Conclusions

N-Mono- and disubstituted α-chloroacetamides exhibit outstanding activity as grass-specific, pre-emergence herbicides.

A three-carbon chain fulfills some requirement for maximum activity.

N-Disubstitution appears superior to *N*-monosubstitution with a straight-chain aliphatic group.

Branched-chain aliphatic groups show less activity in the *N*-disubstituted chloroacetamides than in the *N*-monosubstituted compounds.

A number of heterocyclic derivatives are among the most active compounds tested.

The aromatic compounds are almost inactive unless an aliphatic substituent is attached to the nitrogen atom.

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PESTICIDE RESIDUES

Determination of *O*-(3-Chloro-4-nitrophenyl)-*O*,*O*-dimethyl Phosphorothioate (Chlorthion) Residues in Cottonseed

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An analytical method described for the determination of chlorthion residues in cottonseed is spectrophotometrically sensitive to 0.02 p.p.m. in 200 grams of cottonseed. The isolative procedures include extraction, liquid-liquid partition, and chromatography. Cottonseed from a planting treated with dosages of chlorthion probably greater than that expected in commercial treatment was found to contain no residues when a visual comparative method sensitive to 0.01 p.p.m. was used.

THE USEFULNESS OF *O*-(3-chloro-4-nitrophenyl)-*O*,*O*-dimethyl phosphorothioate (chlorthion) for the control of several cotton pests (*3*) requires an investigation into the possible presence of residues of this material, as the expressed oil of cottonseed may be converted to edible oils and the cottonseed cake may be used as livestock feed.

Cottonseed obtained from a plot, planted and treated with chlorthion especially for this investigation, was mechanically ginned, delinted with concentrated sulfuric acid, ground, and extracted with pentane in the apparatus depicted in Figure 1. Chlorthion was separated from coextracted cottonseed oil by partition between pentane and acetonitrile. Pigments and other interfering materials were then removed from the acetonitrile solution of chlorthion by chromatography through activated alumina. Finally, the colored compound was developed with chlorthion (*6*) in a manner analogous to that with parathion according to Averell and Norris (*1*).

In Figure 2 are shown the spectral curves for chlorthion recovered from fortified cottonseed and for a typical control blank. For chlorthion, the absorption maximum is at 545 mμ; 5-cm. cells were used. Control blanks are faintly yellow with absorption at 545 mμ

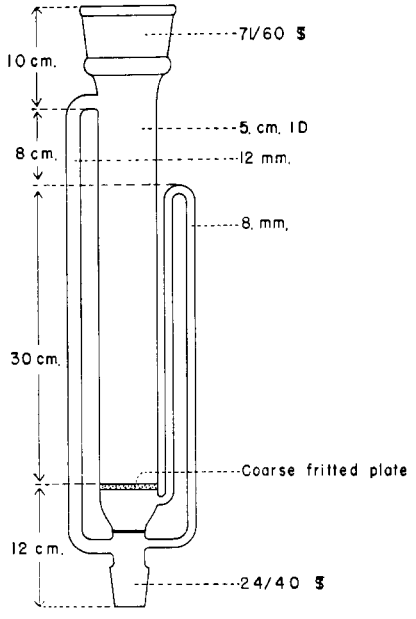


Figure 1. Apparatus for continuous extraction of cottonseed

equivalent to 3 to 4 γ of chlorthion (0.02 p.p.m.). This blank is nearly independent of the quantity of cottonseed control sample used, as illustrated in Table I. In this investigation, however, the control samples were equal in weight to the treated.

In Figure 3 are shown the absorbance-concentration plots for pure chlorthion and for chlorthion recovered from fortified cottonseed. Four micrograms of chlorthion from 200 grams of fortified cottonseed in a final volume of colored solution of 50 ml. were easily detected with the naked eye and possessed a transmittancy reading of 90% when 5-cm. cells were used. In visual comparison with Nessler tubes filled to 25-cm. depth, 0.01 p.p.m. could be detected. There was no indication of the presence of chlorthion in the treated cottonseed.

Field Treatment

A small planting of cotton, variety Acala 4-42, on Citrus Experiment Station property was used for this chlorthion residue test. The cotton was planted in five small blocks, each eight

rows wide. Three of the five blocks received no insecticidal applications of any kind; the remaining two blocks received applications of chlorthion only. In picking the cotton, each block was divided into two plots of four rows each, and each plot was picked separately to simulate, so far as possible, four replications.

Six applications of 5% chlorthion dust were made by rotary hand duster at an average rate of approximately 35 pounds per acre per application. Thus, a total of approximately 10.5 pounds of technical chlorthion was applied to the treated cotton. The first application was made on July 30, when the cotton was about 24 inches tall; subsequent applications were made at approximately weekly intervals, on August 6, 17, 23, and 30, and September 7. The cotton was about 5 feet tall at the time of the last treatment. Lint was harvested and ginned during the period from October to December, the normal picking period in southern California.

Special Apparatus

Grinder. Any grinding apparatus may be used that is capable of reducing whole cottonseed to a fine granular form. In this work a commercial motor-driven coffee grinder was used.

Extractor. The extractor depicted in Figure 1 was made for this work.

Chromatographic Column. A column 18 mm. in inside diameter and 330 mm. long with base of coarse fritted glass was used. The column was expanded at the top to a bulb of 250-ml. volume.

Kuderna-Danish Evaporative Concentrator (*4*). A Beckman Model DU spectrophotometer, Model DR recording attachment was used for the spectral curves; Model B for absorbance-concentration readings.

Reagents

Light petroleum ether (pentane, Skellysolve F, boiling point 30° to 60° C.).

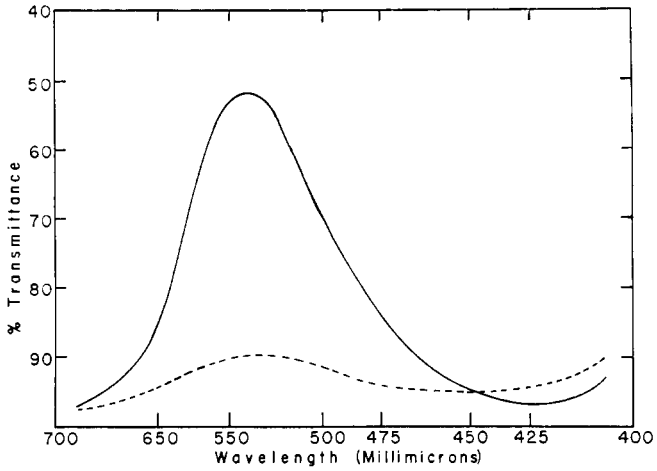
Anhydrous sodium sulfate, granular reagent grade.

Chlorthion, purified sample (supplied by Farbenfabriken von Bayer).

Acetonitrile, practical grade, boiling point 80–82° C. This solvent, if basic, must be acidified with 85% phosphoric acid until neutral or slightly acid.

Table I. Chlorthion Equivalence of Cottonseed Control Samples

Grams of Cottonseed	Transmittance, % at 545 mμ	Chlorthion Equivalence, γ
75	91.2	3.3
125	89.1	4.0
200	89.0	4.0
200	89.7	3.9



——— 200 grams of cottonseed fortified with 39 γ of chlorothion (control blank is reference)

– – – – 200 grams of cottonseed (reagent blank is reference)

Beckman DU spectrophotometer, DR recording attachment, 5-cm. cells volume 50 ml.

Figure 2. Spectral characteristics of final colored solutions in analysis of cottonseed

Phosphoric acid, 85% reagent grade.

Sodium nitrite solution, 0.25%.

Ammonium sulfamate solution, 2.5%.

N-1-Naphthylethylenediamine dihydrochloride solution, 1% (older preparations must be purified).

Alumina, Aluminum Ore Co. activated alumina, Grade F-20, −80 mesh.

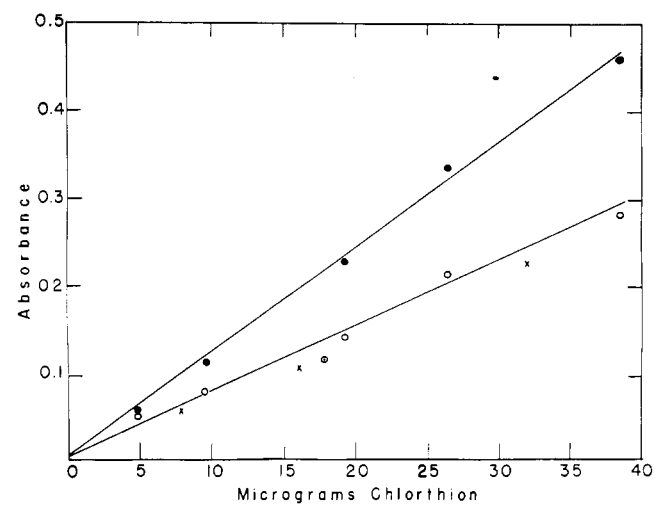
Analytical Procedure

Delinting. Stir mechanically ginned cottonseed with concentrated sulfuric acid until the adhering cotton is decomposed (about 5 minutes), then pour the mass into tap water. Pour this mixture onto a large wire screen and immediately wash with large quantities of tap water, and, finally, with distilled water. Dry the seed overnight on the same screen placed over a drying oven (temperature approximately 40° C.).

Grinding. A commercial, motor-driven coffee grinder adjusted to its finest setting is used to grind the cottonseed. The operation proceeds smoothly without gumming or the production of heat.

Extraction. Attach a 500-ml. round-bottomed flask containing boiling chips and 350 ml. of pentane to the extractor (see Figure 1). Cover the porous plate of the extractor first with a wad of fine glass wool and then with a 1-inch layer of coarse anhydrous sodium sulfate. Insert a length of 10-mm. glass tubing, extending above the top of the extractor, and add 200 grams of ground cottonseed through a 6-inch powder funnel. Trickle pentane from a separatory funnel through the glass tube so that the extractor becomes filled from the bottom until overflow at the side arm begins. Remove the glass tube and rinse with pentane. Place the assembly on a heating mantle and extract the cottonseed by reflux for 6 hours. After the extract solution has cooled (filter through a fine porous glass funnel if murky), evaporate on a water bath to somewhat less than half the volume of the flask. Use a 3-bulb Snyder column to minimize volatilization losses.

Partition Extraction. Transfer the solution to a 500-ml. separatory funnel and wash the flask with small portions of pentane until the volume in the funnel reaches a 250-ml. mark. Equilibrate



● Pure chlorthion
○, X, ⊙ Chlorthion recovered from fortified cottonseed
Volume of colored solutions, 50 ml. Beckman Model B spectrophotometer 5-cm. cells λ = 545 mμ

Figure 3. Absorbance-concentration plots

with 60 ml. of acetonitrile. When the phases have separated, withdraw the lower phase into a second 500-ml. separatory funnel containing 100 ml. of pentane. Equilibrate, then transfer the lower phase into a 250-ml. 24/40 standard-taper Erlenmeyer flask. Repeat the extractions in both funnels with three more 30-ml. portions of acetonitrile and collect in the same Erlenmeyer flask. Add boiling chips to this flask, fit a 3-bulb Snyder column, and boil on the steam bath for several minutes to evaporate the pentane dissolved in the acetonitrile; cool the solution.

Chromatography. Suspend prepared alumina in 50% aqueous methanol and wet-pack a chromatographic column (18 mm. in inside diameter) by vibration and gentle tamping to a depth of 2 inches. Pass 30 ml. of acetonitrile through the column. When this has reached the top of the alumina, transfer the acetonitrile extract solution into the chromatographic column and rinse the Erlenmeyer flask with two 10-ml. portions of acetonitrile. Collect the filtrate from the column in a 1-liter separatory funnel containing 500 ml. of water and 10 ml. of concentrated hydrochloric acid. When the acetonitrile has almost reached the top of the alumina, add 30 ml. of acetonitrile; repeat with another 30 ml. Then extract the aqueous solution in the separatory funnel with 100 ml. of pentane, and transfer the lower phase to a second 1-liter separatory funnel, where it is extracted again with 100 ml. of pentane. Pass the pentane from each funnel directly into a Kuderna-Danish evaporative concentrator (*4*) fitted with a 19/38 50-ml. standard-taper round-bottomed flask, and rinse each funnel with 25 ml. of pentane conveniently delivered from a pipet. Add a few boiling chips, evaporate on a steam bath through a 3-bulb Snyder column to about 10 ml., add 10 ml. of acetonitrile, and evaporate again to 10 ml.

Reduction and Color Development. Remove the Snyder column, rinse the bulb of the evaporator with about 15 ml. of pentane, and add 5 ml. of water, 1 ml. of 85% phosphoric acid, and 0.5 gram of powdered zinc to the flask. Fit a 19/38 standard-taper male joint, lengthened to 15 inches, to serve as air condenser to reduce losses by volatilization, and heat on the steam bath for 30 minutes. Add 20 ml. of water through the condenser and replace with a standard-length joint (about 5 inches). Bring to boil for a few minutes to expel last traces of pentane. Cool the colorless turbid solution.

Filter by suction through 19/38 standard-taper joint (inner connection with reduced lower tube) packed with a wad of glass wool, a $^1/_2$- to $^3/_4$-inch layer of packed Celite, and finally with about a 1-inch wad of cotton coated with petroleum jelly–paraffin. Collect the filtrate in a 50-ml. volumetric flask. With the aid of an eye dropper, rinse the reaction flask with three 4-ml. portions of 0.3*N* phosphoric acid solution and pass the washings through the filter. Treat the clear, faintly colored solution according to the method of Averell and Norris (*7*) to produce color. Add 0.5 ml. of 0.25% solution of sodium nitrite (10-minute wait), 0.5 ml. of 2.5% solution of ammonium sulfamate (10-minute wait), followed by 1 ml. of 1% solution of *N*-1-naphthylethylenediamine dihydrochloride. After allowing color to develop for 10 minutes, add 3 ml. of concentrated hydrochloric acid and make to volume with water. The absorbance may be determined immediately. The color is stable for many hours.

Preparation of Standard Curve. Pipet aliquots (1, 2, 4, 6, and 8 ml.) of a hexane solution containing 5 γ per ml. of chlorthion into 50-ml. round-bottomed $^{19}/_{38}$ standard-taper flasks. Add 1 ml. of 85% phosphoric acid, 5 ml. of water, 10 ml. of acetonitrile, and 0.5 gram of powdered zinc. Put a $^{19}/_{38}$ standard-taper joint extended to 15 inches on the flask and complete the reduction by heating for 30 minutes on a steam bath. Add 20 ml. of water. Replace the joint with the usual 5-inch length and boil the solution for a few minutes to displace last traces of hexane. Cool the solutions, then filter through medium-porosity fritted funnels into 50-ml. volumetric flasks. Wash the reaction flask with three 4-ml. portions of 0.3*N* phosphoric acid and pass the washings through the filter. Color development is the same as for the cottonseed extracts. Determine absorbance at 545 mμ in 5-cm. cells.

Preparation of Activated Alumina. Suspend 500 grams of alumina in 800 ml. of distilled water and acidify with 5 ml. of concentrated hydrochloric acid. Let stand for 2 hours, shaking vigorously from time to time. Wash five times by decantation, allowing the fine materials to be poured off. Transfer to a large Büchner funnel and wash until filtrate is only faintly acid. Finally, wash three times with 300 ml. of methanol and dry in a flat pan in an oven at 180° C. overnight (16 hours).

Preparation of Filter Cotton. Dry the cotton (10 grams) in an oven at 150° C. for 2 hours; then immerse in 25 ml. of a pentane solution of 3 grams of white petroleum jelly and 3 grams of household paraffin. Rotate the mass by hand in the air until the solvent is nearly evaporated. Evaporate in air for about an hour, then in an oven at 150° C. for 30 minutes. This cotton is useful for removing the small quantity of oil remaining in the aqueous solution of reduced chlorthion during the filtration prior to color development.

Fortification and Recovery. Chlorthion is added as a hexane solution to the top of the charge of cottonseed in the extractor and extraction begun at once. In this manner, opportunity for adsorption or decomposition is afforded as the chlorthion passes through the entire sample of cottonseed. Recoveries are shown (○) in Figure 3.

A fine stream of chlorthion in hexane (1.6 γ per ml.) is impinged onto a stream of ground cottonseed being poured into a jar. The jar is tumbled for several hours, then stored for 2 weeks in a cold room (45° C.) before extraction and analysis. These recoveries (×) are shown in Figure 3.

Chlorthion dust (25% wettable powder) is successively diluted with Attaclay till it contains 5 γ of chlorthion per gram. This is done by weight dilution and prolonged tumbling (48 hours) to ensure uniformity. In this instance, 3.6 grams containing 18 γ of chlorthion was tumbled with 200 grams of cottonseed and stored for 1 month at 45° C. After analysis, the recovery is shown in Figure 3 (⊙).

Discussion

Extraction of Cottonseed. The choice of solvent for the extraction of cottonseed was governed by the fact that solvent partition seems to be the method of choice to separate chlorthion from the large quantity of coextracted cottonseed oil. Although chlorthion is much more soluble in most usual laboratory solvents than in petroleum ether, its solubility has been found to be 25 mg. per ml. in Skellysolve F and 34 mg. per ml. in Skellysolve B (60° to 70° C. fraction). These solubilities are more than sufficient to expect complete extraction of the quantities of chlorthion that could be expected from usual field treatments. Pentane is a very good solvent for cottonseed oil, which is, in turn, miscible with chlorthion.

Pentane was, therefore, chosen because it was the solvent required in subsequent liquid-liquid partition, and its low boiling point allowed rapid circulation through the extractor by reflux. The authors' laboratory is equipped with a water cooler fitted with a circulating pump to furnish condenser water below 10° C., and losses of the low boiling pentane are almost completely eliminated.

Soxhlet extraction is unsatisfactory because the siphon action removes the solvent exterior to the extraction thimble, while the solvated mass within undergoes only slow solvent exchange. Equilibration by shaking ground cottonseed with solvent produces a difficultly

filterable mixture and relatively poor recovery of solvent.

The extractor depicted in Figure 1 is very satisfactory. The extracting liquid level above the cottonseed minimizes the possibility of incomplete extraction by channeling. The overflow arm does not siphon but flows continuously, and the sample being extracted is always immersed in the solvent.

Proper charging of the extractor is necessary to ensure satisfactory operation. If the petroleum ether is added by pouring atop the cottonseed, there result a large number of dead-air spaces. If the cottonseed is added to pentane contained in the extractor, the "fines" of the grind float to the top, later settle, and form an almost impervious mat. However, filling from a separatory funnel through a glass tube (ca. 10 mm.) reaching to the bottom of the cottonseed results in a charge that contains no air spaces and is sufficiently pervious to accommodate the usual rate of reflux used, which is such that a steady stream falls from the cooling condenser. Measurement of a similar flow rate indicated that about 15 liters of solvent passed through the sample in 6 hours.

Cottonseed subjected to this extraction suffered the same loss in weight (24%) as it did when exhaustively extracted with Skellysolve B for 48 hours and with chloroform for 16 hours in Soxhlet extractors. In the authors' extraction, the yellow overflow liquid became colorless within 3 hours, but as this may not be the criterion for completeness, the process was continued for a total of 6 hours.

Murkiness in the extract results in difficult phase separation in subsequent partition extraction. The sodium sulfate aids in preventing this, but the turbidity is easily removed by filtering the extract through a fine porous-glass filter.

Partition Extraction. Jones and Riddick (*5*) and Burchfield and Storrs (*2*) have demonstrated the advantages of separating insecticides from fatty coextractives by partition between *n*-hexane and polar solvents such as acetonitrile or dimethylformamide. For partition extraction of chlorthion from a pentane solution of cottonseed extractive, methanol and formamide possess unfavorable partition coefficients. That of dimethylformamide is best, but troublesome quantities of oil are also extracted. Acetonitrile proved to be a satisfactory compromise; it extracts very little oil but sufficient chlorthion to allow for a sensitive reproducible colorimetric analysis. The acetonitrile used must be tested for basicity and, if necessary, be made acid; otherwise, serious losses of chlorthion occur.

On considering the densities of the major components present—namely, cottonseed oil (0.92), acetonitrile (0.78), and pentane (0.62)—it is readily seen that some dilutions of cottonseed oil in pentane will not allow ready separation of acetonitrile as the lower phase. Tests with various ratios showed that a solution of a ratio of 1 to 4 (by volume) of cottonseed oil and pentane, respectively, allowed separation into two clear phases in about 5 minutes after this solution was shaken with acetonitrile. Occasional spinning of the funnel released droplets of acetonitrile clinging to the walls of the separatory funnel in the pentane phase.

The efficiency of extraction was demonstrated when 200 ml. of commercial cottonseed oil in 800 ml. of pentane was fortified with 233 γ of chlorthion. Extraction with 100 ml. of acetonitrile (about 50 ml. in the first extraction is recovered, owing to solution in the pentane phase), followed by three more extractions each with 50 ml. of acetonitrile, resulted in a recovery of 190 γ or 81%. This was considered satisfactory in view of the reproducibility of recovery data; furthermore, the quantities of solvents involved were conveniently handled.

Since the samples of cottonseed in this work contained about 25% of oil and the method proved sufficiently sensitive to determine less than 0.02 p.p.m. in 200-gram samples, this amount was taken as the standard sample, and equilibration was performed with volume of 250 ml. of pentane extract solution. Extraction with 60 ml. (ca. 40 ml. recovered) of acetonitrile, followed by three more extractions with 30 ml. of acetonitrile, is an extractive solvent ratio slightly greater than that in the example cited above.

The collected deep-yellow acetonitrile extractive was heated on the water bath to remove dissolved pentane, as the presence of the latter during chromatography causes formation of gas bubbles within the alumina and results in breakup of the column and in channeling.

Chromatography. The deeply colored extract contained yellow pigments that caused variable and sometimes serious losses of chlorthion if they were not removed. Shaking with a number of adsorbents such as activated charcoal, alumina, silene, Attaclay, and magnesium oxide, either in the original pentane extract or in the acetonitrile after partition extraction, proved unsatisfactory. Alumina has a strong affinity for the pigments, probably chiefly gossypol, but losses of chlorthion will occur in this step if the alumina is basic. The column prepared as described above is sufficient to remove the pigments from 200 grams of cottonseed. The upper half of the alumina column will retain the pigments as three orange-brown zones. A pale straw-colored pigment appeared in the filtrate, but this was decolorized in the reduction stage of the analysis. Losses of chlorthion in this stage of the analysis are negligibly small.

The acetonitrile filtrate from the column could be evaporated directly from a Kuderna-Danish evaporator with only negligible loss of chlorthion; this process, however, is annoyingly slow. The acetonitrile was therefore diluted with 500 ml. (2 volumes) of water (acidified to aid phase separation), and the chlorthion was extracted with pentane. The second extraction is chiefly precautionary, as separate analyses on the two extracts rarely showed even faint traces of chlorthion in the second.

These pentane extracts were not washed, as emulsions formed at this stage. Washing is unnecessary, however, because acetonitrile is a completely satisfactory solvent in the reduction of chlorthion and it does not interfere in the color formation stage in the analysis for chlorthion.

Reduction. Reduction with the usual reagents used with parathion (*7*)—namely, hydrochloric acid, and either ethyl or isopropyl alcohol (*7*)—at the color development stage often produced yellow colors even in reagent blank and control sample solutions. Purification of all reagents and, in addition, substitution of methanol or acetonitrile for the organic solvent did not completely remove this difficulty, although with acetonitrile the yellow was consistently more faint. However, if phosphoric acid is used in place of the hydrochloric, blank solutions remain colorless. Because acetonitrile was already present in small amounts, it was chosen as the solvent for the reduction. The action of phosphoric acid with zinc is brisk in this solvent.

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