Use of Chromous Chloride for the Confirmation of Heptachlor Residues by Derivatization

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Introduction

Gas chromatographic detection of multicomponent chlorinated pesticide residues cannot provide an unequivocal identification when based on the single parameter, retention time. One or more tests by another technique are usually required for confirmation of residue identity. Among the available methods, such as thin-layer chromatography (1,2), flash-heater modification of pesticides (3), multi-column gas chromatography (4) and derivatization techniques (5), the last method is often more convenient and more specific.

Previous derivatization investigations have yielded specific methods for the confirmation of heptachlor and its epoxide, cis- and trans-chlordane (6,7) and thiodan (8). This paper describes the preliminary findings on the specific reductive dechlorination of heptachlor using a chromous chloride reagent as an alternative method for confirming residue identity.

Materials and Method

Materials

Petroleum ether (b.p. 30-60°) and acetone were redistilled in glass using a fractionation column (10 cm x 2 1/2 cm o.d.) packed with glass helices. Aqueous chromous chloride solution was obtained from Fisher Scientific Company and used without further purification.

Apparatus and Instruments

All glassware used in this work was heated in a sulphuric-nitric (2:1) acid bath above 80°C for at least one hour and then thoroughly rinsed and dried before use.

An Aerograph Hy-Fi Model 600-D with electron-capture detector and a 5' x 1/8" o.d. coiled glass column packed with a 1:1 mixture of 4% DC-11/6% QF-1 on 60-80 mesh Chromosorb W was used. The column was conditioned at 240°C for 24 hours prior to use. Operating conditions: temperature (°C) - column and detector, 200°; injection block, fitted with Pyrex insert, 210°; nitrogen carrier gas at 80 ml/min.

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Sample Extraction and Cleanup

After the usual sub-sampling of agricultural feeds, dry samples (10 gm) and wet samples (25 gm) were each extracted with petroleum ether (b.p. 30-60°), partitioned with aqueous acetonitrile and finally cleaned up by passage through a basic alumina (deactivated with 6% water) column (9).

Derivatization Method

0.1 µg heptachlor or a suitable aliquot of sample extract was transferred to a 15 ml glass-stoppered graduated tube. Any solvent was evaporated just to dryness with a stream of nitrogen. Approximaterly 1 ml acetone was added and the tube again flushed with nitrogen. As a gentle flow of nitrogen was applied to the open tube, 0.5 ml chromous chloride solution was added and then the contents were tightly stoppered under the nitrogen atmosphere (a spring-loaded clamp was found effective in securing the stopper to the mouth of the tube). The tube was transferred to a water bath (55-60°C) for 45 min. After reaction was complete the tube was removed from the bath, cooled, then 5 ml distilled water and 5 ml petroleum ether added to the reaction mixture. After shaking and subsequent separation of the two layers, the upper organic portion was analyzed by gas chromatography.

Results

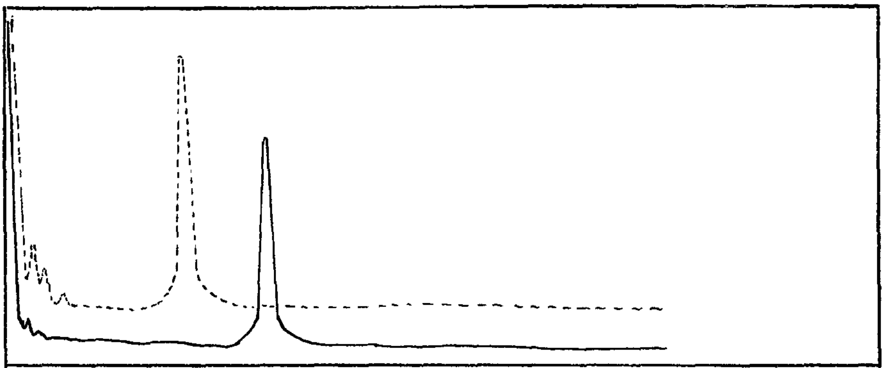
Heptachlor was reduced, by monodechlorination, to chlordene in high yield (Figure 1) by treatment with chromous chloride. The product was verified as chlordene by comparative melting point, infrared spectra and thin-layer chromatography after its isolation from a preparative scale reaction. Production of chlordene is understandable in terms of the greater activity of the allylic chlorine as compared with the other chlorine atoms present in the molecule. Other pesticides tested were p,p'-DDE, p,p'-DDD, p,p'-DDT (and their isomers), lindane, dieldrin, methoxychlor and the cis- and trans-chlordanes. Only lindane reacted significantly under the conditions described. When the reaction time was prolonged p,p'-DDT and p,p'-DDD (and their isomers) yielded a variety of degradation products the identity of which will be reported later.

Discussion

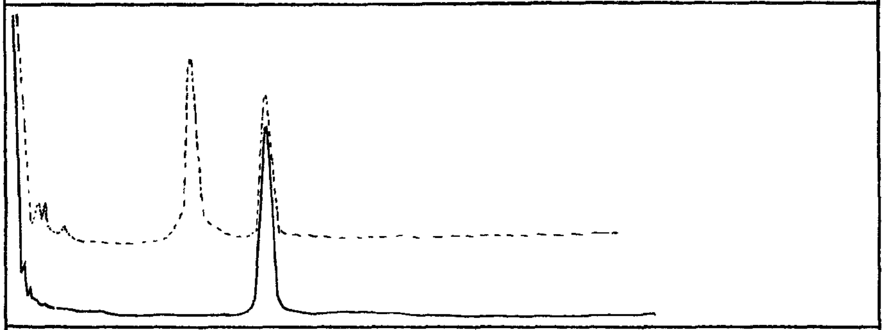
Chromous chloride is a powerful reducing agent. It readily absorbs oxygen from the atmosphere to turn the blue chromous ion to green chromic ion. Therefore, handling of this reagent should be carried out under an inert atmosphere such as nitrogen or CO_2.

Among the chemical derivatization methods (6,7) developed for the confirmation of identity of heptachlor residues, the chromous chloride method is the easiest to perform. The method is also very sensitive and, hence, a level of heptachlor down to 0.01 ppm for a 10 gm sample extract can be readily confirmed. For samples containing low levels of fat or pigments, e.g. beet pulp, as low as

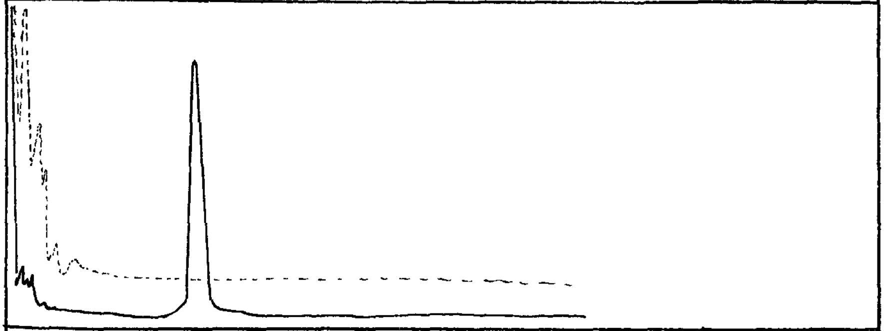
Figure 1



Chromatogram of heptachlor before (——) and after (---) treatment with CrCl$_2$ solution



Chromatogram of heptachlor and 2-chlorochlordene before (——) and after (---) treatment with CrCl$_2$ solution



Chromatogram of lindane before (——) and after (---) treatment with CrCl$_2$ solution

0.005 ppm heptachlor can be confirmed.

As reported earlier (7) 2-chlorochlordene and heptachlor have similar gas chromatographic retention times, i.e. 0.83 and 0.82, respectively, relative to aldrin. It has also been observed that heptachlor and 2-chlorochlordene have similar Rf values on either alumina or silica gel thin-layer plates with a variety of developing solvents. 2-chlorochlordene is the dehydrochlorinated product of *trans*-chlordane (7) and has been found in crops treated with technical chlordane. If this product is present together with heptachlor, only one peak will be observed in the chromatogram (Figure 1); therefore, the identity of these two compounds based on retention time alone can be misleading. The present chromous chloride test easily differentiates between these compounds since 2-chlorochlordene is unaffected by this reagent while heptachlor is converted to chlordene which has a significantly different gas chromatographic retention time, i.e. 0.57 relative to aldrin, from that of 2-chlorochlordene (Figure 1).

The chromous chloride test can be used in the presence of the other chlorinated pesticides mentioned above since these compounds do not interfere with the test. Although lindane reacted under the same reaction conditions, no interference from this pesticide was experienced due to its conversion into more volatile derivatives which emerged with the solvent front in the final gas chromatograph (Figure 1).

This method is not applicable for the confirmation of heptachlor residues if the amount of chlordene produced is small in relation to that already present in the sample. Under these circumstances, alternative methods (6,7) are recommended.

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