Rapid Identification and Determination of Residues of Chlorinated Pesticides in Crops by Gas-Liquid Chromatography*

By E. S. GOODWIN, R. GOULDEN and J. G. REYNOLDS

(*Woodstock Agricultural Research Centre, "Shell" Research Ltd., Sittingbourne, Kent*)

The development of a rapid "sorting test" for identifying traces of chlorinated pesticides in crops by means of gas - liquid chromatography with electron-capture ionisation detection is outlined. In this method, the crop is macerated with acetone and the extract partitioned into hexane before gas - liquid chromatography in nitrogen on a 2-foot column of 100- to 120-mesh kieselguhr supporting 2·5 per cent. by weight of E301 silicone elastomer and 0·25 per cent. by weight of Epikote 1001 maintained at 163° C. Only conventional gas - liquid chromatographic equipment is required, and neither preliminary "clean up" nor concentration of the extract solution is necessary. The seven insecticides lindane, heptachlor, aldrin, Telodrin (1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methanoisobenzofuran), dieldrin, endrin and DDT can be identified when in admixture in fifteen varieties of crops representative of top fruit, leafy vegetables and root crops, the first six insecticides in concentrations generally down to 0·1 to 0·25 p.p.m. and the last generally down to 1 p.p.m. Chlordane, toxaphene and methocychlor can also be identified, but only in rather higher concentration. The procedure, which requires about 50 minutes for a single analysis and 30 minutes for serial analyses, can readily be made fully quantitative and improved in sensitivity for any particular insecticide by adjustment of sample volumes, operating conditions and/or the introduction of an extract "clean-up" stage. Interference present in extracts from grain samples can be resolved by the use of a polar gas - liquid chromatographic column or removed by liquid - solid chromatography. Some indication is given of the considerable potentialities of the technique in allied fields.

The continued growth in the use of pesticides on edible crops coupled with the increased attention being paid to consumer safety has produced a pressing need for rapid "sorting tests" by means of which residues of these chemicals can be identified and determined. The importance of this need was recognised by the Pesticide Residues in Foodstuffs Sub-Committee set up by the Analytical Methods Committee of the Society for Analytical Chemistry in the commissioning of an investigation by Needham[1] into the use and potentialities of bioassay methods for the determination of pesticide residues in foodstuffs.

As a complement to the bioassay approach, techniques based on the use of gas - liquid chromatography show much promise for this purpose. With katharometer detection, Coulson, Cavanagh and Stuart,[2] Zweig and Archer[3] and also Dimick *et al.*[4] were able to separate, identify and determine mixtures of pesticides or pesticide isomers on the milligram or decimilligram scale. The application of such gas - liquid chromatographic methods to residue analysis on the microgram scale with more sensitive means of detection, *e.g.*, argon ionisation, is made difficult by chromatographic interference resulting from material co-extracted from the crop, unless a "clean-up" stage is included. The need for such a "clean up" was overcome by

* Presented at the meeting of the Society on Wednesday, November 1st, 1961.

Coulson *et al.*[5] by the use of a gas - liquid chromatographic - combustion - coulometric titration procedure applicable to both chlorinated and thiophosphate pesticide residues and by Zweig, Archer and Rubenstein,[6] who used a gas - liquid chromatographic - infra-red spectrophotometric method.

To avoid the necessity for these somewhat complex combination methods, a simpler means of detection is required that possesses not only great sensitivity but also a high degree of selectivity towards the pesticides to be identified and determined. These requirements are met to a considerable extent by the electron-capture ionisation detector of Lovelock and Lipsky,[7,8] which can be made to exhibit exceptional response to halogenated compounds. This selective response, which is such that nanogram (10^{-9} g) amounts of chlorinated compounds can readily be determined, permits the identification and determination of traces of chlorinated pesticides in crop extracts without the need either for prior "clean up" or for the preliminary concentration of the extract solution.

In an earlier note[9] we indicated that a readily obtainable argon-ionisation detector could be used for electron-capture ionisation detection and outlined the results of some preliminary work on its application to the analysis of crop extracts for traces of chlorinated pesticides. This paper reports the extension of this work, with particular emphasis on the development of a simple rapid "sorting test" for identifying and determining residues of chlorinated pesticides in crops. At the same time, the wide scope of the electron-capture gas - liquid chromatographic technique in the analysis of agricultural, atmospheric and industrial samples for traces of halogenated pesticides is indicated.

EXPERIMENTAL AND RESULTS

A. Development of the "sorting test"

1. Extraction procedure—

In order to make the "sorting test" as rapid as possible and to take full advantage of the speed of the gas - liquid chromatographic stage of the method it was considered essential to employ a quick simple extraction procedure. The use of non-polar - polar solvent mixtures, *e.g.*, hexane - isopropanol or hexane - acetone, was found unsatisfactory in that, with crops containing a high proportion of water, emulsification problems were often encountered and two phases obtained. This resulted in partition of the polar solvent between the aqueous and organic phases; in consequence, the latter had to be completely freed from polar solvent by washing with water before a quantitative aliquot could be taken for analysis. Further, since the extraction time was to be kept as short as possible, it was desirable to use the most effective solvent available. For this reason acetone was chosen as the crop-maceration solvent. The minimum volume consistent with obtaining a fairly fluid macerate was used, the mixture then being filtered and washed with acetone and the filtrate adjusted to volume. Direct gas - liquid chromatography of the acetone extract was, however, found to be impracticable, since the massive amounts of co-extracted crop material present resulted in swamping of the detector, despite its comparative insensitivity to non-halogenated compounds. The insecticide present in the acetone extract was therefore partitioned into hexane in the presence of an excess of 2 per cent. aqueous sodium sulphate solution, much of the interfering co-extracted material being left behind. This process took up little time, since separation of the phases was rapid and the aliquot for analysis could be taken directly from the supernatant hexane layer.

2. Gas - liquid chromatographic procedure—

(*a*) *Apparatus used*—The Shandon Universal Gas Chromatograph employed comprised a U-shaped chromatographic column heated by boiling liquid under reflux and surmounted by an argon-ionisation detector, the signal from which was fed via a d.c. amplifier to a 10-inch chart width recorder (full-scale deflection, 1 mV) having a pen-response time of 2 seconds. The detector was modified only to improve its insulation, this being achieved by substitution of the Sindanyo and silicone-rubber components by polytetrafluoroethylene. This effected a three-fold improvement in detector sensitivity. The range of potentials that could be applied to this detector was extended by the use of high-tension batteries to below the minimum of 300 volts available from the instrument.

(*b*) *Column packings*—In all of this work kieselguhr was used as supporting medium. Initially, columns up to 8 feet long of 60- to 100-mesh material packed in $\frac{5}{32}$-inch internal diameter copper tubing were employed, but the need to eliminate insecticide decomposition and to reduce retention times led to the use of 2-foot (and even shorter) columns of 100- to 120-mesh material. Kieselguhr from several sources appeared equally satisfactory and the use of specially acid-washed material effected no improvement. No advantage was obtained by the use of Ballotini glass micro beads, whether plain, acid or water-washed, in place of the kieselguhr.

The number of stationary phases that can be employed in gas - liquid chromatography at temperatures in excess of 200° C is limited. One of these, which is widely used, is E301 silicone elastomer (obtained from Imperial Chemical Industries Ltd.), which was employed in our earlier experiments at concentrations up to 10 per cent. of the weight of supporting medium. Under these conditions and at even lower temperatures, columns containing the stationary phase mentioned above gave satisfactory chromatography immediately for aldrin, but some conditioning with insecticide was needed (compare Coulson *et al.*[5]) before the chromatography of either lindane or dieldrin could be achieved. This effect was thought to be due to adsorption of these insecticides on to the kieselguhr, and it was for this reason that the glass micro beads referred to above were examined. Stable, involatile polar compounds have been used with success[10,11] for reducing the adsorptive properties of supporting media. The addition to the E301 silicone elastomer of 10 per cent. of its weight of Reoplex 400 (Geigy Co. Ltd.) or Epikote 1001 (Shell Chemical Co. Ltd.) was therefore studied and found to obviate the need for column conditioning. Further, the decomposition encountered during the chromatography of some of the insecticides examined (compare Goodwin *et al.*[9]) was either reduced or eliminated. Of the above two polar additives, Epikote 1001 was found preferable on account of its greater adsorption-suppressing efficiency and thermal stability under the conditions employed. Some tests were carried out in which the whole of the E301 silicone elastomer was replaced by Epikote 1001, but the insecticide separations obtained were much less satisfactory than with E301. As the work developed, the amount of stationary phase employed was progressively decreased. This resulted in shorter retention times and a reduction in the tendency of the stationary phase to "bleed," with its consequent adverse effect on the detector. In most of the later work, 2·5 per cent. of E301 silicone elastomer *plus* 0·25 per cent. of Epikote 1001 on plain 100- to 120-mesh kieselguhr was used.

(*c*) *Carrier gas*—Initially, argon was employed as carrier gas at flow rates of about 6 litres per hour and was dried before use by passage through indicating silica gel. Subsequently, argon was replaced by oxygen-free nitrogen, dried as above, in order to eliminate any residual argon-ionisation response from the detector still obtaining at the low potentials employed. In addition, flow rates were raised to 12 litres per hour, so as to reduce insecticide retention times and also effect some improvement in sensitivity.

(*d*) *Column temperature*—In conformity with earlier published work on the gas - liquid chromatography of chlorinated pesticides, column temperatures in the region of 230° C were employed at first. Under these conditions, some of the insecticides examined, when chromatographed in submicrogram amounts, decomposed with the production of multi-peak chromatograms. Further, decomposition of co-extracted crop material coupled with "bleeding" of the stationary phase resulted in the gradual de-sensitisation of the detector. Lowering of the column temperature helped to overcome these failings, but at the same time resulted in increased retention times, which were counteracted, as already described, by employing faster gas-flow rates and lower concentrations of stationary phase. Column temperatures as low as 163° C were used, this being practicable with compounds having such low vapour pressures as the chlorinated insecticides, provided that the loads injected do not exceed a few micrograms.

(*e*) *Detector characteristics*—The Shandon argon-ionisation detector is a metal-cased detector having a radium D source. When employed in the conventional manner at a potential of 1200 volts and with argon as the carrier gas, positive chromatographic peaks were obtained for both chlorinated and organo-phosphorus insecticides when injected in microgram amounts. As the potential was decreased, the peaks for the chlorinated insecticides became negative, indicating that the detector was exhibiting electron-capture ionisation response. This response increased steadily as the applied potential was decreased to 300 volts, the lowest potential obtainable from the power pack of the instrument. At this voltage

significant peaks were given by nanogram amounts of the chlorinated insecticides. It was under these conditions that much of the earlier work[9] was done, and it is probable that the lack of interference shown by co-extracted crop material on the injection of comparatively large volumes (100 μl) of extract was due in part to the balancing out of any negative peaks owing to electron-capture ionisation response for the non-chlorinated material by the residual positive argon-ionisation effect still obtaining at an applied potential of 300 volts. Subsequently, lower potentials were applied by the use of high-tension batteries, with consequent increase of sensitivity, until a point of maximum sensitivity was reached. After the detector had been cleaned with an abrasive and washed with solvent, the optimum potential for maximum sensitivity was still further reduced (to less than 40 volts), and at the same time an additional increase in sensitivity occurred. This fact, coupled with the replacement of argon by oxygen-free nitrogen, resulted in the elimination of any argon-ionisation effect and necessitated a reduction in the volume of extract injected in order to bring the crop background interference down to an acceptable level. When argon, a potential of 300 volts and injections of 100 μl volume were used, there was a tendency for premature contamination of the column and for drift in the balance point between electron-capture ionisation and argon-ionisation effects due to de-sensitisation of the detector, *e.g.*, by decomposition products coming from the column. Accordingly, it was considered preferable to employ electron-capture ionisation as the sole means of detection.

By using the Shandon detector for this purpose it was found that the amplifier gain control could be set at up to $\times 200$ before the "noise" level became significant. In the "sorting-test" procedure, therefore, the gain was normally set at $\times 50$, so as to give adequate sensitivity and yet leave some amplification in hand. The response of the Shandon detector when used under conditions of electron-capture ionisation showed reasonable linearity over a limited range only, which varied with the insecticide under test. For example, the response of the detector was fairly linear for aldrin in amounts up to about 0·0025 μg, for dieldrin up to about 0·006 μg and for DDT up to about 0·02 μg, equivalent in the present work to about 2, 5 and 20 p.p.m., respectively, on the crop. Under the standardised conditions employed for the "sorting test," loss of linearity became significant when the chromatographic peak due to the insecticide exceeded about 70 per cent. of the full-scale deflection of the recorder. Thus, by setting the gain at $\times 50$, the most effective use could be made of the recorder chart width under conditions of near-linear detector response.

The success of the Shandon argon-ionisation detector when employed as an electron-capture ionisation detector resulted in the construction and study of an argon-ionisation detector of the small diode pattern described by Lovelock.[8] When used at its optimum potential for electron-capture ionisation detection (about 100 volts), this detector, which contained a tritium source, exhibited a sensitivity to chlorinated compounds about ten times greater than that of the Shandon detector. By means of this Lovelock detector, chlorinated insecticides could be determined on the decinanogram scale. Despite its greater sensitivity and lower "noise" level, it was decided for the present work not to employ this detector in place of the Shandon model, since the latter had adequate sensitivity for the purpose in hand and, moreover, had the merit of being typical of detectors readily obtainable commercially.

A brief study was made of the behaviour of the Shandon detector towards organo-phosphorus insecticides when used at the optimum potential for maximum electron-capture ionisation response for the chlorinated insecticides. The sensitivity of the detector to the organo-phosphorus compounds was found to be very much lower than to the chlorinated compounds. For example, the response to parathion was fifteen times lower, to malathion forty times lower and to Phosdrin three hundred times lower than it was to aldrin.

B. Procedure recommended for "sorting test"

1. Instrument preparation—

(*a*) *Column packing*—Weigh out amounts of 100- to 120-mesh chromatography-grade kieselguhr, E301 silicone elastomer and Epikote 1001 to give 2·5 per cent. by weight of silicone and 0·25 per cent. by weight of Epikote on the weight of support. Dissolve the mixed stationary phases in AnalaR ethyl acetate, and add the kieselguhr to the solution. Remove the organic solvent on a bath of hot water, stirring the mixture throughout the evaporation. Sift the dried material, collecting the portion between 100 and 120 mesh. A yield of about 70 per cent. is generally obtained. Fill a 2 foot long $\frac{5}{32}$-inch internal diameter

copper gas - liquid chromatographic column with 4·0 to 4·5 g of the freshly prepared graded column filling, tapping the column repeatedly during the addition in order to achieve uniform and dense packing.

(*b*) *Column temperature*—Maintain a constant column temperature of 163° C by boiling cyclohexanol under reflux in the surrounding vapour jacket.

(*c*) *Carrier gas*—By the use of a soap-film flow meter, prepare a plot of the flow rate of oxygen-free nitrogen through the packed column against the column inlet pressure as indicated on the nitrogen cylinder reducing valve. By using this calibration, adjust the flow rate of nitrogen to a room temperature value of 12 litres per hour. The usual inlet pressure for this flow rate is about 8 lb per sq. inch gauge.

(*d*) *Column pre-treatment*—Condition the new column at 163° C by passing nitrogen through it at 12 litres per hour for some hours before fitting the detector in place. This process avoids contaminating detector parts during the high initial "bleed" of stationary phase.

(*e*) *Control settings*—Set amplifier and recorder zero pre-set controls so that the recorder pen is 2 inches from the normal full-scale deflection side of the chart. Arrange for the chart to run at 24 inches per hour, and set the gain control at ×50. Adjust "backing off" until the pen position coincides with that for amplifier and recorder zeros when the instrument is switched to "READ."

(*f*) *Detector potential*—Potentials are applied to the detector from a battery source connected with its positive terminal earthed. Record the detector response at applied potentials in the range 4 to 40 volts for a constant injection of a standard solution of lindane in hexane. By inspection, select the optimum potential for maximum detector response. In this connection it may be noted that the point of inflexion on the curve produced by plotting "backing-off" readings against applied potential gives an approximate value for the optimum potential for maximum detector response. Some slight drift from the optimum potential occurs with column use. For maximum sensitivity it is therefore advisable to check the optimum position by measurement of the detector response to lindane when the potential is varied a few volts either way.

2. SAMPLE PREPARATION—

From the field sample supplied, weigh a fully representative 50-g sub-sample of the crop into the 200-ml maceration jar of a high-speed top-drive macerator. Chop the crop material into small pieces with a long-bladed knife, and add sufficient redistilled AnalaR acetone just to cover the sample. The volume of solvent normally required is 60 ml, but for dry crops, such as tea and flour, and some bulky materials, such as cabbage, the volume may be increased to a maximum of 80 ml. Macerate the sample for 3 minutes, and then set aside for 5 minutes. Transfer the contents of the jar as completely as possible to a 7-cm diameter No. 3 sintered-glass funnel. Filter under reduced pressure directly into a 100-ml graduated cylinder, and press the crop material as dry as possible by means of a flattened glass rod or small beaker. Wash the crop residue on the filter with acetone so as to make the filtrate up to 100 ml. Remove the filter, insert the stopper in the cylinder, and shake well.

By pipette, place 5 ml of the acetone extract into another 100-ml graduated stoppered cylinder. Add 10 ml of redistilled laboratory-reagent grade n-hexane by pipette, and mix thoroughly by swirling. Measure 85 ml of a 2 per cent. aqueous solution of sodium sulphate into the cylinder, insert the stopper, and shake vigorously for 30 seconds. Allow the two phases to separate, then remove 5 μl of the supernatant hexane layer by means of a 10-μl fixed-needle Hamilton syringe pipette.

3. GAS - LIQUID CHROMATOGRAPHY OF SAMPLE—

Check and, if necessary, adjust the gas-flow rate, column temperature and instrument control settings. Start the chart drive, and inject the 5-μl sample via the silicone-rubber septum at the inlet to the column. After about 22 minutes, stop the chart drive. Examine the chromatogram in comparison with one obtained from a control crop (if available) processed under identical conditions. When peaks are obtained compare their retention times with those obtained for mixtures of chlorinated technical insecticides run under identical chromatographic conditions.

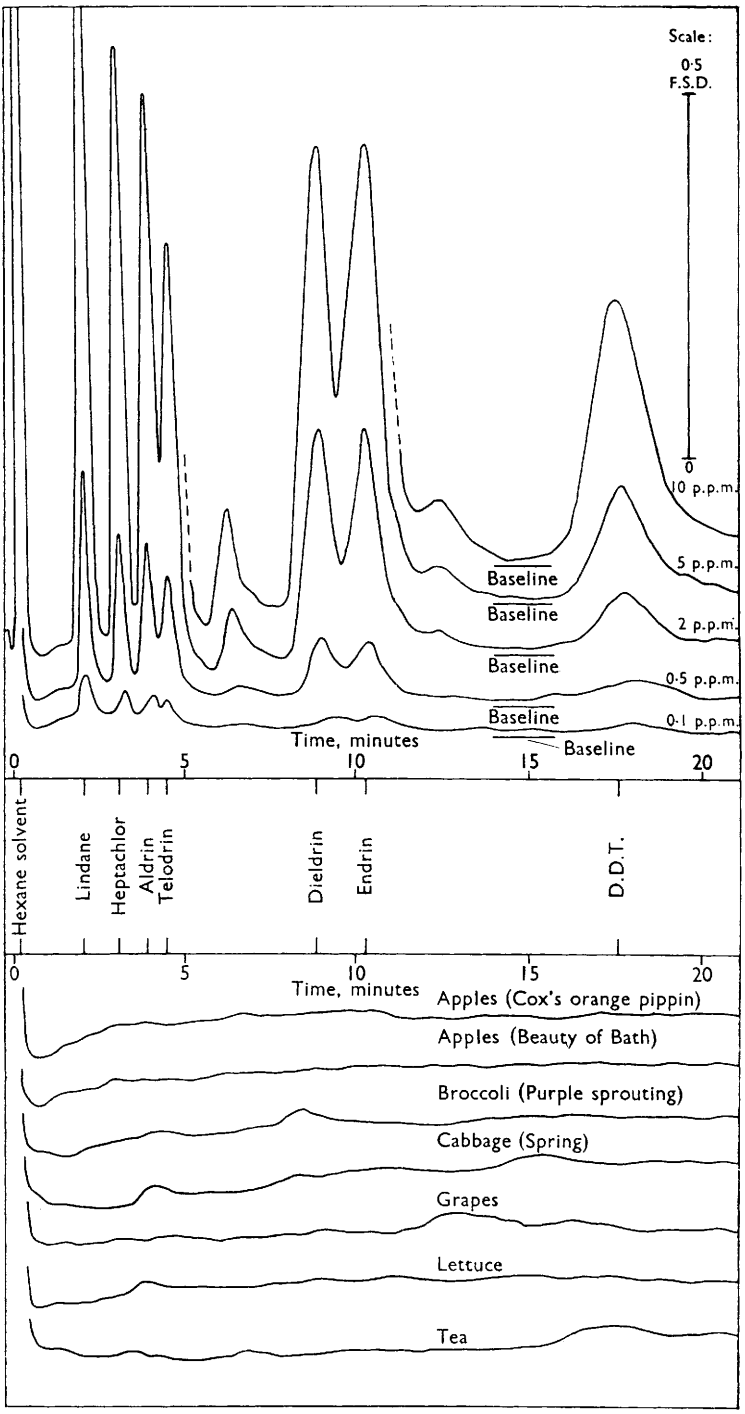


Fig. 1. Chromatograms of technical-grade insecticide mixtures and control-crop extracts

C. Results obtainable by "sorting test"

1. Chromatograms of insecticides against control crops—

Control samples of the crops listed below, taken as being representative of top fruit, leafy vegetables and root crops, were processed according to the above procedure and in all instances gave chromatograms in which the level of background interference was acceptably low—

Apples (Blenheim)	Cabbage (spring)	Lettuce
Apples (Cox's orange pippin)	Cabbage (winter)	Potatoes
Apples (Beauty of Bath)	Carrots (English)	Swedes
Broccoli (purple sprouting)	Carrots (Italian)	Tea
Broccoli (spring heading)	Grapes	Tomatoes.

Examples of these control chromatograms are shown in Fig. 1, together with the chromatograms produced by a mixture of the technical insecticides lindane, heptachlor, aldrin, Telodrin,* dieldrin, endrin and DDT in amounts equivalent to 0·1, 0·5, 2·0, 5·0 and 10·0 p.p.m. on the weight of the crops. As would be expected, the peak heights given by the insecticides decrease as their retention times increase. With lindane, for example, 0·1 p.p.m. of insecticide is readily detectable in the crops shown, full-scale deflection of the recorder being obtained when about 1·5 p.p.m. are present. With dieldrin, on the other hand, the minimum amount generally detectable in the "sorting test" is about 0·25 p.p.m., full-scale deflection of the recorder being obtained when about 8 p.p.m. are present. The general limits of detection for the seven insecticides in the above-mentioned fifteen crop varieties are given in Table I. Sometimes, however, the control chromatograms are such that the limit of detection for one of the insecticides may be less satisfactory, *e.g.*, aldrin in spring cabbage and DDT in tea.

Table I

General limits of detection of seven technical insecticides in apples, broccoli, cabbage, carrots, grapes, lettuce, potatoes, swedes, tea and tomatoes, by the standard "sorting-test" procedure

Insecticide	Lindane	Heptachlor	Aldrin	Telodrin	Dieldrin	Endrin	DDT
General limit of detection, p.p.m.	0·1	0·1	0·1	0·2	0·25	0·25	1·0

In the insecticide chromatograms shown in Fig. 1 a further two small peaks are evident, particularly so at the higher concentrations. The first of these peaks, which has a retention time of 6·3 minutes, is due to heptachlor epoxide. This epoxide, which is not the biological metabolite, was present in the technical heptachlor used in this work and could be isolated from it by liquid - solid chromatography on Florisil. The second peak, having a retention time of 12·2 minutes, is due to *o*,*p*'-DDT present in the technical DDT employed.

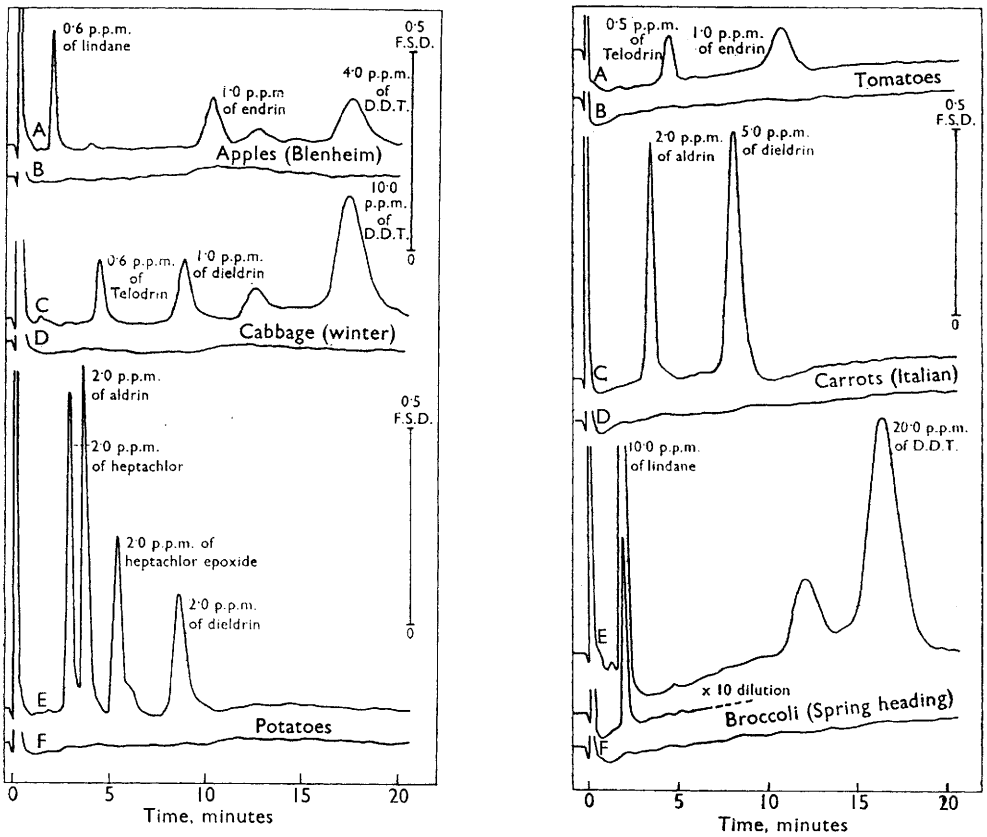
In addition to the seven insecticides referred to above, chromatograms were run on technical samples of chlordane, methoxychlor and toxaphene. The first of these gave a well defined chromatogram having eight peaks with retention times ranging from 1·6 to 12·8 minutes. Two of the peaks had retention times not far removed from that of heptachlor, but of only about one-tenth the sensitivity of the latter. Methoxychlor produced only one major peak of 35-minutes retention time and one minor peak of retention time between that of lindane and heptaclor, but had a peak sensitivity of only about one fifth that of chlordane. Toxaphene gave a multi-peak parabola of equally low intensity, having a retention time up to about 20 minutes.

2. Typical "sorting-test" results—

Known amounts of three or four technical insecticides in acetone solution were added at the maceration stage to samples of apple, cabbage and potato, which were then processed as described. The chromatograms produced, which are shown in Fig. 2, indicate the kind of results generally obtained by the use of the standard "sorting-test" procedure. In the test on apple, shown in Fig. 2A, the smaller peak, of 12·3-minutes retention time, is due to the *o*,*p*'-DDT present in the technical DDT used. This peak is shown again, more clearly, in Fig. 2C in the test on cabbage. In the treated potato sample (Fig. 2E) the heptachlor

* Telodrin is the Shell Trade Mark name for 1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methanoisobenzofuran.

epoxide added was the biological metabolite, not the one present in the technical heptachlor. The two epoxides have different retention times and are thus readily distinguishable from each other and from the heptachlor itself.



Figs. 2 and 3. Chromatograms of toxicant-treated and control crops obtained by using the standard "sorting-test" conditions

3. Quantitative aspects—

When, in the "sorting test," chromatographic peaks due to insecticide are less than about 70 per cent. of the recorder full-scale deflection, it is possible to make a rapid approximate assessment of the amount of insecticide present. When peaks exceed 70 per cent. of the recorder full-scale deflection, with the result that detector response is no longer reasonably linear, a repeat injection is necessary before making any quantitative assessment. This repeat injection is best carried out with a 5-μl sample after appropriate dilution of the hexane extract. Alternatively, a smaller sample (say down to 1 μl) can be injected, but this leads to reduced precision. Conversely, when peaks are very small, a higher gain setting can be employed, a larger sample—say up to 25 μl—can be injected or a bigger aliquot of the acetone extract can be partitioned into the hexane. Success with any of these procedures depends on the level of crop-background interference which obtains.

For quantitative work, measurement of peak areas was found, as expected, to give more reproducible results than measurement of peak heights. In the preparation of calibration graphs, the use of different volumes (4 to 40 μl) of a single standard insecticide (generally 0·1 μg per ml) solution is preferred to a fixed volume (*e.g.*, 5 μl) of several standard solutions, since preparation of standards in the latter method can be time-consuming when many insecticides are being studied. At the same time, it must be borne in mind that, with the smaller Hamilton micro-syringe pipettes, significant over-injection occurs owing to the

"flashing off" of a portion of the solution contained in the syringe needle. As an example, in this work the over-injection on a 5-μl sample in the 10-μl fixed-needle syringe pipette is +0·4 μl. The use of a standardised injection procedure makes this increment fairly reproducible, so that allowance for it can be made when the syringe is filled.

An indication of the recoveries obtainable by the standard "sorting-test" procedure was obtained in the following experiment. Known amounts of pairs of insecticides in acetone solution were added at the maceration stage to 50-g samples of control tomato, carrot and broccoli, which were then processed and analysed as described. The areas under the peaks in the chromatograms, which are shown in Fig. 3 (A, C and E), were determined, and the recoveries were calculated from the calibration graph resulting from the direct injection of known amounts of the insecticides on to the column. The results obtained are shown in Table II. For lindane and DDT in broccoli, two chromatograms were run, one under the standard conditions to determine DDT and the other after ten-fold dilution of the hexane extract to determine lindane.

TABLE II

RECOVERIES BY THE STANDARD "SORTING-TEST" PROCEDURE OF PAIRS OF TECHNICAL INSECTICIDES ADDED AT THE MACERATION STAGE TO THREE CONTROL CROPS

Crop treated	Insecticide added	Amount present, p.p.m.	Amount recovered, %
Tomato	Telodrin	0·5	90
	Endrin	1·0	93
Carrot	Aldrin	2·0	78
	Dieldrin	5·0	77
Broccoli	Lindane	10·0	80
	DDT	20·0	83

4. GENERAL OBSERVATIONS—

At the comparatively low temperature of 163° C and with the small loadings used in the "sorting test," column life can be up to several weeks. Detector performance, on the other hand, falls off more rapidly, owing to progressive contamination resulting from a slight "bleed" of stationary phase or crop-decomposition products. This deterioration in sensitivity is due to a drift in the optimum potential for maximum sensitivity to a higher value. When this deterioration becomes serious, high performance can be restored by a combination of abrasive cleaning and solvent washing of the detector.

With careful standardisation of the "sorting-test" conditions, insecticide retention times are generally reproducible to within ± 2 per cent. As might be expected, slight increases in retention times do, however, take place when larger (*e.g.*, 25 μl) aliquots of the hexane extract are used. Reference chromatograms should therefore be run under identical volume-injection conditions.

The time required to carry out one "sorting-test" analysis from receipt of a representative field sample to semi-quantitative assessment of results is about 50 minutes. Of this time about 20 minutes is required for the gas - liquid chromatographic stage, which requires no supervision. In consequence, serial analyses can be completed in about half an hour.

D. EXTENSION OF METHOD TO INCREASE SCOPE

1. TREATMENT OF CROPS SHOWING CHROMATOGRAPHIC INTERFERENCE—

On examining control grain samples by the "sorting test," sharp interference peaks having retention times of 4·2 to 4·3 minutes were observed in the chromatograms. This interference was found present in wheat, oats, barley, maize (slight only) and rice. The rice also gave a large peak at 1·0 minutes; a sample of control onion gave a peak at 0·7 minute. These two last-mentioned peaks are, however, of no serious consequence in that they occur well before the most volatile of the insecticides studied (lindane) appears. The interference peaks appearing in the grain samples at about 4·25 minutes, on the other hand, could be confused with aldrin. Two methods of resolving this problem were examined.

(*a*) *Two-column gas - liquid chromatography*—In the "sorting-test" procedure, gas - liquid chromatography is effected on an essentially non-polar stationary phase, with the result that compounds are eluted in order of volatility. By use of a polar stationary phase, however, the order and times of elution become more dependent on the polarities of the compounds

being chromatographed. Thus, in this instance, a 5-μl aliquot of the control oat sample was chromatographed on a 2-foot column of 100- to 120-mesh kieselguhr supporting 2·5 per cent. by weight of Epikote 1001 maintained at 188° C by refluxing propylene glycol. Chromatograms from both columns were also obtained from the oat sample after the addition at the maceration stage of 0·25 p.p.m. of aldrin. The results obtained, which are shown in chromatograms A, B, C and D in Fig. 4, indicate that, although on the standard non-polar column the oat-interference peak is indistinguishable from aldrin, on the polar column complete resolution is effected.

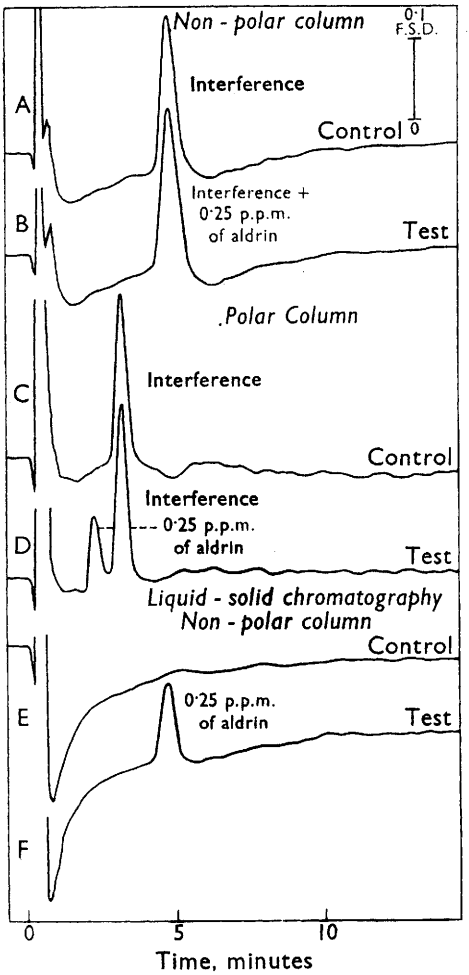


Fig. 4. Chromatograms of control and aldrin-treated oats

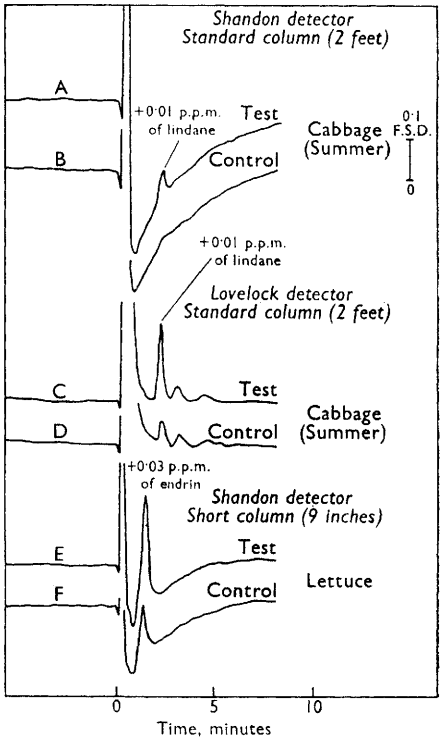


Fig. 5. Chromatogram of crops treated with toxicants at low concentrations

(*b*) *Liquid - solid chromatography*—Differences in compound polarity form the basis of liquid - solid (adsorption - elution) chromatography. Accordingly, 5-ml aliquots of the hexane extracts of the two above-mentioned samples were chromatographed on columns 6 cm long by 1 cm bore containing 5 g of Brockmann grade I/II alumina of about 1 per cent. adsorbed moisture content and eluted with n-hexane until the eluates reached a volume of 25 ml. Gas chromatography of 25-μl aliquots of these eluates on the standard non-polar column at 163° C gave the chromatograms E and F shown in Fig. 4. From these it can be seen that liquid - solid chromatography on alumina has eliminated from the control sample the interference peak at 4·25-minutes retention time and that, in the chromatogram of the treated sample, only the peak due to aldrin remains. Recovery of the insecticide put through this "clean-up" process was found to be quantitative.

2. PROCEDURES FOR MAXIMUM SENSITIVITY—

When small amounts (*e.g.*, <0·1 p.p.m.) of insecticide have to be detected, the level of chromatographic interference due to the crop must be reduced, *e.g.*, by liquid - solid chromatography, and more sample must be used in the analysis, as shown in the following example.

Summer cabbage (50 g) was treated at the maceration stage to contain 0·01 p.p.m. of lindane and processed to give a 100-ml acetone extract. A 20-ml aliquot of this was partitioned into 10 ml of n-hexane, a 5-ml portion of which was chromatographed on a calibrated 5-g column of alumina with hexane - 1 per cent. acetone solution; a 15-ml eluate fraction was taken. Aliquots of 50 μl of this solution were then gas-chromatographed on the standard 2-foot column. In the first instance the Shandon detector was used with a gain-control setting of $\times 100$ and later the Lovelock detector already referred to was employed with a gain-control setting of $\times 20$. The gas chromatograms obtained, which are shown in Fig. 5 (A, B, C and D), indicate that, although some control-crop interference at the retention time of lindane is still evident after liquid - solid chromatography, the presence of 0·01 p.p.m. of lindane in the crop is clearly visible.

For insecticides having longer retention times, increase in sensitivity can be achieved by the use of a shorter column supporting less stationary phase and by the employment of a faster gas-flow rate. A lettuce sample was treated to contain 0·03 p.p.m. of endrin and then processed and "cleaned up" as described above. Gas - liquid chromatography was carried out on a 9-inch column of 100- to 120-mesh kieselguhr supporting 1 per cent. of E301 silicone elastomer and 0·1 per cent. of Epikote 1001. The nitrogen flow rate employed was 21 litres per hour. With the Shandon detector and a gain-control setting of $\times 50$, the chromatograms E and F shown in Fig. 5 were obtained, which indicate that endrin is readily detected at the 0·03 p.p.m. level, despite the presence of some crop interference, and is eluted from the column in 1·5 minutes.

GENERAL DISCUSSION AND CONCLUSIONS

Some of the limitations in the use of bioassay techniques for the screening of foodstuffs for the presence of pesticide residues have recently been discussed by Chilwell and Hartley,[12] who observed that the only serious attention that had been given to the screening aspect of residue analysis was the possibility of identification by dual-system paper chromatography,[13] so far most completely developed for the chlorinated hydrocarbons. Notwithstanding its shortcomings, a bioassay method involving, say, house-flies, fruit-flies or mosquito larvae constitutes at the present time the most potentially useful type of "catch-all" screening procedure. After preliminary screening by such a method, those edible crops exhibiting toxicity must be examined for the identification and determination of the pesticidal residues present. These residues will generally be either organo-phosphorus compounds or chlorinated hydrocarbons. For the latter, gas - liquid chromatographic methods of analysis show much promise and have the advantage over paper-chromatographic procedures of being both rapid and quantitative.

The chief merits of gas - liquid chromatography with electron-capture ionisation detection over other gas - liquid chromatographic methods for determining chlorinated pesticide residues are that it requires no ancillary equipment to effect selectivity, no preliminary concentration stage and, in general, no "clean up" of the extract solution. These factors result in both simplicity and speed. The electron-capture gas - liquid chromatographic "sorting test" described herein could therefore be a useful supplement to the primary bioassay screen and, when only chlorinated pesticides are involved, might even replace it.

From the results obtained it can be seen that the "sorting test" employed in standard form permits the detection of the six chlorinated insecticides lindane, heptachlor, aldrin, Telodrin, dieldrin and endrin in concentrations down to 0·1 to 0·25 p.p.m. in most of the crops examined. For DDT, however, the general limit of detection is rather higher at 1 p.p.m., although for screening purposes this is not serious in view of the comparatively high tolerance limit normally set for this compound. The limit of detection attainable in the standard procedure is obviously dependent on the nature of any chromatographic interference arising from the crop itself. Experience with the method may show that the majority of top fruit, leafy vegetables and root crops produce satisfactorily low chromatographic backgrounds and that only a few types of foodstuffs, *e.g.*, the grains, require special

treatment. If this proves to be so, then most samples could be analysed by the standard "sorting-test" procedure and the two-column gas - liquid chromatographic technique or a simple liquid - solid chromatographic "clean-up" process applied only to the few crops known to be atypical. If, however, interference *is* exhibited in some samples of a wide variety of crops then the "sorting test" should at least serve as a rapid method of screening samples for the *absence* of chlorinated pesticide residues, all crops showing chromatographic peaks being examined by the above-mentioned supplementary techniques. The preliminary screening could then be made still more rapid, at the expense of individual toxicant identification, by reduction of retention times to, say, 5 minutes over-all.

One limitation to the standard "sorting-test" procedure is that chlordane, toxaphene and methoxychlor would not be detected unless present in comparatively high concentration, the two first-mentioned because of their multi-component nature and the last because of its long retention time under the conditions employed. Of these three insecticides, however, the two last-mentioned have high tolerance limits.

In the quantitative work, recoveries from three crops of six insecticides added at the maceration stage in concentrations ranging from 0·5 to 20 p.p.m. average 80 to 90 per cent. and show good reproducibility for any one crop. The use of an appropriate correction factor would therefore seem to be reasonable. Since experiments have shown that the partition stage of the sample-preparation procedure is over 95 per cent. efficient, the losses encountered may well be due to retention of toxicant by the crops. Nevertheless, a rapid crop-preparation process resulting in reasonably high and reproducible recoveries is considered to be preferable for the present purpose to a longer but more exhaustive extraction procedure. When complete maceration of the sample is unnecessary, then the use of some simpler insecticide-removal process, *e.g.*, the solvent stripping of whole fruit, should effect a further saving in time and result in a substantial reduction in the limits of detection, owing to the comparatively small amount of co-extracted crop material present.

As has been shown, the general limits of detection attainable by the standard procedure can be significantly reduced by introducing a simple "clean-up" stage and by altering such variables as partition and injection aliquots and the conditions of gas - liquid chromatography employed. It should be emphasised here that the standard "sorting-test" procedure is necessarily a compromise method designed to give adequate results for the widest possible range of chlorinated insecticides. It follows that in more limited circumstances, when only one or two insecticides need be sought for, operating conditions can be tailored as appropriate to achieve greater sensitivity in the method. As already indicated, for example, reduction in column length and amount of stationary phase, coupled with increase of gas-flow rate, permit an appreciable lowering in the limit of detection of endrin, as well as a marked reduction in the time required for gas chromatography.

The commercial detector used in this work under conditions of electron-capture ionisation detection shows exceptional response to halogenated compounds. This, Lovelock has recently pointed out,[14] is due to their highly "electrophoric" nature. The limits of detection in the "sorting test" are governed by the relative amounts of pesticide and co-extracted crop material present and by their respective "electrophoric" characteristics rather than by any limitation in the sensitivity of the commercial detector. Full advantage can be taken of the even greater sensitivity of the more recent detectors (see Lovelock[8]) only if the ratio of crop interference to pesticide is reduced. The proposed method appears to be comparatively insensitive to the organo-phosphorus insecticides, since they do not in general possess high electron affinity. One exception to this is parathion, in which the presence of the highly "electrophoric" $-NO_2$ group could explain why its limit of detection is no more than fifteen times greater than that for aldrin.

It will be obvious from the foregoing discussion that gas - liquid chromatography with electron-capture ionisation detection is a technique of some potential value for the identification and determination of traces of halogenated compounds. Evidence of compound identity can be made almost certain by use of the two-column gas - liquid chromatographic technique, whereas the analysis of samples containing more heavily interfering extractables than do crops can generally be effected after the application of suitable "clean-up" procedures. As an example, the method has been used with success for determining traces of chlorinated insecticides in "cleaned-up" extracts of animal, avian and insect tissue. The last-mentioned analysis, made possible only by the ability of the method to determine nanogram amounts

of insecticide in very dilute solution, indicates the potentialities that this technique may have in the study of the mechanisms of insect resistance. In the same way have traces of halogenated insecticides, nematicides and herbicides been determined in soil by the method, which readily permits metabolic conversions such as aldrin → dieldrin or heptachlor → heptachlor epoxide to be followed. Finally, it may be noted that preliminary work indicates that the technique could have much value in the determination of traces of chlorinated insecticides in contaminated atmospheres and in samples as varied as wool, wood, hardboard and plastics.

We thank Mr. I. G. Blackwell for carrying out much of the experimental work outlined, Mr. A. Taylor, of the Infestation Control Laboratory, M.A.A.F., Tolworth, Surrey, for a gift of pure heptachlor epoxide (biological metabolite), Dr. R. A. E. Galley, Director of the Woodstock Agricultural Research Centre, for his interest and encouragement and the Directors of "Shell" Research Ltd. for their permission to publish this paper.

References

1. Needham, P. H., *Analyst*, 1960, **85**, 792.
2. Coulson, D. M., Cavanagh, L. A., and Stuart, J., *J. Agric. Food Chem.*, 1959, **7**, 250.
3. Zweig, G., and Archer, T. E., *Ibid.*, 1960, **8**, 190.
4. Wilkens Instrument and Research Inc., Walnut Creek, California, "Aerograph Research Notes," Winter Issue, 1959.
5. Coulson, D. M., Cavanagh, L. A., De Vries, J. E., and Walther, B., *J. Agric. Food Chem.*, 1960, **8**, 399.
6. Zweig, G., Archer, T. E., and Rubenstein, D., *Ibid.*, 1960, **8**, 403.
7. Lovelock, J. E., and Lipsky, S. R., *J. Amer. Chem. Soc.*, 1960, **82**, 431.
8. Lovelock, J. E., *Anal. Chem.*, 1961, **33**, 162.
9. Goodwin, E. S., Gouldon, R., Richardson, A., and Reynolds, J. G., *Chem. & Ind.*, 1960, 1220.
10. Harva, O., Kivalo, P., and Keltakallio, A., *Suomen Kemistilehti*, 1957, **32B**, 71; *Chem. Abstr.*, 1959, **53**, 21359.
11. Bohemen, J., Langer, S. H., Perrett, R. H., and Purnell, J. H., *J. Chem. Soc.*, 1960, 2444.
12. Chilwell, E. D., and Hartley, G. S., *Analyst*, 1961, **86**, 148.
13. Mills, P. A., *J. Ass. Off. Agric. Chem.*, 1959, **42**, 734.
14. Lovelock, J. E., *Nature*, 1961, **189**, 729.

Received *May 17th*, 1961