S-(1,2-dicarboxyethyl)*O,O*-dimethyl dithiophosphate is not converted to *O,O*-dimethyl dithiophosphoric acid by the alkali treatment and, therefore, does not interfere.

Cuprous ion interferes by forming with the dithiophosphate a colorless complex that is more stable than the cupric complex. Readily oxidizable materials—e.g., mercaptans(thiols)—if not removed prior to addition of the copper reagent, will reduce the cupric ion and thus give low results. In the procedure described, mercaptans and other acidic impurities are removed by carbon tetrachloride extractions made on acidified aqueous solution just before addition of copper reagent.

Small amounts of the following metallic ions do not interfere: iron(II), iron (III), zinc, nickel(II), cadmium, aluminum, tin (II), and lead. Wettable sulfur, fermate, parzate, cuprocide, aramite, toxaphene, parathion, ovotran, DDT, and methoxychlor do not interfere.

Plant materials which have been analyzed by the method described include alfalfa, apples, barley, beets, broccoli, cauliflower, cottonseed, cranberries, cucumbers, eggplant, grapes, green beans (foliage and pods), kale, lettuce, lima beans (fresh and canned), mustard (fresh and canned greens), onions, peaches (canned), peas (canned), peppers, potatoes, spinach, strawberries, string beans (fresh and canned), Swiss chard, tobacco, tomatoes, and tomato juice (canned).

Ginsburg, Filmer, and Reed (*1*) have applied the method to the analysis of corn, lima beans, lettuce, and onions; Kolbezen (*2*) to analysis of dates, walnut meats, pears, oranges, lemons, milk, avocados, and cantaloupe; and Westlake and Butler (*3*) to analysis of apples, pears, peaches, spinach, snap beans, cucumbers, broccoli, potatoes, strawberries, and peas.

Acknowledgment

Acknowledgment is made of the assistance of E. W. Easter and G. D. Sharkin in carrying out the analyses of the plant materials.

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PESTICIDE RESIDUES

Chemical Determination of Aldrin in Crop Materials

A. E. O'DONNELL, M. M. NEAL[1], and F. T. WEISS
Emeryville Research Center, Shell Development Co., Emeryville, Calif.

J. M. BANN, T. J. DeCINO, and S. C. LAU
Agricultural Research Division, Shell Development Co., Denver, Colo.

Analytical methods for the determination of aldrin residues on the order of 0.1 p.p.m. in agricultural crop materials were needed to obtain data for government requirements. Two methods have been developed. Both involve the extraction of aldrin from the plant matrix by means of a hydrocarbon solvent, followed by separation of the aldrin from dissolved glycerides, if necessary, by saponification and from most of the other dissolved biological materials by adsorption chromatography. The aldrin is then determined in the concentrate either by an improved modification of the phenyl azide–photometric procedure of Danish and Lidov or by determination of chlorine by combustion procedure of Agazzi, Peters, and Brooks. Tests have been made on a wide variety of insecticide-free plant materials and apparent aldrin values of less than 0.08 and 0.05 p.p.m. have been obtained by the chlorine and photometric methods. Recovery of aldrin added in known amounts to extracts of the plant materials generally has been found to be accurate to a few hundredths part per million, calculated on the basis of the crop material. The photometric method has a high degree of specificity for aldrin, whereas the chlorine method is influenced by certain other common chlorine-containing insecticides which are not separated from aldrin.

RELIABLE METHODS for the determination of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydroendo-exodimethanonaphthalene (aldrin) at concentrations on the order of 0.1 p.p.m. in plant materials were needed to satisfy requirements for government registration. Two specific methods, an infrared absorption method (*5*) and a phenyl azide–photometric method (*4*), had been proposed for the determination of microgram quantities of aldrin and were potentially of use. The infrared method appeared less promising for use with plant materials than the photometric method because of the much lesser absorbance of aldrin in the infrared region than of aldrin as the colored product in the visual region in the photometric method (molar absorptivities of approximately 3.6×10^2 and 5.4 and 10^4 liters per mole cm., respectively). In addition, the interference due to absorption of radiation by many biological materials is greater in the infrared region than in the visible region.

Two less specific methods have also been proposed for the determination of microgram quantities of aldrin—the microbioassay method of Sun and Sun (*10*) employing insects, and the quartz tube combustion–amperometric chloride ion titration method of Agazzi, Peters, and Brooks (*1*). The bioassay technique for the determination of aldrin residues in biological materials requires facilities not

[1] Present address, Baxter Laboratories, Morten Grove, Ill.

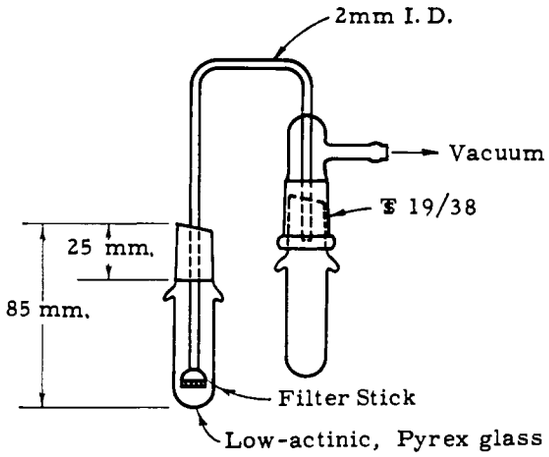


Figure 1. Reaction tube and filter apparatus

generally available at most laboratories. The chlorine determination, on the other hand, is relatively simple and appeared useful provided the aldrin is separated from interfering amounts of naturally occurring (plant) halogen compounds prior to combustion.

In view of their potentialities, the photometric and chlorine methods were investigated further for applicability to the determination of 0.1 p.p.m. of aldrin in plant materials. Success in making the desired determination was first achieved by determining chlorine after extraction of the aldrin from the sample with a hydrocarbon fraction and chromatography of the extract to remove naturally occurring halogen and objectionable crop extractives. Early tests were made using a reduced-scale combustion procedure (*8*). Later applications, made with larger samples and the more versatile macro quartz tube apparatus (*1*) to obtain greater sensitivity, are described in this paper.

Difficulties were encountered with the phenyl azide–photometric method in initial work; the reagent blanks were high and erratic and the recoveries of known amounts of pure aldrin were low and variable. Subsequent detailed investigation of the method, discussed below, showed that by using phenyl azide which was free of phenol impurity and by employing somewhat more drastic reaction conditions, aldrin could be accurately determined. The modified method was found to be applicable to the determination of aldrin in plant materials, using solvent extraction of the sample and chemical and/or chromatographic treatment to prepare the sample for analysis.

Apparatus

Reaction tube for photometric method as shown in Figure 1.

Quartz combustion capsule, as shown in Figure 2.

Evaporation flask, as shown in Figure 2.

Chromatographic column, as shown in Figure 3.

Vacuum evaporator, as shown in Figure 4.

Filter apparatus, as shown in Figure 1.

Spectrophotometer, a Beckman Model B or a Coleman Junior, Model 6A.

Air evaporator, bath, and tube holder, consisting of a manifold for directing a number of streams of clean, dry air downward into a number of reaction tubes, a rack for holding the tubes in place, and a vessel to contain the rack and warm water.

Oven, atmospheric, capable of maintaining a temperature of 85° ± 2° C. or an oil bath capable of maintaining a temperature of 75° ± 2° C. A rack or shelf for holding tubes above the heated bottom of the oven or bath should also be provided.

Equipment suitable for the maceration of crop materials. A commercial food chopper and laboratory mill have proved satisfactory.

Tumbling machine, for tumbling jars containing macerated plant material and solvent.

Reagents

Attasol-Hyflo Super-Cel Adsorbent Mixture (3 to 2 by weight). Heat the mixture for 16 hours at 180° to 200° C. to activate. Attasol is a treated clay manufactured by the Attapulgus Clay Co., Philadelphia, Pa. Hyflo Super-Cel is a filter aid manufactured by the Johns-Manville Co.

Nuchar-Columbia Activated Carbon–Silicic Acid–Attasol Adsorbent Mixture (1:5:5:5 by weight). Nuchar is available from A.S. LaPine and Co., Chicago, Ill. Columbia activated carbon is available from the National Carbon Co., Carbide and Carbon Chemical Corp., Fostoria, Ohio. Silicic acid used was Baker and Adamson Code No. 1169, reagent grade.

Aldrin, melting point 100.5° to 110.0° C. and 99.5% or better purity,

Figure 2. Evaporation flask and quartz combustion capsule

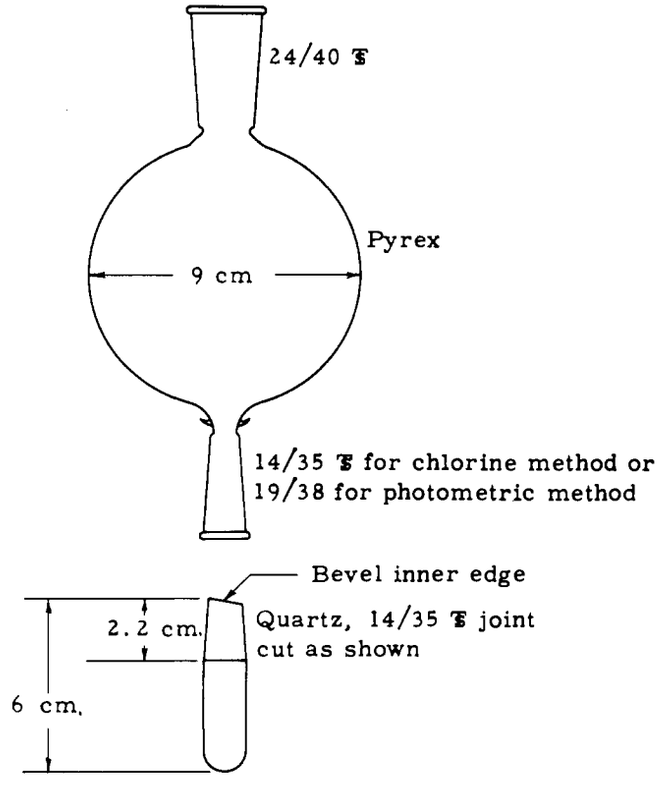
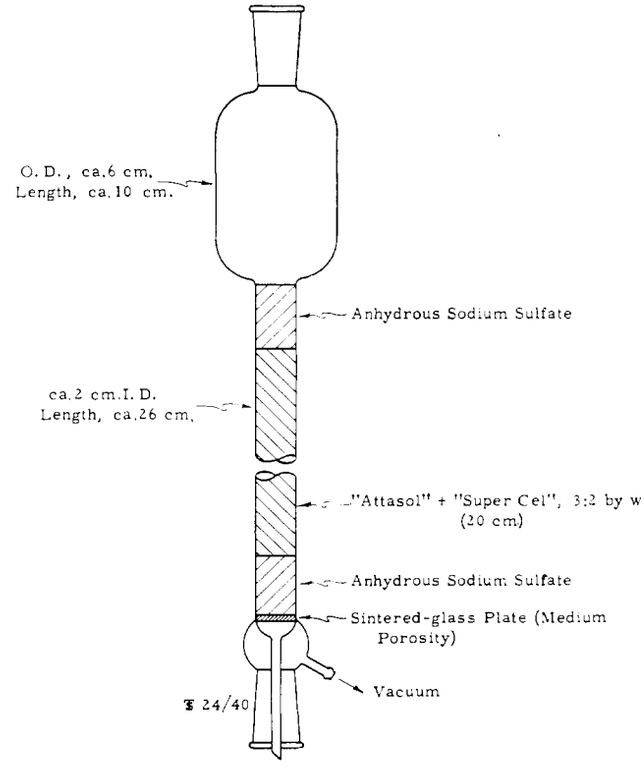


Figure 3. Chromatographic column



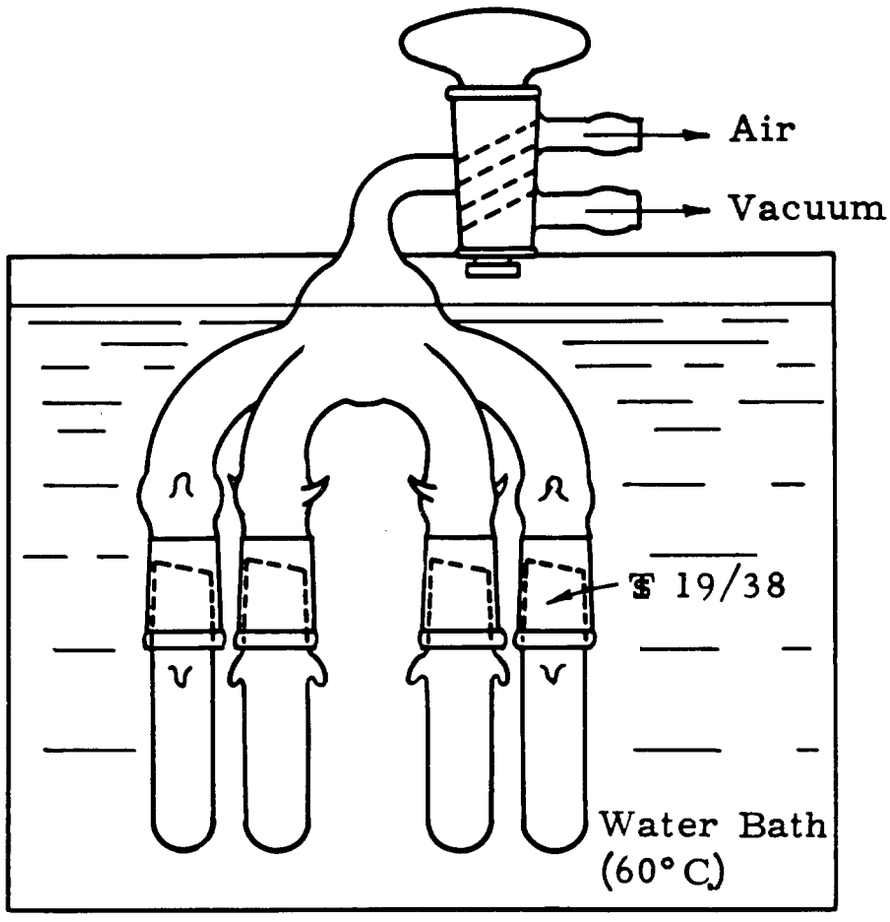


Figure 4. Vacuum evaporator

available from Shell Chemical Corp., Denver, Colo.

Extraction Solvent. A commercial C_6 petroleum fraction having a boiling range of 65° to 72° C., a saturates content of at least 98%, and an evaporation residue of less than 0.0016%. A generally satisfactory solvent is manufactured by the Skelly Oil Co., Tulsa, Okla., under the trade name Skellysolve B and is available from chemical supply houses.

Some lots of this solvent may contain interfering substances, nonvolatile halogen compounds or materials giving absorbance in the photometric method; hence the solvent should be tested by the pertinent method prior to use. If it contains interferences, it can usually be purified by a simple flash distillation, discarding a 10% forecut and leaving 15% bottoms. Contact of the solvent with rubber should be avoided as the solvent dissolves materials which give interference in the photometric method.

Diazotized 2,4-Dinitroaniline. Add 1.5 ±0.05 grams of 2,4-dinitroaniline to 30 ±0.01 ml. of concentrated sulfuric acid and heat to 90° C. to dissolve. Cool the solution in a salt and ice bath, slowly sift in 0.7 ±0.01 gram of finely powdered sodium nitrate, and allow to stand for 1 hour. Transfer the flask to an ice bath and allow to stand for an additional 2 hours.

While stirring the solution, slowly add 40 ± 0.1 ml. of 85% phosphoric acid, being careful to keep the temperature of the solution below 40° C. Allow the solution to stand at room temperature for an additional 2 hours before using. Discard the solution if it darkens to a deep orange color on prolonged standing.

Phenyl Azide Reagent. Prepare according to the method of Lindsay and Allen (*6*).

Prepare a solution of phenyl azide in extraction solvent to contain 0.3 ml. of phenyl azide per milliliter of solution. Wash the solution with two 50-ml. portions of 4% aqueous sodium hydroxide solution and then with 25-ml. portions of water until a washing colorless to phenolphthalein is obtained. Dry the azide solution with anhydrous sodium sulfate filter, and store in an amber bottle in a refrigerator below 5° C. until needed.

Each day, prior to use, withdraw a portion of phenyl azide reagent as required, wash with 10-ml. portions of 4% sodium hydroxide and water, and dry as described above.

Procedure

Maceration of Sample And Extraction of Aldrin Wash the surface of edible crops with water, drain until dry, and discard stones from fruit. Macerate the crop with a food chopper or, for dried grains, a laboratory mill.

Extract a weighed portion of the macerate with a measured volume of extraction solvent by tumbling for 1 hour in a jar. For most materials of low water content use 2 ml. of solvent per gram of material. Use 6 ml. of solvent per gram in the case of alfalfa, clover, ensilage, foliage, hay, range grass, tobacco, and the like. With materials of high water content use 2 ml. of extraction solvent and 0.3 ml. of 99% isopropyl alcohol per gram. Decant the supernatant liquid through a filter paper. When alcohol is used, remove it by washing the filtrate three times with portions of distilled water equal to the alcohol used. Dry the alcohol-free phase over anhydrous sodium sulfate and filter.

Removal of Glycerides Evaporate the solvent from a measured volume of the extract and saponify the residue by refluxing for 1 hour with potassium hydroxide in 95% ethyl alcohol. Use 0.25 gram of potassium hydroxide and 3 ml. of alcohol (minimum of 50 ml.) for each gram of residue. Add water equal to the volume of alcohol and separate the aldrin from the glycerol and soap by extracting six times with volumes of extraction solvent equal to the alcohol used. Wash the combined hydrocarbon phases with portions of distilled water to remove alcohol and caustic.

Swirl, but do not shake the phases in the first washing to avoid emulsification. Dry the washed solution over anhydrous sodium sulfate, filter, and evaporate to 100 ml.

Chromatography Of Extracts **Preliminary Preparation.** For materials to be analyzed for chlorine, take a measured volume of the filtered extract which is equivalent to 200 to 300 grams of the original sample and concentrate to 100 ml. by evaporation on a steam bath.

With fruits and most vegetables to be analyzed by the photometric method, concentrate a measured volume of the filtered extract equivalent to 100 to 200 grams of the original sample to 100 ml. by evaporation on a steam bath. For grasses, forage crops, and certain vegetables including beans, peas, spinach, and root crops transfer a measured volume of extract equivalent to 100 grams of sample to a glass-stoppered Erlenmeyer flask. Add 30 grams of the carbon–silicic acid–Attasol mixture, stopper the flask, and shake vigorously for 1 to 2 minutes. Allow the solids to settle and decant the liquid through a filter paper into a flask having a standard type ground-glass joint, retaining as much of the adsorbent mixture in the flask as is possible. Add 50 ml. of extraction solvent to the flask, stopper, shake, and allow the solids to settle. Filter through the original filter, adding the wash to the original filtrate. Repeat the washing

four additional times, combine the filtrate and washings, and evaporate on the steam bath to a volume of 100 ml.

Columnar Chromatography. Attach a flask to the bottom of the chromatographic column and apply a vacuum (approximately 20 cm. of mercury) to the side arm. While tapping the column, add anhydrous sodium sulfate until a layer 0.5 cm. thick is formed, and then add the adsorbent mixture to a depth of 20 cm. Add a top layer of 3 cm. of anhydrous sodium sulfate and lightly press the surface of the adsorbent, using a flat-ended glass rod. Add 100 ml. of extraction solvent to the column and allow it to pass through the column until the liquid level falls to within 1 cm. of the top of the upper sodium sulfate layer. Release the vacuum, remove the flask from the column, and replace it with a clean, dry flask.

Quantitatively transfer the concentrated extract or the concentrated filtrate from preliminary treatment with adsorbents or from saponification to the column, using several small portions of extraction solvent to complete the transfer. Apply a vacuum and allow the solution to pass through the column until the liquid level drops just below the top of the upper sodium sulfate layer. Wash down the sides of the column with 10 ml. of extraction solvent and draw the liquid level just below the top of the sodium sulfate. Repeat the washing and then add 100 ml. of extraction solvent and draw it through the column until the liquid level drops just below the top of the sodium sulfate layer.

Determination of Aldrin by Chlorine Determination

Attach a combustion capsule securely to the evaporation flask, quantitatively transfer the effluent and washings from the chromatographic column to the assembly, and add approximately 100 mg. of chlorine-free white oil. Insert a distillation trap and evaporate the solvent on a steam bath until the solution, on cooling, is contained in the combustion capsule. Disconnect the tube from the evaporation flask, partially immerse the tube in a water bath at 50° C., and evaporate the remaining solvent using a gentle stream of air.

Determine the chlorine content of the residue in the capsule using the macro quartz tube combustion–reduced scale amperometric titration procedure of Agazzi, Peters, and Brooks (*1*). Avoid contamination of the capsule by handling it only with forceps and washing the exterior first with acetone and then with distilled water prior to inserting it into the combustion tube. Make blank determinations using 100 mg. of the chlorine-free white oil. Subtract the average value of the chlorine found in the blank determinations from that found in the analysis of the sample and calculate the difference as apparent aldrin in the original sample of crop material.

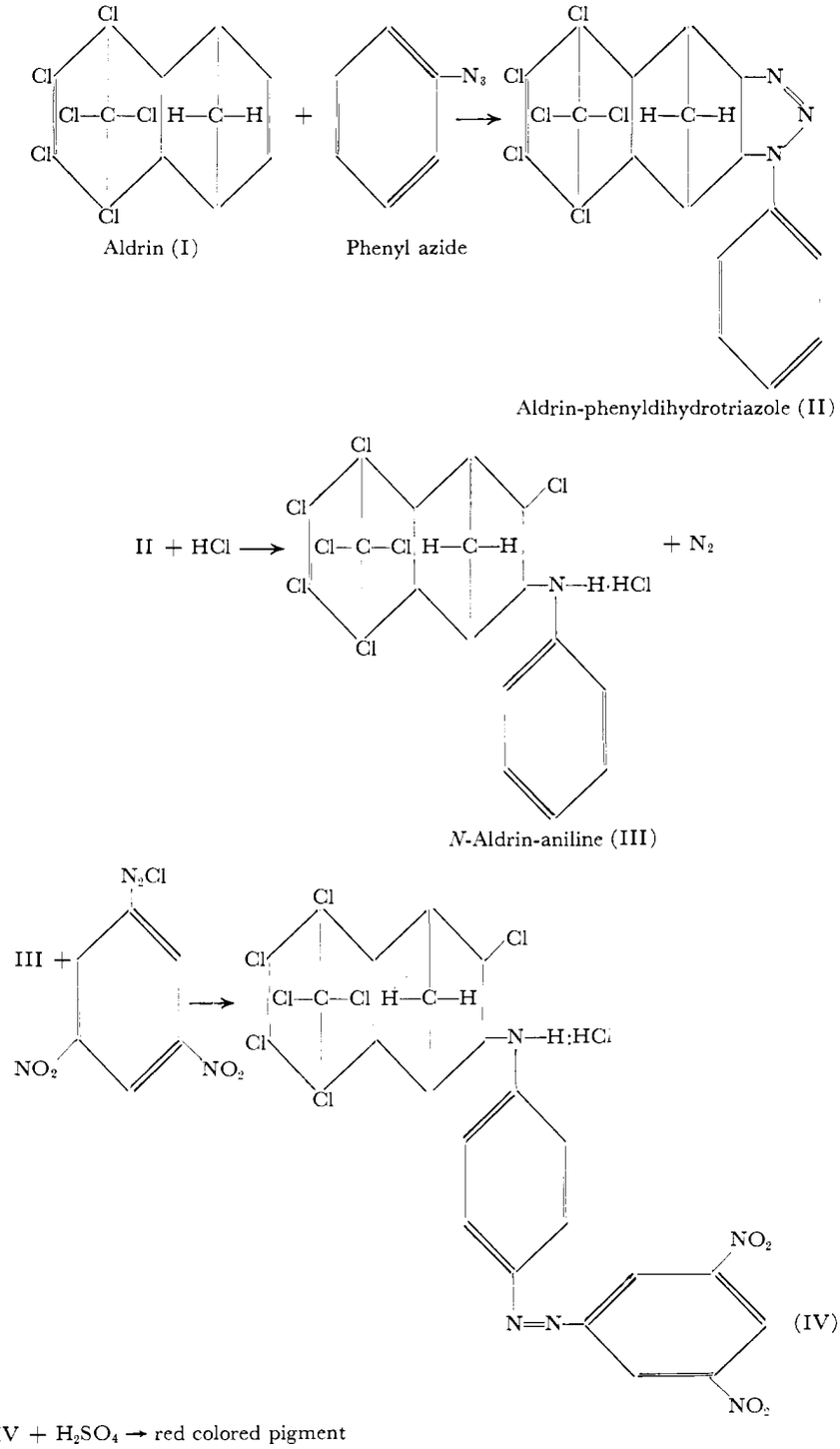


Figure 5. Chemistry of phenyl azide method

Determination of Aldrin by Photometric Method

Calibration of Apparatus. Prepare a graph showing the relationship between absorbance and amount of aldrin as follows:

Prepare standard solutions of aldrin in extraction solvent containing 0, 5, 10, 20, 30, and 40γ per ml. Pipet triplicate 1-ml. aliquots of the solution into separate low-actinic reaction tubes, add 1 ml. of freshly caustic-washed phenyl azide reagent to each of the tubes, and place the tubes in a 40° C. water bath. Direct a gentle stream of air on the surface of the solution until all of the solvent is evaporated. (Approximately 5 minutes should be required to evaporate 1 ml. of solvent.) Do not prolong the evaporation beyond that necessary for removal of solvent, because phenyl azide is volatilized by prolonged blowing with air. Remove the tubes from the bath, and heat in an oven at 85° ± 2° C. for 1.5

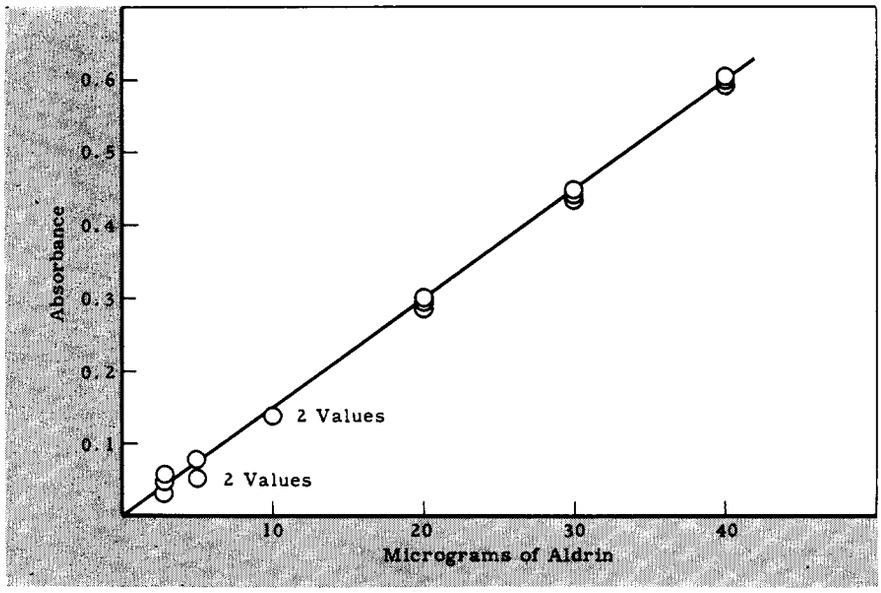


Figure 6. Typical calibration data obtained by photometric method

Beckman Model B spectrophotometer
1-cm. light path
515-mμ spectral position
Reference, water

hours, or in an oil bath protected from light at 75° C. for 30 minutes.

Attach the tubes to the vacuum evaporator and slowly evacuate the tubes until a vacuum of 1 to 2 mm. of mercury is reached. Completely immerse the vacuum evaporator in a 60° C. water bath and allow to remain in the bath until all of the excess phenyl azide is removed from the tubes and the manifold (10 minutes is usually sufficient).

Remove the tubes from the manifold and pipet 5.0 ml. of absolute ethyl alcohol into each tube, and from a buret add 1.0 ml. of concentrated hydrochloric acid. Also from a buret add 0.30 ml. of the diazotized 2,4-dinitroaniline solution, cap the tube, and mix. Allow the tube to stand for 20 minutes, cool in an ice bath, and slowly add 3.7 ml. of 2 to 1 sulfuric acid from a buret. Cap tube, mix solution, and allow to stand for at least 3 minutes but not more than 1 hour.

Transfer the solution to an absorption cell or to a cuvette and measure the absorbance of the solution relative to distilled water at 515 mμ using the spectrophotometer. Subtract the average absorbance of the zero aldrin standards from the absorbance of each of the other aldrin standards. Plot the net absorbances as ordinates against the micrograms of aldrin as abscissas and draw a straight line through the points.

Determination of Aldrin in Sample. Attach a reaction tube securely to the evaporation flask and quantitatively transfer the effluent and washings from the column to the assembly. Insert a distillation trap and evaporate the solvent on a steam bath until about 5 ml. of solution remains in the tube when cooled.

Disconnect the tube from the evaporation flask, and add 1.0 ml. of freshly caustic-washed phenyl azide reagent. Evaporate the solvent and continue as described under Calibration of Apparatus through the removal of excess azide.

Accurately pipet 5.0 ml. of anhydrous alcohol into the tube and suspend the residue by warming in a 40° C. water bath, adding a glass bead if necessary to aid solution. Cool to near room temperature, add the hydrochloric acid, and proceed with formation of the colored solution as in the calibration. Filter the mixture into a clean, dry tube using a clean, dry filter apparatus. Transfer the clear filtrate to a 10-mm. absorption cell or to a cuvette and measure the absorbance relative to distilled water at 515 mμ, using the spectrophotometer.

Make triplicate blank determinations using 100-ml. portions of extraction solvent, commencing at the columnar chromatographic procedure.

Correct the absorbance of the sample by subtracting the average absorbance of the blanks. From the calibration curve determine the weight of aldrin equivalent to the net absorbance and calculate as parts per million in the original sample of crop material.

Investigation of Variables in Phenyl Azide–Photometric Method

Chemistry of Method The reactions involved in the phenyl azide–photometric method are shown in Figure 5. Danish and Lidov showed the product of the reaction between aldrin and phenyl azide to be the aldrin-phenyldihydrotriazole, II (*4*).

The product from the reaction of II with hydrochloric acid was characterized by elemental analysis and by ultraviolet and infrared examination and shown to be the anilino derivative, III. The azo compound, IV, has not been identified, but the structure indicated is that which would be most probable from coupling of the diazonium salt with III.

Results with Pure Aldrin. A typical calibration curve obtained by the method with pure aldrin is given in Figure 6. Essentially identical curves were obtained by five operators who used different batches of reagents and performed their work at various times over a period of several months. Examination of their 66 observations over the range of 0 to 40γ of

Figure 7. Spectral absorbance curve for aldrin product

Beckman Model B spectrophotometer
1-cm. light path
Reference, water

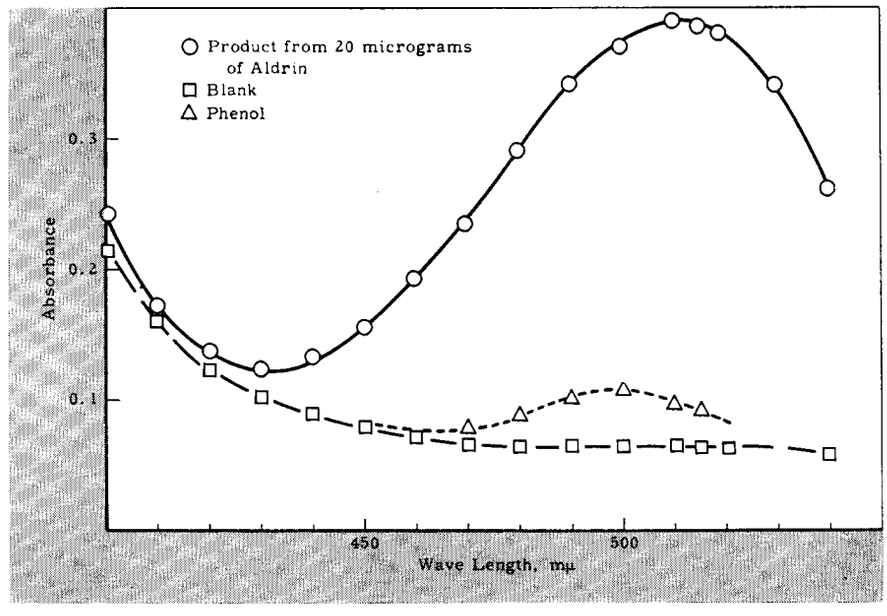


Table I. Determination and Recovery of Added Aldrin in Crops by Chlorine Method

Crop	Aldrin, P.P.M. Added	Determined	Recovery
Beans, snap	0.00	0.02, 0.02, 0.03	...
	0.10	0.09, 0.10, 0.11	0.07, 0.08, 0.09
	0.30	0.24, 0.24, 0.25	0.22, 0.22, 0.23
Cantaloupe	0.00	0.01, 0.04, 0.14	...
	0.10	0.16, 0.12, 0.12	0.13, 0.09, 0.09
Carrots	0.00	0.05, 0.05, 0.05	...
	0.10	0.16, 0.18, 0.18	0.11, 0.13, 0.13
	0.30	0.34, 0.36, 0.37	0.29, 0.31, 0.32
Cherries, Royal Ann	0.00	0.04, 0.02, 0.03	...
	0.10	0.08, 0.11, 0.14	0.06, 0.09, 0.12
	0.30	0.29, 0.34, 0.36	0.27, 0.32, 0.34
Clover, Ladino	0.00	0.00, 0.02, 0.03	...
	0.10	0.12, 0.15, 0.17	0.06, 0.09, 0.11
	0.30	0.33, 0.35	0.27, 0.29
Onions	0.00	0.01, 0.03, 0.04	...
	0.10	0.11, 0.11, 0.11	0.07, 0.07, 0.07
	0.30	0.30, 0.33, 0.59	0.26, 0.29, 0.55
Pineapple	0.00	0.02, 0.02, 0.03	...
	0.10	0.11, 0.11, 0.14	0.09, 0.09, 0.11
Tomatoes	0.00	0.02, 0.02, 0.02	...
	0.10	0.10, 0.11, 0.13	0.08, 0.09, 0.11

aldrin revealed the standard deviation of the method to be 1.0γ of aldrin.

Purity of Phenyl Azide The spectral absorbance curve for a typical reagent blank is shown by the lower curve in Figure 7 and that for a typical aldrin product by the upper curve. The intermediate curve is characteristic of that obtained with phenol by the method and is similar to that encountered with some batches of distilled but not caustic-washed phenyl azide found to contain phenol as an impurity.

Conditions for Formation of Aldrin-Phenyldihydrotriazole

Amounts of Phenyl Azide. The volume of phenyl azide reagent used (1 ml. of 30%) for each determination is in large stoichiometric excess to assure complete reaction in the presence of biological extractives. The absorbance of the blank with this amount of reagent usually corresponds to 3 to 4γ of apparent aldrin.

Time and Temperature. The heating of aldrin and phenyl azide mixture, from which all solvent had been removed, in an oven at 75° to 80° C. for exactly 30 minutes as proposed by Danish and Lidov was found to give low and erratic results. To find the conditions necessary for more complete and uniform reaction, mixtures of aldrin and purified phenyl azide were heated in an oven and in an oil bath at different temperatures for varying periods and the aldrin-phenyldihydrotriazole was measured by the photometric procedure. The results obtained are plotted in Figure 8. Further tests showed that reproducible and essentially complete reaction could be achieved by heating in an oven at 85° C. for 1.5 hours. Heating at temperatures in excess of 95° C. in either oven or oil bath was found to cause erratic results, probably due to gradual decomposition of reagent.

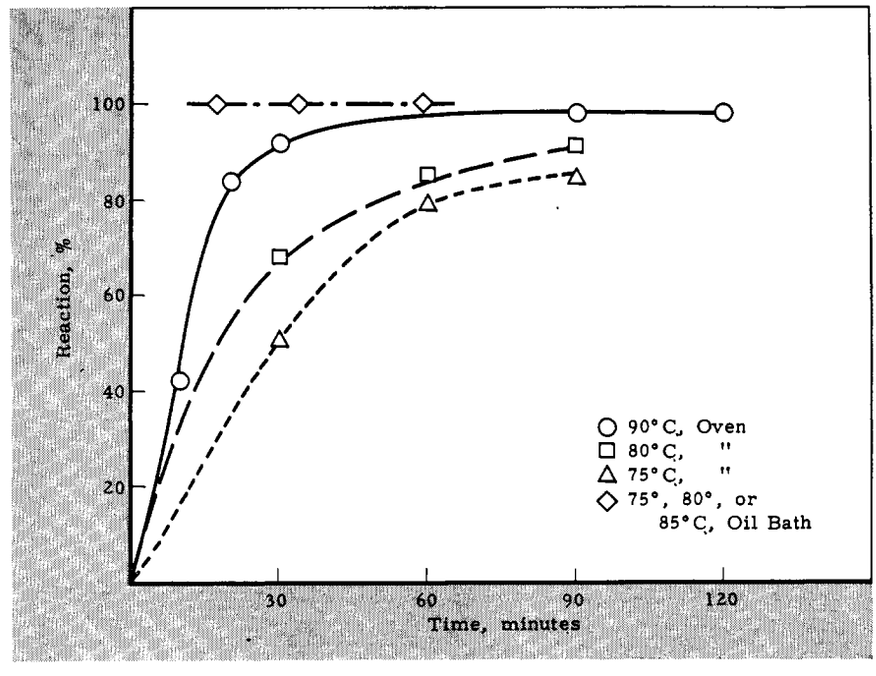
Presence of Nonreactive Diluents. Tests with 0.3 ml. of unremoved extraction solvent showed the presence of diluent to be without effect on the extent of formation of the aldrin-phenyldihydrotriazole on heating for 1.5 hours in an oven at 85° C. Most, if not all, of the solvent probably evaporated during the heating period. Tests with 25 mg. of a *n*-paraffin wax (melting point approximately 60° C.) showed it to be without effect on the reaction of 0 to 40γ of aldrin. However, the results with 50 mg. of wax were variable and tended to be low by as much as 25%.

Conditions for Removal of Excess Reagent. The effect of 3 mg. of unremoved azide corresponds to approximately 1γ of aldrin. Reduction of residual azide below the amount of 3 mg. is desirable, therefore, and is achieved in the method.

Conditions for Coupling And Dye Formation The effect of minor variations in the coupling and color forming steps was found to be negligible, provided the correct order of addition of reagents was followed and thorough mixing achieved. When waxy diluents were present, it was necessary to warm the alcohol before adding the hydrochloric acid to assure that the aldrin-phenyldihydrotriazole was all in solution. The standing of the coupled product for 40 minutes rather than the recommended 20, prior to the addition of sulfuric acid, was without deleterious effect.

When materials were present which were not soluble in the colored solutions—for example, plant and paraffin waxes—it was necessary to clarify the solution before measuring the absorbance. This was done either by filtration, as described in the method, or alternatively the aldrin-phenyldihydrotriazole was reacted with hydrochloric acid in ethyl alcohol for 20 minutes to form the *N*-

Figure 8. Effect of time and temperature of oven or oil bath on extent of reaction of aldrin with phenyl azide



aldrin aniline salt (see Figure 5). The solution was then extracted with 3 ml. of extraction solvent and the hydrocarbon phase drawn off, using a hypodermic syringe, and discarded. The aniline salt was then coupled and colored in the usual way, the solution centrifuged (2500 r.p.m.) to remove turbidity, and the absorbance of the clear solution measured.

Separation of Aldrin from Plant Materials

The procedures used to extract the aldrin from the plant materials were essentially those used in earlier investigations of bioassay methods for aldrin (*9*). A single equilibrium extraction of the macerated crop was made with a measured volume of extraction solvent, and, as in the AOAC method for DDT (*3*), it was assumed that the concentration of aldrin in recovered extract was the same as that in the solvent held by the macerate. Isopropyl alcohol was used together with the hydrocarbon for crops of high moisture content to prevent emulsification and the alcohol was washed from the recovered extract with water. Tests have shown that the distribution of aldrin between water-alcohol and extraction solvent, as used in the method, was entirely in favor of the hydrocarbon phase.

The amount of biological material dissolved from glyceride-free materials was within the range of 0.03 to 3%. Glycerides were dissolved in large amounts by the extraction solvent. The chlorine content of the extractive materials was found to be from 0.07 to 3 p.p.m., calculated as apparent aldrin in the crop and was both inorganic and organic in nature.

Aldrin had to be separated from dissolved plant materials before being determined. The purpose was to reduce plant chlorine compounds to a negligible amount and to remove metal-containing compounds, such as chlorophyll. Metals are known to retain chlorine to some extent in the combustion tube, thus reducing the accuracy of chlorine determination. The separation of aldrin from colored pigments and most of the waxes (<25 mg. for best results) was necessary before the photometric method was applied.

Tests involving partitioning with solvents, including extraction solvent and acetonitrile, steam distillation, and dewaxing by crystallization, had shown that, in general, these procedures were less useful than chromatography for separating aldrin from crop extractives. Attasol adsorbent was particularly effective in making the needed separations, because of its good capacity for most crop extractives. Certain crops, however, such as grasses and forage crops, as well as certain vegetables, including beans, peas, and spinach, gave extracts containing such a high amount of crop extractives that the extract could not be cleaned by use of the Attasol column alone. In such cases, shaking of the extract with an adsorbent mixture containing carbon, silicic acid, and Attasol was found to remove a large amount of the plant materials from the extract and to give a solution from which the remaining biological materials could be adequately removed by subsequent columnar chromatographic treatment. Glycerides were not retained by the adsorbents but could be hydrolyzed with caustic without affecting the aldrin. The glycerol and soap formed were separated from the nonsaponifiables and aldrin by partitioning between aqueous alcohol solution and extraction solvent, as in biosassay methods for aldrin (*9*). Subsequent chromatography over Attasol of the hydrocarbon phase then removed most of the nonsaponified extractives.

Applicability to Determination of Residues in Crops

To test the applicability of the methods to the determination of aldrin residues in crops, known amounts of the insecticide were added to extracts of insecticide-free crops and the solutions analyzed. Aldrin levels in the "recovery" samples ranged from 0.03 to 0.3 p.p.m. expressed as concentration in original sample

Samples of 300 grams of crop material were used in tests employing analysis for chlorine to permit determinations of concentrations as low as 0.04 p.p.m. of insecticide in the crop. For test by the photometric method, samples of 100 to 300 grams of crop were used. Concentrations as low as 0.02 p.p.m. of aldrin can be determined with samples of 100 grams or more, but the larger samples were preferred because of the greater reliability that could be obtained.

Results representative of those obtained on nineteen crops by the chlorine method are given in Table I, and results representative of those obtained on 44 crops by the photometric method are given in Table II. Values of 0.08 p.p.m. or less apparent aldrin were obtained, with minor exceptions, for the insecticide-free crops by the chlorine method, as shown in Table I. Recovery of added aldrin by the chlorine method generally was accurate to a few hundredths of a part per million, calculated as concentration in the crop. By the photometric method, apparent aldrin values of 0.05 p.p.m. or less were obtained for the insecticide-free crops (see Table II) and the values for recovery of added aldrin

Table II. Determination and Recovery of Added Aldrin in Crops by Photometric Method

	Aldrin, P.P.M.		
Material	*Added*	*Determined*	*Recovery*
Alfalfa, meal	0.00	0.02, 0.04, 0.05	...
	0.10	0.14, 0.15, 0.16	0.10, 0.11, 0.12
Beans, snap, fresh	0.00	0.00, 0.01, 0.01	...
	0.05	0.05, 0.06, 0.05	0.04, 0.05, 0.04
	0.10	0.09, 0.09, 0.08	0.08, 0.08, 0.07
Cantaloupe	0.00	0.00, 0.00, 0.01	...
	0.03	0.03, 0.04	0.03, 0.04
	0.10	0.10, 0.11	0.10, 0.11
Cherries, Bing	0.00	0.01, 0.01, 0.00	...
	0.03	0.03, 0.04, 0.04	0.02, 0.03, 0.03
	0.10	0.10, 0.08, 0.11	0.09, 0.07, 0.10
Clover, Ladino	0.00	0.03, 0.03, 0.05	...
	0.05	0.08, 0.08, 0.09	0.04, 0.04, 0.05
	0.10	0.14, 0.13, 0.15	0.10, 0.09, 0.11
Corn	0.00	0.04	...
	0.10	0.13	0.09
	0.30	0.03	0.26
Wheat	0.00	0.02, 0.03, 0.03	...
	0.10	0.11, 0.12	0.08, 0.09
Onions	0.00	0.04, 0.03, 0.01	...
	0.10	0.13, 0.13, 0.14	0.10, 0.10, 0.11
	0.30	0.29, 0.31	0.25, 0.28
Cabbage	0.00	0.04	...
	0.10	0.12	0.08
	0.30	0.31	0.27
Tomatoes	0.00	0.01, 0.01, 0.01	...
	0.10	0.09, 0.09, 0.10	0.08, 0.08, 0.09
Turnips	0.00	0.01, 0.02, 0.03	...
	0.10	0.10, 0.11, 0.12	0.08, 0.09, 0.10

Table III. Interference of Insecticides and Other Compounds as Aldrin by Chlorine and Photometric Methods

Compound	Interference as Aldrin, %		
		Photometric Method	
	Chlorine method	Without chromatography	With Attasol
Chlordan (technical)	39	0.0	...
Dehydrohalogenation product	22	1.3	0.1
Dieldrin (99.5%)	0.0	0.1, 0.2	...
DDT	4.7	0.2	...
Dehydrohalogenation product	64	0.0	...
DDD	<1.0	0.1, 0.2	...
Dehydrohalogenation product	60	0.0	...
Endrin (97%)	0.1	0.3, 0.6	...
Heptachlor	...	0.1, 0.3	...
Isodrin (spatial isomer of aldrin)	90, 95	2.1, 2.4	2.1, 2.4
γ-Benzene hexachloride	62	0.0	...
Dehydrohalogenation product	58	0.0	...
Toxaphene (technical)	54	0.1, 0.1	...
Dehydrohalogenation product	40	0.3	0.2
Allethrin (technical)	...	6	0.0
Octacide 264	...	48	0
Parathion (technical)	...	0.1	...
Piperonyl cyclonene (technical)	...	0.0	...
1-Dodecene (b.p. 213° C.)	...	40	[a]
Cyclopentene (b.p. 45° C.)	...	0.7, 0.9	[a]
Cyclohexene (b.p. 83° C.)	...	0.1	[a]
3-Methylcyclohexene (b.p. 102° C.)	...	3.3, 4.8	[a]
Tricyclopentadiene	...	122	[a]
2,5-Endomethylene-Δ^4-tetrahydrobenzoic acid	...	198, 200	0.8
3,6-Endomethylene-Δ^4-tetrahydrophthalic acid	...	165, 178	0.0
D-Limonene (b.p. 177° C.)	...	2	[a]
α-Ionene (b.p. 148° C.)	...	14	[a]
Pinene (b.p. 154° C.)	...	4.5	[a]
α-Carotene (70%)	...	3	0.1[b]

[a] In general, Attasol would have but limited capacity, if any, for retaining the compound. [b] 3.1 mg. of carotene (70%) used in test. Carotene in amounts usually present in crop extracts is removed by Attasol.

generally were accurate to 0.03 p.p.m. or better, expressed as concentration in the crop.

The results of the above tests indicate that to obtain most reliable values for residues of 0.1 p.p.m. or less of aldrin, the apparent aldrin value for the sample should be corrected for the apparent aldrin value for an insecticide-free control sample determined by concurrent analyses. The importance of making this correction will probably be greater with the chlorine method than with the photometric method because of the lower general specificity of the chlorine method. Certain constituents, such as olefinic or chlorine-containing compounds of some crops, may not be separated from aldrin by the procedures described and may cause interference in the methods. For example, mint was not analyzed successfully at the 0.1 p.p.m. level by either method.

Specificity of Methods

A variety of insecticides, generally in 3-mg. amounts, and other compounds which might react with phenyl azide or be encountered in determining aldrin in plant materials were tested for interference as apparent aldrin in the phenyl azide–photometric method, omitting the chromatographic step. Those materials which were found to interfere appreciably were then tested again using the Attasol chromatographic step. A number of chlorine-containing insecticides and the products from dehydrohalogenation of these insecticides with alcoholic-caustic were analyzed by the chlorine method.

The results of these tests are presented in Table III. Several of the chlorine-containing materials were found to interfere to a large extent in the analysis by the chlorine method. The data on interference of the dehydrochlorination products (Table III) are noteworthy in view of the ease of dehydrochlorination of certain insecticides. Mattson and coworkers (7) found indications of dehydrochlorination products of DDT in human fat when DDT was included in the diet. Because of its limited specificity, chlorine method (see Table III) is useful primarily with crops whose history of treatment with chemicals is known.

None of the insecticides interfered critically in the analyses by the photometric method, although octacide interfered to 48% when the chromatographic step was omitted; this is attributed to its bicycloheptene structure. The small interference of isodrin, the spatial isomer of aldrin, is attributed to failure of the isodrin–phenyl dihydrotriazole to couple with the diazotized aniline (9) (see Figure 5). The interference by 1-dodecene in the photometric method was unexpected, considering information previously available on the reaction of phenyl azide (2). The lesser interference of the olefins of lower molecular weight (see Table III) is probably due in part at least to volatilization of the olefin during the heating step. Fortunately, no interference from nonvolatile simple olefins in crops has been observed. Interference from terpenes and carotenes, omitting the Attasol step, was of the order of a few per cent, except with α-ionene, which interfered to 14% as aldrin. Carotene, except in excessive amounts, is adsorbed by Attasol and does not interfere in the determination of aldrin in crops. Terpenes, if present in more than small amounts in a crop extract, would pass through the Attasol column and partially interfere as aldrin.

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