Estimation of DDT and Related Compounds Together with Polychlorobiphenyl Compounds, in Biological Media

1. INTRODUCTION

DDT, its various isomers and degradation products (including DDE) are detected and measured in biological media by multi-detection systems based on chromatography. The systems used also respond to polychlorobiphenyl (PCB) compounds (which represent a complication in DDT residue analysis): where these are encountered they usually comprise a complex mixture of many different PCB compounds of varying composition according to nature of the particular technical material (or materials) from which they derive and possibly in addition varying on account of subsequent weathering procedures which may have removed or degraded some individual constituents at differing rates. There is no single analytical procedure which is applicable to all substrates; or to a single substrate for all possible residue patterns (9.1).

By way of example, the pattern of PCB isomers from samples of wildlife as judged from their gas chromatograms is not identical to that observed in commercial PCB preparations, some of the isomers having apparently been wholly or partly metabolised. Consequently the amount of PCB in a wildlife sample cannot be estimated by direct comparison with a standard substance and some further form of approximation must be used. By injecting known amounts of PCB on to an Apiezon L column it can be shown that the ratio of the Total Peak Area to the Total Weight Injected for most of the higher chlorinated commercial preparations is roughly the same as that for pp′-DDE, and all calculations can be based on this compound as standard.

The procedure outlined below must be regarded only as illustrative of the approach to DDT and PCB residue analysis. Individual analytical procedures must be verified in the light of the considerations outlined above and with due regard to the general points set out in the introduction to this volume.

2. SCOPE

Provided due regard is had to the matters outlined in the introduction above, this method is applicable to biological tissues which can be sufficiently comminuted to allow quantitative extraction of the compounds analysed.

3. PRINCIPLE OF METHOD

The sample is comminuted with a solvent at room temperature in a glass filter column, from which the extract is obtained by pressure. It is then

subjected to clean-up and examined quantitatively by gas chromatographic analysis using an electron capture detector. Measurements are made relative to standard solutions analysed at the same time.

Some of the alternative clean-up methods may be adequate for certain samples, but the more drastic procedures may remove some of the less stable organochlorine compounds by decomposition.

4. REAGENTS

Acetone, column distilled; magnesium sulphate, washed with anhydrous ether; light petroleum boiling range 69°-71°C, treated with conc. sulphuric acid, washed with water, dried over magnesium sulfate and column distilled; ether; dichloromethane (column distilled); acetonitrile (column distilled), saturated with light petroleum; sodium sulphate, anhydrous; florisil, activated at 600°C, add 2% water w/w; silica gel, Kieselgel G (Stahl); sulfuric acid mixturė, 38ml fuming (20% SO_3), sulfuric acid 62ml conc. sulfuric acid; sodium chloride solution, 2% w/v; aldrin standard solution, 0.02ng/μl.

5. APPARATUS

Homogeniser, 20,000rpm; glass extraction column with G3 sinter glass filter and polytetrafluoroethylene stopcock, 150mm x 11mm; centrifuge; gas chromatograph, with electron capture detector. Column, all glass, 1.6m x 3mm OD; evaporation manifold; column packings (1) 5.1% QF + 1.4% SF – 96 on 100/120 mesh Gaschrom P silanized; (2) 4.0% SF – 96 on 100/120 mesh Gaschrom P silanized.

6. PROCEDURE

6.1 Estimations are usually made on a series of samples, one of which is a blank (usually a previously analysed sample shown to be satisfactory for this purpose) and one a standard. The standard is also prepared from a blank sample by adding a standard solution containing known amounts of the compounds to be analysed. At intervals in between the gas chromatographic analyses, standard injections of aldrin, or similar low retention compound, are made to check sensitivity.

6.2 *Cleaning of glassware*

Clean all smaller glassware items by heating in a special detergent solution. Rinse with water, then with distilled water and dry. Clean extraction

columns with the detergent, then heat to 70°C in a 4 + 1 mixture of conc. sulfuric and nitric acids, rinse with water, then with distilled water. After drying, rinse once with acetone and twice with light petroleum (boiling range 60°-70°C). From the final solvent rinse, remove a sample for examination by gas chromatography.

6.3 *Sample preparation*

Weigh between one and three grams of sample directly into the extraction column. Add 5ml acetone and comminute using the homogeniser. Rinse the homogeniser rotor in 5ml light petroleum in a centrifuge tube, and transfer the solvent to the column. Cover the top of the column with aluminium foil. Rinse the rotor once in water then in two tubes containing acetone and two tubes light petroleum before comminuting the next sample. Leave the columns to stand 2.5 hours or overnight. Place a 25ml measuring cylinder under the column and run the liquid into the flask, applying pressure to the column with a handpump until all the liquid has run out. Fill the measuring flask with sodium chloride solution until the level is about 2cm above the mark, invert carefully a few times. Remove the stopper and centrifuge slowly at first and finally at 2,000rpm. Transfer the upper layer, after freezing out if necessary, with a pipette to a weighed, graduated 10ml centrifuge tube. Add further small quantities of light petroleum to the flask and transfer to the tube. Place the centrifuge tube in a water bath at 45°C and reduce to 2ml (or some other convenient volume) with a stream of air or nitrogen.

6.4 *Clean-up*

A number of procedures, of which the following examples are typical, may be used according to the nature of the sample and the extract obtained. Special procedures may be necessary for samples with a high moisture content or for those high in sulfur (which gives a false positive response).

6.4.1 Column clean-up

Place 5.0g florisil in a chromatographic column, add a 2cm layer of oven-dried sodium sulphate. Rinse the column with about 40ml light petroleum before adding 1ml of solvent extract. When the meniscus has almost reached the column, add light petroleum + ethyl ether (4 + 1) initially only in small portions. Collect 50ml eluate in a weighed ground glass stoppered flask and evaporate to less than 5ml. Adjust the solution volume with light petroleum to 5ml by weighing. Remove an aliquot for analysis by gas chromatography.

6.4.2 Thin layer clean-up

Carefully apply a small amount, about 100µl, of solution containing not more than 20mg fat, spotwise to form a band on a small silica gel plate

(28 x 98mm) coated with 1.2g silica gel which has previously been rinsed with methanol and water and heated to 200°C before and after application to the plate. Develop with dichloremethane until the solvent reaches the top of the plate. Dry quickly and examine in UV light. Scrape the layer 5mm above the fat zone into a small packed column (internal diameter 7mm), and add light petroleum + ethyl ether (3 + 2) dropwise until 1.0ml eluate is corrected. Remove an aliquot for analysis by gas chromatography.

6.4.3 Acetonitrile partition clean-up

To 1-5ml of light petroleum extract in a glass stoppered tube add an equal volume of acetonitrile. Agitate, transfer the acetonitrile phase with a pipette to a separating funnel. Repeat three times. Dilute the combined extracts with the same volume of light petroleum and six times the volume of water. Shake carefully and discard the water phase. Wash the solvent phase with a few portions of sodium chloride solution, dry by pouring through a short column of sodium sulphate; wash the column with a few ml light petroleum. Concentrate by evaporation and adjust to 5.0ml. Remove an aliquot for analysis by gas chromatography.

6.4.4 Acid treatment

Transfer 1ml light petroleum solution to a 5ml stoppered tube, add 1ml sulfuric acid mixture and invert the tube carefully a few times with adjustment of the glass stopper. Centrifuge 10min, freeze out in solid carbon dioxide–acetone, decant the upper layer into a 3ml tube and stopper. Use an aliquot for gas chromatography.

6.4.5 Alkali treatment

Add 1.0-1.5ml potassium hydroxide solution in ethanol to 1ml solvent extract (or a cleaned up solution) in a centrifuge tube. Mix carefully, avoid wetting the stopper. Place the tube in a water bath at 55° adding additional light petroleum if necessary. Fill the tube with 2% sodium chloride solution (about 11ml) and invert several times. Centrifuge and place tube in a freezing mixture. Decant the upper layer into a 3ml tube, make up to 1.0ml with light petroleum; use an aliquot for gas chromatography.

6.5 *Gas chromatography*

Examine a suitable aliquot by at least two different gas chromatographic systems. Those indicated above are, typically, operated at 180°C with a gas flow-rate of about 25ml per min, giving a retention time for pp′-DDT of 20min.

CALCULATION OF RESULTS

7.1 The gas chromatograms obtained are evaluated by comparison with those of standard solutions of the pure compounds sought. If, as suggested above, the gas chromatographic conditions are adjusted to give standard retention times, peak heights are measured and used for the evaluation. Variations in sensitivity (more than 30% during a day) and a limited linear dose-response relationship of the electron-capture detector may be taken into account by correction factors calculated from frequent analysis of an external standard compound having a short retention time and by consideration only of peak heights which fall into the linear range of each compound. Whenever the chromatographic pattern allows the use of an internal standard this will simplify the calculations and usually improve somewhat the quality of the analysis.

7.2 Only an approximate result can be calculated since commercial PCB preparations differ and the composition of individual preparations "age" in different ways on exposure. When the pp′-DDE and PCB's have been separated from the other chlorinated residues by silica gel column, examine the concentrated extract by GLC, preferably on an Apiezon column. Insofar as their retention times are identical with those of a standard preparation, all the peaks following that of pp′-DDE are assumed to be PCB compounds. If pp′-DDE appears to be present and it is thought that there may be a peak due to a PCB compound at exactly the same retention time which will significantly affect the calculated amount of pp′-DDE, then oxidise the pp′-DDE to pp′-DCBP (9.2). On chromatography, the pp′-DDBP will give an earlier retention time peak and leave the PCB's unaffected. Calculate the amount of PCB's in the sample injection in the following manner:

(a) Determine the retention time (Rt) and peak heights (Ht) of all the compounds which appear to be PCB compounds.

(b) Multiply the individual retention times (Rt) by the peak heights (Ht) and sum all the products so obtained:

$Rt_n \cdot Ht_n = Rt_1 \cdot Ht_1 + Rt_2 \cdot Ht_2 + Rt_3 \cdot Ht_3 + \ldots$ etc.

(c) Divide this sum by the product of the peak height x retention time for 1ng pp′-DDE. This will give an estimate of the total amount of PCB (in ng) in the sample injection.

If a silicone column has to be used for the estimation, divide the sum of the Rt's x Ht's by the product of Rt x Ht for 1ng dieldrin. Because it is usual for the chromatographic patterns of the PCB compounds eluted from samples to be different from those of the manufacturers' preparations, it is impossible to use individual peaks in the standard material and determine the amount of PCB by simple proportion. A determination of the area per nanogram for the whole range of PCB preparations gives a figure which is very close to that determined for 1 nanogram of pp′-DDE on an Apiezon column, i.e. the *mean* electron-capture response for PCB's is very similar to that for pp′-DDE. If only single PCB compounds are being determined, there will in some cases be a large error. On silicone columns, the mean electron-capture response is nearer to that of dieldrin. This is because the

PCB compounds produce different peak patterns on this liquid phase. The view that the mean electron-capturing power of PCB's is similar to that of pp′-DDE is confirmed by Zitko et al. (9.3).

8. SPECIAL CASE

8.1 *Separation of PCB's from DDT and other pesticides*

Prepare a column with silica gel such as Hopkin & William, MFC which has been heated to 110°C for at least 2hr and to which when cool has been added 2.5% v/w of distilled water. Weigh out 5.0g of the prepared gel and cover it immediately with hexane. Shake the mixture to release any air bubbles and wash the preparation into a narrow glass chromatographic column (7mm I.D.), using hexane. Run the surplus hexane through the column until its meniscus is just touching the surface of the silica gel.

Add the sample extract to the column in about 2.0ml of solvent. Allow the solution to run into the column until the meniscus is just touching the top of the gel. Wash the sample container with 1.0ml of hexane and transfer this washing to the column. Elute the column with 45ml of hexane at the rate of 1 drop per second, stopping the elution when the meniscus just reaches the gel. Concentrate the eluate and examine it by GLC. It should contain all PCB-type compounds, pp′-DDE and HCB. After changing the receiver under the gel column, further elute the silica gel with 50ml of hexane containing 10% v/w of diethyl ether. Concentrate this second eluate and examine it by GLC. It should contain pp′-DDT and all the more polar residues as far as dieldrin.

9. REFERENCES

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