phase. General Electric SE-30 silicone gum (5% w./w.) on Chromosorb W (80-100 mesh) packed in a borosilicate glass column (6 feet × 1/4 inch, I.D.).

Syringe. A 10-μl. No. 701N Hamilton syringe was used to inject the samples.

Reagents. Benzene (analytical reagent grade) was used as a solvent for heptachlor (analytical reference grade assaying 99.5%) and lindane (technical grade assaying 99.0%).

Procedure

The internal standard technique introduced by Ray (*3*) was used since it is the most accurate of all methods, ±1% being easily achieved. A detailed discussion of the use of an internal standard was made by Wesselman (*4*).

Standard solutions of lindane and heptachlor were prepared and chromatographed under the following conditions: column temperature, 180° C.; detector temperature, 235° C.; flash heater, 315° C.; argon pressure, 20 p.s.i.; cell voltage, 1000; electrometer gain, 1×10^{-7}; sample size, 1 μl. Under these conditions, lindane has a retention time of 7 1/2 minutes and heptachlor 12 1/6 minutes.

To benzene solutions containing 4 mg. of lindane per ml., heptachlor was added to make concentrations of 5.5, 4.5, 3.5, and 3.15 mg. per ml. In these standard solutions, the ratio of lindane to heptachlor was 1.19, 1.73, 2.59, and 2.93, respectively. The standard curve was obtained by plotting the log of the ratio of the lindane peak height to the heptachlor peak height against the concentration of heptachlor. In all cases, the curve is a straight line.

When assaying materials of unknown concentration, they must be dissolved in the proper volume of benzene containing 4 mg. of lindane per ml. so that the concentrations fall within the limits of the calibration curve; however, the curve can be extended to cover a greater range if desired. In practice, the authors have found it expedient to use the same calibration curve while varying the dilution of the sample being assayed.

To assay heptachlor in a commercial fertilizer (Greenfield Triple Action Crab Grass Killer), 8 grams of the fertilizer was extracted with chloroform for 2 hours in a Soxhlet extractor, the extract was evaporated to dryness, and the heptachlor residue was dissolved in 10 ml. of benzene containing 4 mg. of lindane per ml. of benzene.

Results

After the standard curve was prepared, six lots of heptachlor solution were assayed in duplicate using this method. These solutions having a theoretical concentration of 226 mg. of heptachlor per ml. were found to contain 227, 230, 228, 233, 230, and 224 mg. per ml.

The method was also applied to three lots of technical grade heptachlor (72.0% heptachlor). Duplicate assays showed that the samples contained an average of 71.0, 72.0, and 72.9% heptachlor.

When six lots of commercial fertilizer were analyzed, they were found to contain 0.454, 0.468, 0.471, 0.460, 0.456, and 0.435% heptachlor. These results are in good agreement with the theoretical amount of 0.468% heptachlor.

Precision

To estimate the precision of the method, five samples from the lot of heptachlor solution were independently assayed, giving a 95% confidence interval of 5.56 ± 0.065 mg. per ml. (±1.18%). This test was repeated in the same manner for the fertilizer, giving a 95% confidence interval of 2.81 ± 0.093 mg. per ml. (±3.31%).

At present, the method is being expanded to include simultaneous determination of several pesticides in the same solution, and results of this work will be published as soon as sufficient data have been accumulated.

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INSECTICIDE RESIDUES

Chromatographic Identification of Some Organophosphate Insecticides in the Presence of Plant Extracts

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Two paper chromatographic and detection methods are described for the identification of several organophosphate insecticides in the presence of plant extracts following cleanup on a cellulose-charcoal column. The methods are adequately sensitive for those organophosphates investigated and are relatively rapid and free from interference from plant extracts. These methods have been applied to the identification of only the parent compounds in the presence of apple, lettuce, cabbage, and orange extracts.

WIDESPREAD USE of organophosphate insecticides has created problems for the analyst who is concerned with the detection of undesirable amounts of insecticide residues in foodstuffs of unknown history. There is need for some relatively rapid scheme of qualitative analysis suitable for use as a guide to subsequent quantitative assessment. One approach to this problem would be through a study of the chromatography of plant extracts with the ultimate aim of being able to predict the separation of the insecticide residues from the known chromatographic behavior of the pure substances (*8*). A major problem here is the separation of the insecticide from naturally occurring plant substances that may interfere either with the chromatographic separation or with the final identification tests.

Chillwell and Hartley (*2*) have reviewed the methods for the determination of residual organophosphorus insecticides in foodstuffs. Several paper chromatographic methods for the identification of the pure compounds have been reported (*3–6, 10–12*), but these methods have not been applied generally to the practical problem of identification of organophosphate insecticides in the presence of plant extracts. Recently, Laws and Webley (*9*) reported a method for the determination of organophosphorus insecticides in vegetables, which consists in extraction of the insecticides when added to plant material, separation into petroleum-soluble and water-soluble groups, chromatography of the groups on alumina and activated carbon, respectively, and subsequent determination of the phosphorus by measurement of the molybdenum-blue complex.

The object of a cleanup procedure is to reduce the contribution made to the final determination by natural plant products. The degree of refinement necessary in cleaning up extracts from possible interfering substances, before the final determination, depends to a large extent on the selectivity and sensitivity of the final procedure, the nature of the crop, and the lowest level of insecticide that must be detected with certainty (*2*).

Many cleanup procedures described in the literature are applicable to one type of crop or one specific pesticide. In the present study, a cleanup procedure was sought which would be applicable to paper chromatographic identification of a number of organophosphate insecticides in the presence of a wide variety of plant materials. The methods described have been applied for identification of only the parent compounds when added to apple, lettuce, cabbage, and orange extracts obtained by two extraction procedures. These crops represent waxy plants, leafy vegetables, and citrus fruits, respectively.

Experimental

Apparatus. Chromatographic columns, 40 × 2.5 cm., fitted with stopcock, coarse, fritted-glass disk, and solvent reservoir (ca. 200 ml.) at the top.

Chromatographic tanks. Museum jars, size 11 without specimen supports, Arthur H. Thomas Co., Philadelphia, Pa. Cylindrical jars with covers, size 6 × 18 inches, Canadian Laboratory Supplies Ltd., Montreal.

Chromatographic spray bottles, 50-ml. capacity.

Standard micropipets, graduated in μl. and equipped with a safety pipet filler (Pripipette Instrumentation Associates, New York, N. Y.).

Reagents. All chemicals were analytical reagent grade, and all solvents were redistilled before use.

SOLKA-FLOC. Highly purified wood Cellulose, BW 40 (Brown Co., Boston, Mass.) extracted twice with freshly distilled acetone.

ACTIVATED CHARCOAL. Darco G 60 (Brickman Co., Montreal, Canada).

STANDARD SOLUTIONS. Acetone solutions of the organophosphate insecticides were made up to contain 2 mg. per ml. of solution, and appropriate dilutions were used.

CHROMATOGRAPHIC SYSTEM A. Whatman No. 1 filter paper sheets $8\frac{1}{2} \times 8\frac{1}{2}$ inches; immobile phase, 10% (v./v.) 2-phenoxyethanol in ethyl ether; mobile phase, isoöctane (2,2,4-trimethyl pentane) (*11*).

CHROMATOGRAPHIC SYSTEM B. Acetylated paper sheets 4 × $16\frac{1}{2}$ inches 40 to 45% acetyl (CH_3CO-), Carl Schleicher-Schull, Dassel, Kreis Einbeck, West Germany; immobile phase, 2% (v./v.) U.S.P. mineral oil (light) in ethyl ether, mobile phase, 70% aqueous acetone (*10*).

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