In general, the level of minerals in vegetation from the Talbot terrace is low with respect to higher terraces, although it has not always been possible to show a significant difference from that on the Penholoway terrace.

Conclusions

An intensive study over a period of 2 years of the concentration of cobalt, copper, manganese, calcium, and phosphorus in five native browse and forage species showed that sampling leaves of the swamp blackgum, *Nyssa sylvatica* Marsh. var. biflora (Walt.) Sarg., was, except for calcium, an efficient method of estimating the mineral status of native forage species.

Two samples of blackgum leaves from each soil plot were adequate for grouping soils with respect to the mineral nutrients, cobalt and copper, in broomsedge. Five samples were required for estimating the nutritional adequacy of phosphorus and significant differences in the manganese in broomsedge. Black gum was superior in this respect to other plant species tested.

The best estimate of the calcium status of these soils was obtained from five samples of broomsedge collected from each plot in July of either of two years. Although some relationship of soil cobalt to the cobalt concentration in broomsedge and blackgum leaves was apparent, the cobalt content of the soil was not a sufficiently sensitive estimation of that in the plant to permit its classification in terms of nutrient requirements of animals.

Acknowledgment

The authors wish to acknowledge the work of Allen H. Hasty and Glenn H. Robinson of the Soil Survey, Soil Conservation Service, who delineated and classified the soils used in this experiment. Credit is also due to Stephen G. Boyce, now of the Department of Botany, Ohio University, who collected and identified specimens of each of the species used in this study.

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PESTICIDE RESIDUES

Spectrophotometric Determination of Heptachlor and Technical Chlordan on Food and Forage Crops

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To secure approval from federal and state regulatory agencies for the use of heptachlor and technical chlordan, it was necessary to measure the residue resulting from treatment of food and forage crops with these toxicants. Analytical methods were tested to find which were most specific and which were the least affected by plant extractives and other pesticides. The Davidow method for chlordan and the Polen-Silverman method for heptachlor were modified by microtechniques to detect toxicant in the 2.5- to 5.0-γ range. Methods were developed which used chromatography to separate as little as 2γ (or 0.01 p.p.m.) of toxicant with recovery in the range of 80% from as much as 2 kg. of crop material. Good agreement with bioassay methods was found. Analyses of a large number of crops treated with recommended dosages of heptachlor and chlordan show no significant residue present at harvest time.

THE NEED FOR ACCURATE METHODS for the specific determination of micro quantities of chlordan (technical chlordan, the principal component of which is 1,2,4,5,6,7,8,8-octachloro-3a,4,-7,7a-tetrahydro-4,7-methanoindan) and heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene) on food and forage crops has led to the investigation of the published methods of determining these toxicants. The methods based on total chlorine were examined but were not adopted because of their lack of specificity. The colorimetric methods of Allesandrini and Amormino (*1*), Ard (*2*), Davidow (*3*), Harris (*4*), Palumbo (*8*), and Polen and Silverman (*9*) were investigated. Microtechniques were developed to use the Davidow method for chlordan and the Polen-Silverman method for heptachlor.

The crops are ground, dried chemically with anhydrous sodium sulfate, and extracted with "colorimetric" pentane. The pentane extracts are processed to remove plant pigments and waxes and are treated with the color-forming reagent. The intensity of the color formed with the reagent is determined with a spectrophotometer adapted for sampling on a micro scale. The toxicant content is then interpolated from a

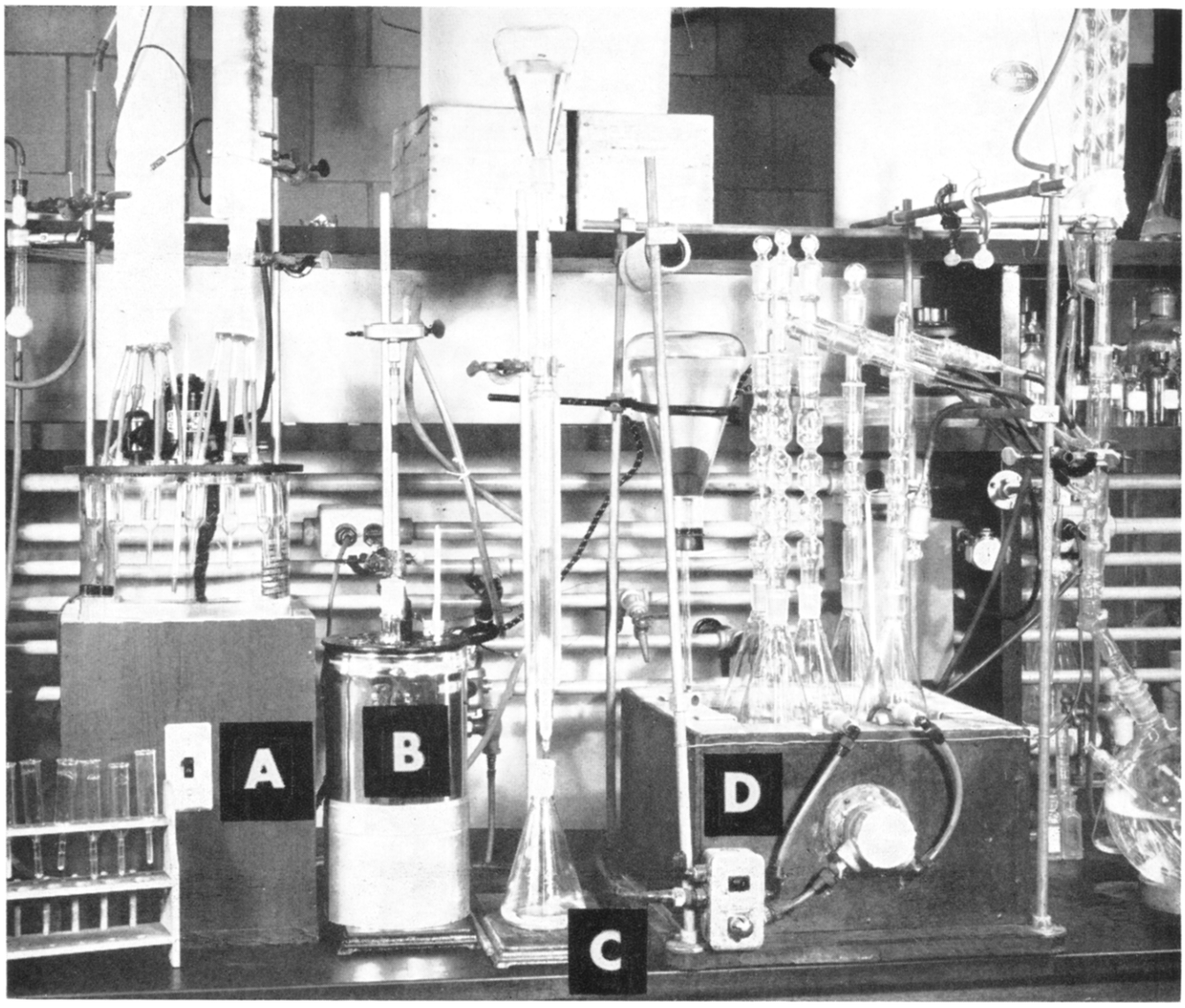

Figure 1. Setup of apparatus

standard calibration curve for the toxicant under consideration.

General Reagents Florisil, 60/100 mesh (Floridin Co., Warren, Pa.) is activated by heating at 130° C. for 24 hours in an oven.

Fuming-concentrated sulfuric acid, 1 to 1 (volumetric) mixture, is made from 15% fuming and concentrated sulfuric acid, reagent grades.

Potassium hydroxide pellets, reagent grade.

"Colorimetric" pentane (Phillips Petroleum Co., Special Products Division, Bartlesville, Okla.) or pure grade *n*-pentane especially purified by washing with concentrated sulfuric acid, followed by 1.0*M* potassium hydroxide solution saturated with potassium permanganate and distillation over potassium hydroxide pellets. The solvent must pass the following interference test.

Two liters of the solvent, evaporated on a 50° C. water bath through a three-bulb Snyder column to near dryness and made to react with Polen-Silverman or Davidow reagent under conditions described in the discussion on preparation of standard curves, shall not exhibit color in excess of the equivalent of 1.5 γ of heptachlor or chlordan nor shall there be any apparent residue from 2 liters of pentane when it is taken to dryness at 50° C.

Colorimetric pentane must not be stored in solvent drums, but must invariably be stored and shipped in glass.

Sodium sulfate anhydrous powder, reagent grade, must pass the following interference test.

One kilogram of anhydrous sodium sulfate extracted for 4 hours in a Soxhlet extractor with colorimetric pentane shall not contribute color in excess of that equivalent to 4 γ of toxicant when the extract is treated as in the interference test for pentane, nor shall color, residual oil, nor cloudiness be present in the concentrated extract before addition of the colorimetric test reagent.

Heptachlor Reagents Florex XXS, 60/90 mesh (Floridin Co., Warren, Pa.) is activated by drying at 130° C. for 48 hours in an oven.

Ethanolamine (Carbide and Carbon Chemicals Corp., New York, N. Y.) is distilled at atmospheric pressure; a center cut is taken from the distillation.

Butyl Cellosolve (Carbide and Carbon Chemicals Corp.) is distilled over potassium hydroxide pellets at atmospheric pressure.

Benzene–isopropyl alcohol solution is prepared from 4 parts of benzene (reagent grade) and 1 part by volume of isopropyl alcohol (reagent grade).

Heptachlor reference standard (melting point 91–95° C., minimum assay 97.5%), can be obtained from the Velsicol Corp., 330 East Grand Ave., Chicago 11, Ill.

Polen-Silverman reagent is prepared by dissolving 33 grams of 85% potassium hydroxide in 28 grams of distilled water, cooling to room temperature, adding an equal volume of butyl Cellosolve and 30.5 grams of monoethanolamine, and diluting to 1 liter with butyl Cellosolve. This solution, after settling for several days, is decanted from any sediment and

diluted with an equal volume of benzene.

The reagent does not darken and the sensitivity does not change appreciably on storage if a center cut of the ethanolamine, freshly distilled at atmospheric pressure, is used and the butyl Cellosolve is distilled from potassium hydroxide pellets at atmospheric pressure before use.

As more or less aging has been observed in this reagent, depending on its method of preparation, accelerated aging as suggested by Polen and Silverman (heating for 5 hours at 50° C. in a closed glass system) should be undertaken if it is necessary to use the reagent before it has been stored for a month (*9*). The sensitivity of the reagent should be frequently checked against the standard calibration curve by running standards.

Chlordan Reagents

Activated alumina F-20 (Aluminum Co. of America, East St. Louis, Ill.) is dried to standard activity by heating at 130° C. for 24 hours under vacuum provided by a water aspirator.

Diethanolamine (Carbide and Carbon Chemicals Corp.) is prepared by distilling 1 liter at a pressure of 20 mm. of mercury. The first 100 ml. of distillate is discarded and the next 50 to 100 ml. used.

Methanol, 99%, reagent grade.

Modified Davidow reagent is prepared by combining volumetrically 2 parts of 1.0*N* methanolic potassium hydroxide and 1 part of diethanolamine and diluting with 9 parts of methanol. The modified reagent has been developed specifically for the micro reaction to provide a more sensitive reagent and to provide a low viscosity reagent that will ebullate evenly in the micro reaction tubes. When macro reactions are run using more than 0.5 ml. of reagent, Davidow's original reagent is recommended (*3*). Aging of the modified Davidow reagent and frequent checking against the standard calibration curve are recommended.

Diethyl ether, absolute, reagent grade.

1*N* potassium hydroxide, in methanol.

Methanol-water solution, 90% methanol.

Reference grade technical chlordan (obtainable from Velsicol Chemical Corp., 330 East Grand Ave., Chicago, Ill., or Wisconsin Alumni Research Foundation, 506 North Walnut St., Madison 1, Wis.) is a batch of technical chlordan arbitrarily chosen and set aside for reference purposes, to which all analytical data are referred.

Special Equipment

Food grinder (Enterprise Model 2-515, U. S. Slicing Machine Co., Chicago, Ill.) or equivalent.

Soxhlet extractors 500, 1500, and 5000 ml., (Ace Glass Co., Vineland, N. J.)

Three sizes of nylon filter disks to fit the above extractors are prepared by fitting No. 10 gage wire rings (7.5, 9, and 15.5 cm. in diameter) with nylon No. 102 filter cloth. The cloth (supplied by Albert Godde, Bedin, Inc., New York, N. Y.) is sewn on the wire rings.

Chromatographic columns are 50-ml. Mohr burets.

Water jackets for chromatographic columns (*C*, Figure 1, and *A*, Figure 2) (W. J. Podbielniak, Inc., 341 East Ohio St., Chicago, Ill.) consist of 17-mm. smooth-bore tube 20 to 21 inches long. A water jacket covers the bottom 14 inches of the tube. In use the Mohr buret is suspended inside the tube of the water jacket and is held in place by a beveled ring cut from Tygon tubing.

Three-ball Snyder columns are fitted with 24/40 ⅋ inner joints.

Polyethylene bags, 3-mil thickness in two sizes, 10 × 20 and 18 × 28 inches, are used.

Heating mantles (Glass-Col) are used for heating the flasks during extraction and stripping of solvent. The temperature is controlled with variable transformers.

The concentrating bath (*D*, Figure 1) consists of a thermostatically controlled electrically heated water bath held at 50° C. A galvanized iron grating is installed to support at least six 500-ml. Erlenmeyer flasks immersed in the bath to a depth of 2 cm.

The pentane evaporation bath for micro reaction tubes (*A*, Figure 1) consists of a 2½-gallon battery jar (Cenco 15202, size 2) containing water maintained at 40° C. for evaporating the pentane in the reaction tubes. Clean, dry air is directed into the top of the reaction tubes by a manifold of jets to promote smooth evaporation.

The reaction bath (*B*, Figure 1) consists of an insulated electrically heated ethylene glycol bath, held at 100° ±

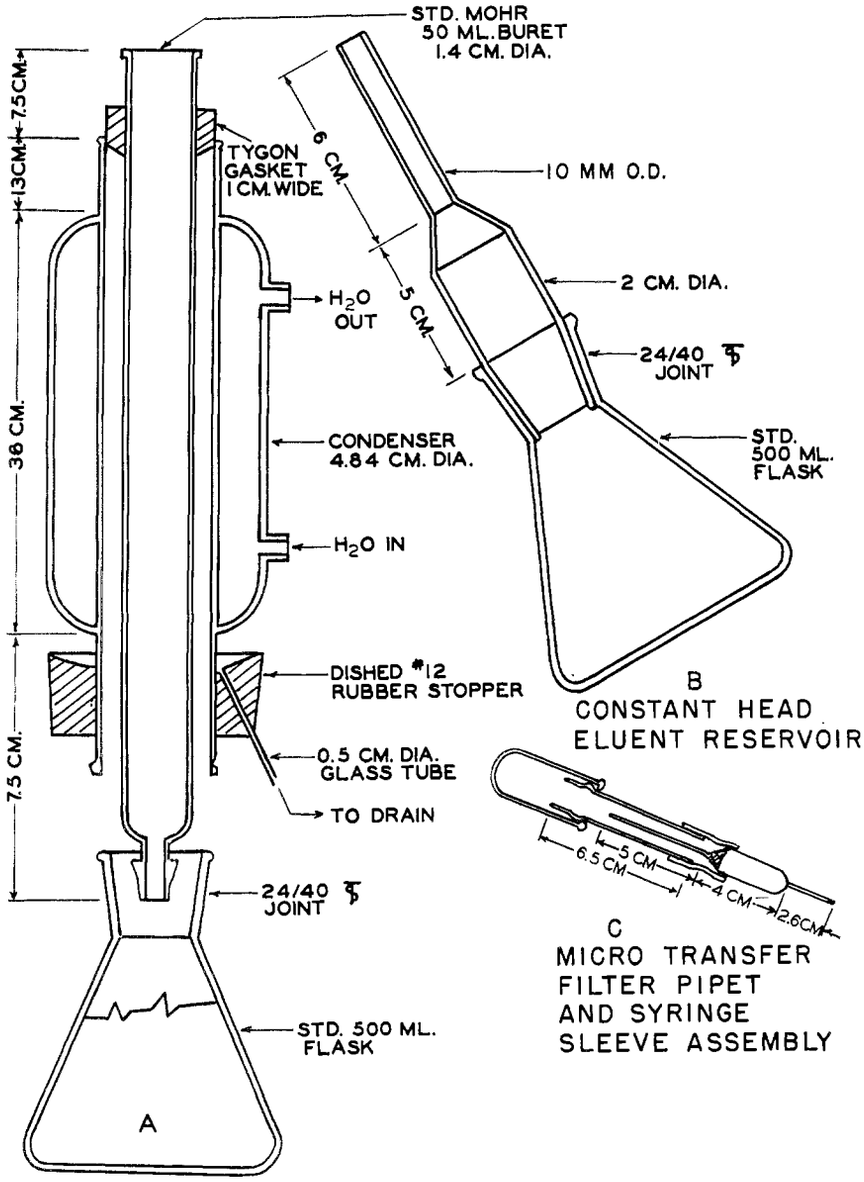

Figure 2. Chromatographic column assembly

0.5° C. by an immersion heater, a mercury thermostat, and an auxiliary relay. The glycol is continually stirred by an air-driven stirrer.

Micro reaction tubes (*A*, Figure 1) are constructed by sealing a capillary tube 4 mm. in inside diameter and 4 cm. long to the constricted bottom of a glass tube 19 mm. in outside diameter and 90 mm. long. The lower end of the capillary is sealed and calibrated at the 0.5-ml. level (Walter J. Podbielniak, Inc., Chicago, Ill.).

The micro transfer filter pipet (*C*, Figure 2) is constructed by blowing an ellipsoidal bulb of 1.5 to 2.0-ml. capacity and about 4 cm. long into a piece of glass tubing 10 ml. in outside diameter. One end is drawn out to a capillary 1.5 to 2.0 mm. in diameter and 5 cm. long to serve as the filling end. A small plug of glass wool is inserted into the glass bulb and packed into the opening where the filling end capillary enters the bulb. The opposite or delivery end is drawn to a capillary of 1.5 to 2.0 mm. and is cut off to a length of 2.6 cm.

The syringe sleeve assembly is made by fitting a piece of 10-mm. glass tubing 6 to 7 cm. long with a piece of rubber tubing $^1/_4$ inch in inside diameter and 2 cm. long, with 1 cm. left projecting beyond the end of the glass connecting tube to act as a sleeve. On the other end of the glass connecting tube is placed a rubber medicine bulb or, preferably, a 5-cm. hypodermic syringe.

The micro transfer filter pipet is inserted into the syringe sleeve assembly, so that the delivery end of the pipet is inside the connecting tube and the bulb of the pipet is held by the protruding rubber sleeve on the connecting tube. In use, the protruding capillary on the micropipet fixed in the sleeve assembly is inserted into the diluted reaction mixture in the micro reaction tube and the contents of the tube are withdrawn into the pipet by the manipulation of the syringe.

The diluent and color-formed reaction complex are mixed by withdrawing and returning the contents of the reaction tube several times by the pipet. The contents of the micropipet are transferred to the microcuvette by removing the delivery end of the micropipet from the syringe sleeve assembly and reinserting the pipet in the reverse direction with the delivery end out. The delivery end of the capillary is inserted into the microcuvette and the desired amount of the contents of the pipet is discharged in the microcuvette. The micropipets are cleaned by running water through them, followed by reagent grade acetone and drying in an oven at 100° C.

The special cell carriage holds four microcuvettes and is equipped with a precision assembled carriage for installation in a Beckman Model DU spectrophotometer (Pyrocell Manufacturing Co., 207–211 East 84th St., New York 28, N. Y.) (7).

Microcuvettes, quartz microcells, 1.5 × 10 × 25 mm. (0.375 ml.) are obtained from the Pyrocell Manufacturing Co., New York, N. Y.

A constant head eluent reservoir (*B*, Figure 2) to keep a constant head of pentane on the chromatographic column is made from a 500-ml. standard-taper flask fitted with a standard-taper (inner part) joint to which has been sealed a tube 6 cm. long by 10 mm. in outside diameter. By inverting this reservoir with the 6-cm. tube entering the buret to the shoulder of the standard-taper joint, the solvent level in the buret is maintained about 6 cm. below the top of the buret until the reservoir is empty. It is necessary to place dry ice or a cold cloth on the bottom of the inverted flask to keep solvent from overflowing the buret.

A Beckman Model DU spectrophotometer (Beckman Instruments Co., Fullerton, Calif.) is fitted with the special cell carriage described above.

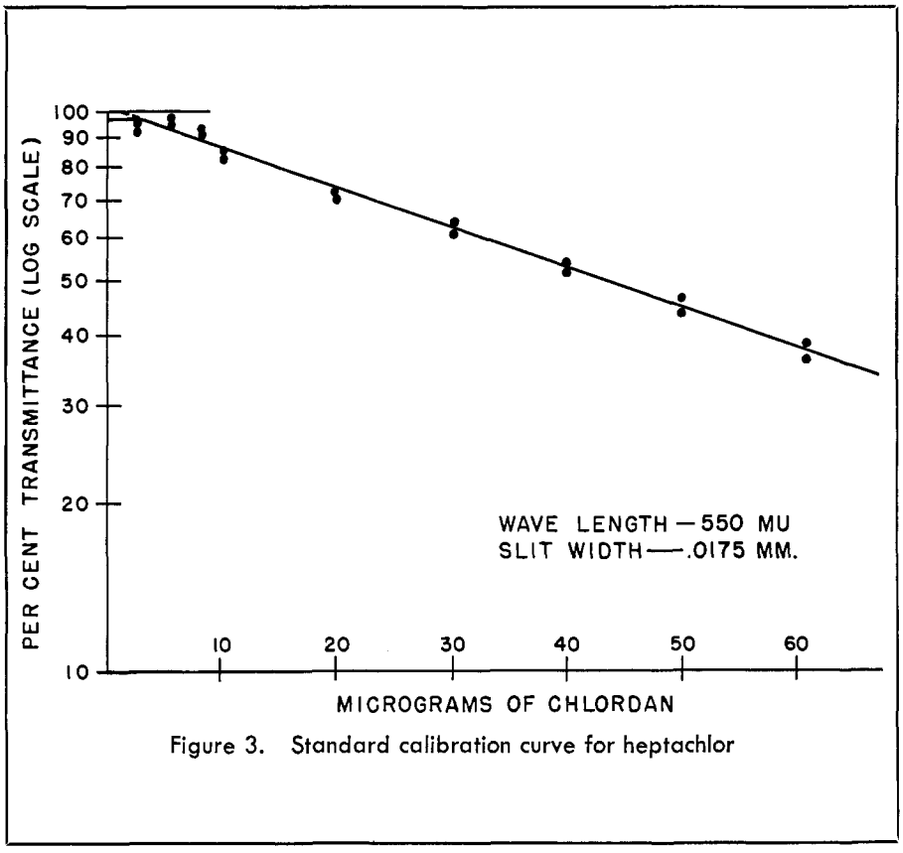

Figure 3. Standard calibration curve for heptachlor

Preparation of Standard Calibration Curves

Heptachlor A standard heptachlor solution containing 4.5 γ of heptachlor per milliliter is prepared by dissolving reference standard heptachlor in colorimetric pentane. This solution is kept in a constant temperature bath at 20° C., to minimize complications arising from a relatively high coefficient of expansion and volatility of pentane.

To obtain points for drawing a standard calibration curve aliquots of 1,2,3,4, and 5 ml. of standard solution representing 4.5 to 22.5 γ of heptachlor are pipetted into the micro reaction tubes, and the solvent is allowed to evaporate to dryness in the pentane evaporation bath at 40° C. To the residue in each reaction tube are added 0.2 ml. of the Polen-Silverman reagent and a small Carborundum chip. The reaction tubes are immersed in the reaction bath, and the color-developing reaction is allowed to proceed at 100° C. for 15 ± 0.5 minutes. The precise timing of the 15-minute reaction period is important to the reproducibility of the results.

The tubes are then cooled to room temperature in a beaker of cold water. The reaction products are diluted to 0.5 ml. with 4 to 1 benzene–isopropyl alcohol solution and are transferred to the microcuvettes by means of the micro transfer filter pipets and the syringe sleeve assembly. The transmittance is determined on the Beckman Model DU spectrophotometer at 567 mμ. The benzene–isopropyl alcohol solution is used as the reference. To obtain reproducible results the photometric read-

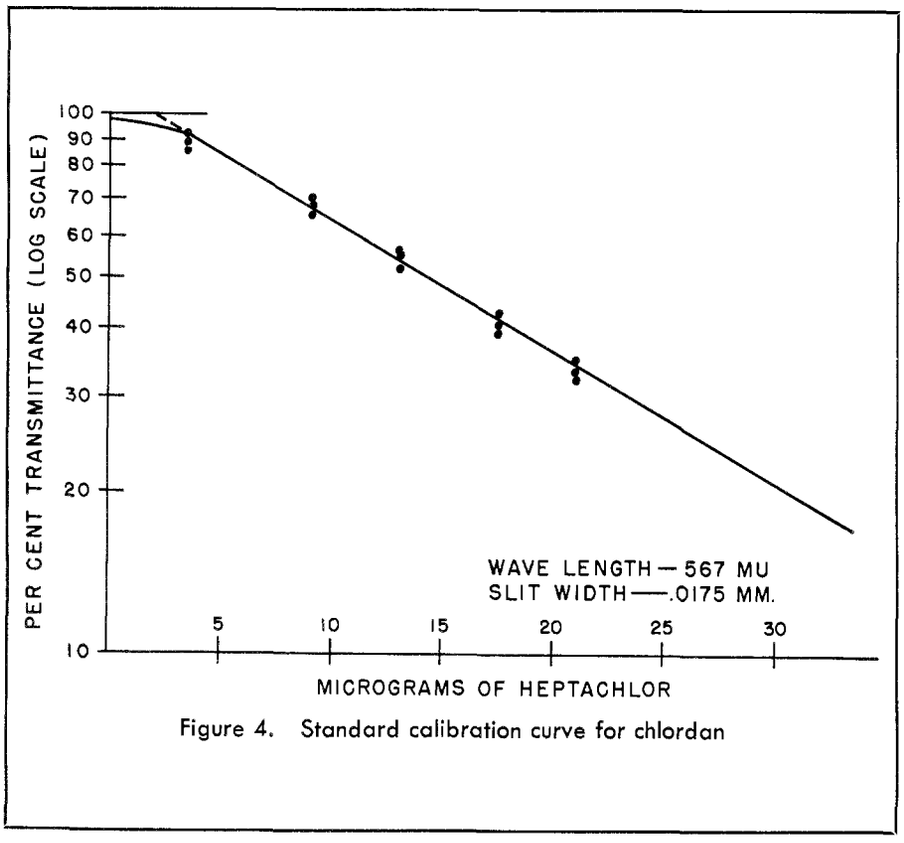

Figure 4. Standard calibration curve for chlordan

ings should be taken at a standard interval (5 minutes) after removal of the tubes from the reaction bath.

The standard curve obtained by plotting per cent transmittance on a logarithmic scale against micrograms of heptachlor is shown in Figure 3. The solid line represents actual data; the dashed line is plotted from the least squares equation prepared from these data. It is evident that the curve does not conform to Beer's law below 4 γ when a benzene–isopropyl alcohol reference sample is used. The equation for the straight line is:

$$\text{Log per cent transmittance} = 2.0397 - 0.02356\ W_H$$

where W_H equals micrograms of heptachlor.

Chlordan A standard chlordan solution having a concentration of 10 γ per milliliter is prepared from reference grade chlordan and pentane and kept in a constant temperature bath at 20° C.

Aliquots of 0.5, 1, 2, 3, 4, and 5 ml. of standard solution representing 5 to 50 γ of chlordan are pipetted into the micro reaction tubes and boiled to dryness in the pentane evaporation bath at 40° C. The residues are heated at 100° C. for 15 minutes with 0.2 ml. of modified Davidow reagent in the reaction bath. The tubes are then cooled to room temperature in a beaker of water and diluted to 0.5 ml. with 90% methanol, and the contents are transferred to the microcuvettes by use of the micro transfer filter pipets and syringe sleeve assembly. The transmittance is determined on a Beckman Model DU spectrophotometer at 550 mμ, using a 90% methanol solution as the reference. The absorbance peak at 550 mμ exceeds the peak at 521 mμ when microgram quantities of chlordan are made to react with modified Davidow reagent for only 15 minutes.

Figure 4 shows the standard curve obtained by plotting per cent transmittance against micrograms of chlordan. The solid line represents actual data; the dashed line represents the least squares equation prepared from these data and is in effect a Beer's law plot. There is good agreement between the two curves above 10 γ of chlordan, but below this value the curve does not conform to Beer's law. The equation for the straight line is:

$$\text{Log per cent transmittance} = 2.0062 - 0.00722\ Wc$$

where Wc equals micrograms of chlordan.

Treatment of Samples

Samples of food or forage crops weighing from 500 to 1000 grams are weighed to the nearest gram (for development work larger samples, multiples of 500 grams, are taken) and cleaned according to the practice that corresponds to the accepted domestic or commercial preparation of the crop. Samples of crops that contain water (except canned goods) are cut into 1-inch cubes (small fruits and vegetables need not be cut) and stored in polyethylene bags for 24 hours at 18° F. to provide a cold material that will substantially reduce the amount of sodium sulfate necessary to dry the crop. Dry crops are reduced in size by grinding or cutting so that the desired weight will fit into the Soxhlet extracting apparatus.

Drying Weighed crop samples which contain water are removed from the 18° F. storage and ground with twice their weight of anhydrous sodium sulfate. Wet-pack canned goods are weighed and ground with sulfate. Any partially dry crop, or one in which there is a possibility of water, should be ground with sufficient sulfate to dry the crop.

Crops that have been ground with sulfate are returned to the 18° F. storage for 24 hours to assure more complete removal of the water into the sulfate complex.

Extraction Samples are extracted in the Soxhlet extractors with pentane for 4 hours. Nylon filter disks, used in place of paper extraction cups, are placed in the extractor to cover the siphon opening. A minimum temperature should be used to maintain an even reflux and cycling in the Soxhlet extractor. At completion of the extraction the extracted crop is removed from the Soxhlet extractor, and the solvent is concentrated to 75 ml. in the extraction flask. The extract is transferred to a 125-ml. Erlenmeyer flask protected with an aluminum foil cap.

The extract may be stored at 18° F. for analysis later, or prepared for analysis immediately by adding a boiling chip and reducing the volume to 5 to 10 ml. on the concentrating bath at 50° C. No measurable loss of toxicant from 75 ml. of a pentane crop extract evaporated from the open flask under these conditions has been observed as long as crop pigments and waxes have not been removed from the extract.

Removal of Interfering Substances from Crop Extracts

Pentane extracts of plant materials may contain not only traces of insecticide residue but also plant pigments, oils, waxes, and other related plant materials which seriously interfere with the color reactions. Heptachlor and chlordan can be separated from these materials by the selective adsorption of these extractives with Florisil, Florex, or alumina. Florisil is used with crop extracts containing little or no oil and wax (Pro-

cedure A), Florex is used for all other crops in the separation of heptachlor (Procedure B) and alumina is used for the chlordan separation (Procedure C). Where an excessive amount of oil, plant pigments, or other interfering substances is encountered a sulfuric acid wash (Procedure D) is used to remove the bulk of this interfering material before the plant extract is chromatographed. Excessive amounts of plant waxes may be removed by cooling and filtering the extracts (Procedure E).

Procedure A **Florisil Chromatography (Heptachlor or Chlordan).** A chromatographic column is prepared by inserting the buret into the special water jacket (*C*, Figure 1), with the Tygon collar holding the buret firmly in place. A small plug of glass wool is placed in the bottom of the buret and 10 grams of activated Florisil is poured into the column. The adsorbent is packed by tapping the column with a wooden dowel rod.

The Florisil is wet with colorimetric pentane and the plant extract concentrated to about 5 ml. is transferred to the column. After the extract has been adsorbed onto the Florisil, the flask from which the plant extract was transferred is thoroughly rinsed with two or three 1-ml. portions of pentane and the pentane rinsings are added to the column. When the washings have penetrated into the Florisil, the column is eluted with 200 ml. of pentane from a constant head eluent reservoir. The total eluate is collected in a 500-ml. Erlenmeyer flask with a 24/40 ground-glass joint.

Procedure B **Florex (Heptachlor).** A chromatographic column is set up as in Procedure A. Ten grams of activated Florex is used in this column. After the adsorbent has been packed by tapping the column with a wooden dowel rod, the Florex is wet with pentane and the plant extract, concentrated to 5 ml., is transferred to the column. When the extract has been adsorbed onto the Florex, the flask is thoroughly rinsed with two or three 1-ml. portions of pentane and the pentane rinsing is added to the column. The column is eluted with 150 ml. of pentane, the first 50 ml. is discarded and the remaining 100 ml. of eluate is collected as above for analysis.

Procedure C **Alumina (Chlordan).** A column is packed with 10 grams of activated alumina; and the eluate from Procedure A is concentrated to 5 to 10 ml. in the 500-ml. flask under a three-bulb Snyder column. The alumina column is wetted with pentane, and the concentrated eluate is added to the column, followed by two or three 1-ml. pentane rinsings of the 500-ml. flask. Fifty milliliters of pentane is used to elute the column and the entire pentane eluate is discarded. The column is then eluted with 50 ml. of diethyl ether. The ether eluate, which contains the chlordan, is collected in a 500-ml. flask.

Table I. Interference and Recovery Levels for Various Crops

	Chlordan			Heptachlor		
Crop	Clean-up procedures	Av. check,[a] p.p.m.	Av. recovery,[b] %	Clean-up procedure	Av. check,[a] p.p.m.	Av. recovery,[b] %
Alfalfa	EAC	0.04	65	EAB	0.02	72
Barley	DC	0.01	75	...	...	...
Beans, string	A	0.02	65	A	0.002	89.8
Beans, lima	A	0.01	71	A	0.002	90
Beets	A	0.03	72	A	0.010	75
Broccoli	AC	0.03	75	B	0.010	75
Cabbage	AC	0.03	78.5	B	0.010	77
Carrots	A	0.011	79.0	A	0.004	91.8
Cherries	A	0.01	78	A	0.01	90
Clover	AC	0.03	65	AB	0.02	72
Corn, stored	A	0.02	65	A	0.01	77
Cucumbers	AC	0.01	70	B	0.01	83.2
Grass	EA	0.05	75	EA	0.05	80
Honey	A	0.01	70	...	...	...
Oats	EAC	0.03	70	...	...	...
Onions	AC	0.006	93.5	B	0.005	76
Peaches	A	0.03	74	A	0.02	78
Peas	A	0.03	68	A	0.015	88
Potatoes	A	0.015	83.3	A	0.007	83.2
Radishes	A	0.03	71	B	0.01	80
Spinach	EAC	0.02	65	EAB	0.01	75
Strawberries	A	0.03	73.5	...	...	...
Sugar beets	...	...	...	B	0.01	76.2
Squash	A	0.02	70	B	0.015	84.5
Sugar cane	...	...	...	A	0.01	80
Sweet potatoes	A	0.02	86.5	A	0.01	98
Tobacco, green	DC	0.01	70	DB	0.01	80.8
Tobacco, cured	D2C	...	...	D2B	0.01	74
Tomatoes	A	0.01	88	A	0.01	86
Turnips	A	0.06	66	B	0.012	84.5

[a] Interference level. Deviations from average interference level of average check sample are in most cases less than ±0.01 p.p.m. expressed as chlordan and 0.003 p.p.m. expressed as heptachlor; in a few crops deviations as high as 0.02 and 0.005 p.p.m., respectively, are encountered.

[b] 0.005 to 0.400 p.p.m. range in 400- to 500-gram samples. Average deviation of recovery samples varies because of effect of average interference level from 15% at 0.01 p.p.m. to 5% at 0.4 p.p.m.

Procedure D **Acid Removal of Interfering Materials (Heptachlor and Chlordan).** The crop extract is concentrated (or diluted) to 300 ml. with pentane and shaken vigorously in a 1-liter separatory funnel with 40 ml. of fuming-concentrated sulfuric acid. The mixture is allowed to settle in the separatory funnel for 16 hours, and the pentane layer is decanted and concentrated to 5 to 10 ml. in a 500-ml. flask under a three-bulb Snyder column. The 5 ml. of extract is next treated by a Florex column for heptachlor analysis as in Procedure B. In the determination of chlordan residues the 10 ml. of extract is added directly to an alumina column as outlined in Procedure C.

Procedure D2 **Double Acid Treatment (Heptachlor and Chlordan).** Concentrated amine extracts from large samples of a crop (such as dried tobacco) react so vigorously with acid that a modification of Procedure D is necessary, involving the following procedure.

The extract is adjusted to a volume of 300 ml. in pentane and cooled in a dry ice–isopropyl alcohol bath. Thirty milliliters of fuming-concentrated sulfuric acid is added dropwise over a period of 5 minutes with constant stirring. The mixture is removed from the bath, allowed to come to room temperature and transferred to a 1-liter separatory funnel. The acid is discharged after 8 hours, the pentane layer is treated with a fresh charge of acid, and Procedure D is followed after the acid is added.

Procedure E In some crops (alfalfa, range grass, oats) such an abundance of plant wax is encountered that the preceding column techniques cannot be used until most of this wax is removed. The plant extract, reduced to 20-ml. volume by heating at 50° C. in the concentrating bath, is cooled in a dry ice–isopropyl alcohol bath until the wax is precipitated. The extract is then filtered through a previously extracted, fluted, filter paper and rinsed with small amounts of cold pentane. This procedure may be repeated for more complete removal of wax, if the volume of pentane is held at 20 ml. and the wax in the funnel is rinsed each time.

The cooling of a plant extract before chromatography (Procedures A, B, and C) will often show whether it is necessary to remove wax. The extract, after concentration to 5 ml. in the 50° C. bath,

Table II. Conditions of Analyses

Insecticide	Reagent	Reference and Diluting Solvent	Wave Length, Mμ
Heptachlor	Polen-Silverman	4:1 isopropyl alcohol–benzene	567
Chlordan	Davidow	90% methanol	550

should first be completely chilled under running tap water. If no sediment (less than 0.1 ml.) is found, the wax-removal step is probably not necessary, but if any significant amount of wax is found, the extract should be diluted to 20 ml. with pentane and treated as in Procedure E.

Summary of Procedures Table I shows a partial list of crops in which heptachlor and chlordan residues have been determined. The second column lists the procedures that have been successful in removing sufficient interference to determine residue. Because of differences in the same crops, alternative procedures may be of value if good results are not found with the procedure listed. Procedure D can probably be used on all crops, followed by a suitable column separation. The third and fourth columns of Table I list the average interference level of the check sample and the average recovery of toxicant added to the crop before extraction.

Determination of Insecticide

The eluates from the chromatographic columns are concentrated to about 10 ml. on the concentrating bath at 50° C. from an Erlenmeyer standard-taper flask fitted with a Snyder three-bulb fractionating column to prevent loss of toxicant by codistillation or mechanical entrainment. The concentrated eluates are transferred to the micro reaction tubes and the remaining pentane is evaporated to dryness in the pentane evaporating bath at 40° C. A stream of filtered dry air may be used to assist in driving off all the pentane.

Two-tenths milliliter of the test reagent indicated in Table II is added to the reaction tube and the tube is placed in the reaction bath at 100° C. for 15 ± 0.5 minutes. On removal from the reaction bath, the tube is immediately cooled to room temperature in a beaker of cold water. The sample is diluted to 0.5 ml. in the reaction tube with the solvent specified in Table II and is transferred to a microcuvette with a micro transfer filter pipet. The pipet is affixed in the syringe sleeve assembly and the solution drawn out of the micro-reaction tube through the filter end of the pipet. Mixing is accomplished by drawing into and discharging the contents of the pipet into the micro reaction tube several times. The filled transfer filter pipet is reversed in the syringe sleeve assembly and its contents are discharged into the microcuvette.

Table III. Determination of Heptachlor

Crop	No. of Samples	Heptachlor, P.P.M. Spectrophotometric[a] Total	Check	Net	Bioassay[b] Total	Check	Net
Potatoes	1	0.030	0.010	0.020	0.033	0.005	0.028
Cabbage	1	0.030	0.010	0.020	0.030	0.009	0.031
Turnips	1	0.026	0.027	0.000	0.004	0.003	0.001
Onions	1	0.008	0.005	0.003	0.006	0.007	0.000
Corn	1	0.005	0.005	0.000	<0.001	0.000	0.000

[a] Velsicol Chemical Corp.
[b] Boyce Thompson Institute.

Table IV. Comparative Results of Analyses

Crop	No. of Samples	Chlordan, Net Av. P.P.M. Spectro.[a]	Bioassay[b]	Samples	Heptachlor, Net Av. P.P.M. Spectro.[a]	Bioassay[b]
Potatoes	17	0.041	0.047	17	0.021	0.008
Yams	3	0.040	0.021	5	0.026	0.003
Cabbage	3	0.029	0.020	1	0.012	0.010
Turnip	4	0.031	0.026	19	0.022	0.006
Onion	13	0.020	0.017	27	0.008	0.006
Corn	4	0.045	0.035	5	0.016	0.010
Carrot	20	0.037	0.036	6	0.030	0.008
Average		0.0345	0.0329		0.0174	0.0067

[a] Velsicol Chemical Corp.
[b] Boyce Thompson Institute.

The transmittance of the sample is determined against the indicated reference on a Beckman DU spectrophotometer which has been adapted for microanalysis with the special cell carriage. With the cells in proper alignment and with the machine set at three full turns off of full sensitivity, a slit width near 0.018 mm. should be obtained (the aperture plate provided with the microcell holder assembly is not used). The transmittance of the sample is used to determine the amount of toxicant in the sample by reading the amount of toxicant from the standard calibration Curve or by solving for this in the equations for the straight-line curves.

Discussion

Recovery of Toxicant from Experimental Samples The percentage recovery of the toxicants from crop check samples was determined by the addition of measured amounts of standard toxicant solutions to the crops before extraction and also to the pentane extracts of other samples of these crops. These two methods of addition of toxicant give essentially the same recovery data. The extraction process is therefore shown to give approximately 100% recovery of the toxicant.

To determine the effect on toxicant recovery of the location of toxicant in different parts of the crop, various methods of adding the toxicant were used. Standard solutions in pentane were sprayed on the outside of crops and acetone solutions of the toxicant were injected hypodermically into the pulp of potatoes. When these samples were ground and extracted, the recoveries were complete and the same as those found when the standard solution of toxicant was added to the ground pulp or to the pentane extract before clean-up procedures. The principal loss of the analytical process is in the chromatography step of the clean-up procedure. A list of recoveries and interference levels for many crops is shown in Table I. The recoveries and interference levels are based on the use of colorimetric pentane for the extraction of the crops and the chromatography of the crop extracts (chlordan is removed from the alumina column with diethyl ether, Procedure C). Higher boiling solvents such as hexane ought not to be used (9) because of excessive toxicant loss when the solvent is concentrated. Oxygenated solvents are not advisable for extraction (even fractions of 1%) because of their effect on the adsorption properties of the chromatographic adsorbents in these column techniques and because in many cases they remove an unmanageable amount of plant extractives from the crops.

Comparison of Spectrophotometric and Bioassay Tests

The Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, N. Y., using a bioassay test involving photomigration of mosquito larvae (*5*), has analyzed duplicates of many of the authors' samples. The agreement on a large number of samples from a wide variety of crops was very good. Some typical results are listed in Tables III and IV.

On the basis of the results shown in Tables III and IV, it was concluded that the averages obtained by the spectrophotometric and bioassay methods for the determination of chlordan agreed very well, considering the small amounts of residue present. The agreement between the methods in the determination of heptachlor was less satisfactory, probably because of the presence of residual lipides which masked the action of the insecticide in the bioassay test, causing low residue results, and acted as background interference in the colorimetric analyses, causing high residue results. Subsequent work showed that heptachlor can be separated from nonvolatile lipides by steam distillation, and better correlation with the chemical test was obtained by bioassay after the lipides were removed (*5*).

The Wisconsin Alumni Research Foundation, Madison, Wis., using a bioassay modification of the method of Laug (*6*) and Sun (*10*) with the common housefly as the test insect, has analyzed duplicates of samples analyzed by the Velsicol Chemical Corp. Good agreement was found on a wide variety of fruit and forage crops (Table V).

Typical Analyses of Heptachlor Residues on Food Crops

When massively excessive dosages of heptachlor (250 pounds per acre) were applied experimentally to the furrow at the planting time of potatoes, a residue was found on the peel of the potatoes at harvest time but virtually no residue was found in the pulp of the potato (Table VI). This is regarded as very significant evidence that the heptachlor does not transmigrate into the vegetable or fruit. The heptachlor residue on the skin of the crop was completely destroyed by cooking.

The analytical methods devised in this work were perfected to show specifically and reliably the absence of chlordan or heptachlor residues above 0.01 p.p.m. in check samples of crops known to contain no trace of either toxicant. Samples weighing 500 grams are necessary to minimize sufficiently the effect of traces of interference not removed by the various clean-up techniques. In development work where large samples (up to 2500 grams) have been used, the residue could frequently be estimated in the range of 0.001 p.p.m.

Table V. Comparison of Spectrophotometric and Bioassay Analyses

Crop	Insecticide	Time between Application and Harvest	Toxicant, P.P.M. WARF[a] bioassay[b]	Toxicant, P.P.M. Velsicol spectro.
Alfalfa	Heptachlor	48 hours	0.91	0.912
		3 days	0.07	0.033
		11 days	0.06	0.048
		14 days	0.025	0.025
		15 days	0.003	0.002
	Chlordan	24 hours	0.08	0.112
		48 hours	0.23	0.307
		72 hours	0.02	0.010
		Treatment		
Sugar beet tops	Heptachlor	Seed treat.	0.005	0.004
		Soil	0.000	0.004
		Soil band	0.005	0.013
Sweet corn	Heptachlor	...	0.002	0.003
Cherries	Heptachlor	7 days	0.32	0.23
		21 days	0.004	0.009

[a] Wisconsin Alumni Research Foundation.
[b] Bioassay with houseflies according to method of Laug (*6*, *10*).

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Table VI. Typical Heptachlor Residues[a]

	Part	Methods of Application	Rate of Application, Lb./Acre	Heptachlor Residues, P.P.M. Total	Check	Net
Potatoes	Whole	Soil treatment	250[b]	0.346	0.032	0.314
	Peels	Soil treatment	250[b]	0.372	0.032	0.340
	Pulp	Soil treatment	250[b]	0.073	0.032	0.041
Boiled	Whole	Soil treatment	250[b]	0.029	0.032	0.000
Baked	Whole	Soil treatment	250[b]	0.056	0.032	0.024
Boiled	Pulp	Soil treatment	250[b]	0.009	0.032	0.000
Potatoes	Whole	Soil treatment	9	0.003	0.015	0.008
			6	0.024	0.022	0.002
			3	0.013	0.013	0.000
			1	0.007	0.008	0.000
		Foliage spray	8	0.005	0.005	0.000
			5	0.005	0.006	0.000
			1/4	0.016	0.015	0.001

[a] Treated and check crop samples were received from a wide variety of sources. Whenever possible, a check or untreated sample of the same variety and from the same geographical area (plot on same farm) was obtained for each treated sample. This explains the apparent variation in check samples.
[b] Approximately 100 to 200 times the dosage required for economic control of wireworms.