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RAPID DETERMINATION OF DDT AND RELATED COMPOUNDS IN SOILS VIA CARBON SKELETON GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

The levels of DDT and related compounds in soil samples from an apple orchard have been determined. Extraction of residues was achieved by cyclic steam extraction and by conventional solvent extraction methods allowing comparison of the two methods. Determination of the organochlorine residues present in the extracts was performed by gas–liquid chromatography–electron-capture detection and by carbon skeleton gas chromatography–mass spectrometry. Excellent agreement between the two determination techniques was achieved.

Prior to application of *p,p'*-DDT, a value of approximately 0.4 ppm was obtained for ΣDDT in the soil. The major component of this total was DDE. After spraying the apple trees with technical grade *p,p'*-DDT, ΣDDT for the soil rose to 0.6 ppm. This increase was due to translocation of *p,p'*-DDT from the trees,

INTRODUCTION

Although the use of such well known organochlorine species as DDT* and polychlorinated biphenyls (PCBs) has been discontinued, or much reduced, in countries such as the U.S.A. and Great Britain such compounds are of continuing significance to the analytical chemist. The industrial usefulness of PCBs depends on their thermal stability and chemical inertness and hence it is likely that a considerable quantity of these compounds remain in circulation. DDT, a cheap effective pesticide, receives wide agricultural use, particularly in tropical and semi-tropical countries where ambient conditions assist dispersal. It is of considerable importance also in the control of the malaria-carrying mosquito.

The decline in use of DDT and PCBs has promoted the use of other organochlorine species. Thus aldrin, dieldrin, mirex and toxaphene are current alternatives

* DDT = 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDD = 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; DDE = 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene.

to DDT. Likewise, PCBs have been superseded by polychlorinated naphthalenes (PCNs) and polychlorinated alkanes (PCAs). Since these latter species possess similar properties, and act in a similar manner, to their respective forerunners their presence in the environment is deserving of equal attention[1].

Even in areas where the use of compounds such as DDT and PCBs have been largely discontinued the persistence of these chemicals in sediments and soils provides a reservoir from which translocation along food chains may be achieved. Thus beef cattle raised on feed-lots which have not received DDT or related compounds for eight years still contain considerable levels of DDE in body-fat[2]. It is relevant to note that after eight years of a DDT moratorium the residue consisted almost exclusively of DDE. As DDT and its major metabolite DDE together with DDD are usually found together it is conventional to quote ΣDDT values which are the sums of the concentrations (including isomers) of DDT, DDE and DDD in cases where more than one compound is present[3].

The current recommended procedure for the determination of organochlorine residues in environmental samples consists of four distinct steps: the extraction of the residue from the matrix, the simplification or "cleaning-up" of this extract, the estimation of the residue of interest and finally the confirmation of the identity of the compound(s) under study.

The removal of the residue from the matrix is normally achieved by hexane extraction after the sample has been dried or blended with sodium sulphate. The primary cleanup is accomplished by liquid–liquid partitioning into acetonitrile or acetone followed by back extraction into hexane. This final hexane solution should contain only organochlorine species. It is then fractionated on a Florisil column to obtain the extract of interest[4]. Determination is normally performed on a gas chromatograph fitted with an electron-capture detector (ECD)[4]. Confirmation of the identity of the various species present in the fraction may be achieved by thin-layer chromatography, infrared spectroscopy, chemical dehydrochlorination using strong alkali, polarography or mass spectroscopy[4].

However, the volatility and hydrophobicity of organochlorine compounds in general and of DDT and related compounds in particular renders them ideally suited to steam extraction with concomitant partition into hexane. The co-distillation of DDT with water has long been known[5] and the feasibility of extracting organochlorine compounds by exhaustive steam extraction has recently been reported[6], and applied[7].

In this paper we report our studies of the levels of DDT and congeners in agricultural soil. Samples were taken from an orchard plot which has received several DDT applications over recent years. Due to infestation with Codling Moth, *Cyida pomonella*, the apple trees were treated with technical grade *p,p'*-DDT (active ingredient 1 lb/acreon April 6, 1978). Hence pre-application and post-application soil samples were available for study. Soil samples were extracted by the solvent method (hexane → acetonitrile → hexane) and by exhaustive steam distillation. The extracts were determined by gas–liquid chromatography (GLC)–ECD and by carbon skeleton gas chromatography–mass spectrometry (CS–GC–MS)[8] allowing comparison of both the extraction and the determination procedures.

EXPERIMENTAL

Samples and sample preparation

Soil cores (4 cm^2 × 6 cm) were collected by hand-operated auger before and after application of DDT and stored in plastic containers at 0° until analysed.

Solvent extraction

Soil (10 g, undried) was extracted with hexane–acetone (3:1, v/v, 20 ml) by shaking for 4 h. A known volume of solvent was removed and washed with doubly distilled water to remove acetone. The hexane layer was dried over anhydrous sodium sulphate and removed. The sodium sulphate was then extracted with 2 × 30 ml of hexane and the combined hexane extracts reduced to 25 ml under nitrogen.

The extract (25 ml) and acetonitrile (50 ml) were shaken together for 1 min in a separating funnel and the acetonitrile (lower) layer retained to be further extracted by 3 × 20 ml of acetonitrile-saturated hexane. Subsequently the acetonitrile layer was reduced to 10 ml and 50 ml hexane added. The resultant volume was reduced to 10 ml, this process being repeated twice more to remove all acetonitrile from the final extract prior to fractionation on Florisil.

Florisil was heated (650°, 2 h) before use. Activation (130°, 5 h), de-activation (5%, w/w, water) and equilibration (shaking, 3 h) preceded column packing. Florisil (10 g) was placed in a column (20 cm × 10 mm) and topped with 10 mm anhydrous sodium sulphate. Elution with hexane (100 ml) preceded extract application. Elution with diethyl ether–hexane (6:94, v/v) recovered DDT and related compounds. The eluate was reduced to 1 ml (to remove ether) and made up to 10 ml with hexane to give a suitable sample for analysis.

Steam extraction

Using apparatus built to $\frac{1}{3}$ scale of that previously described[6,7], soil (10 g) was steam extracted for 2 h into hexane (10 ml). A water condenser was fitted to the top of the apparatus to prevent loss. The hexane extract was clear, odourless (apart from hexane) and suitable for immediate analysis by either GLC–ECD or CS–GC–MS techniques.

Chromatography

GLC–ECD determinations were carried out on a Pye Series 104 gas chromatograph equipped with a nickel-63 ECD. A 2 m × 4 mm I.D. glass column packed with the mixed liquid phase OV-17 (1.5%)–QF-1 (1.95%) on Chromosorb W AW DCMS (85–100 mesh) was used with the following parameters: oven temperature, 195°; carrier gas, nitrogens 40 ml min^{-1}; detector oven temperature, 300°; injection port temperature, 220°. Septum bleed was counteracted by washing with acetone, conditioning (250°, 12 h) and storing septa in aluminium foil until required.

CS–GC–MS studies were carried out on a V. G. Micromass 16B2 mass spectrometer interfaced to a Pye Series 104 gas chromatograph via a single-stage jet separator. Heterogeneous catalytic hydrodechlorination of DDT and congeners was carried out on a 3% palladium catalyst as previously described[8].

Quantitation for GLC–ECD was achieved from calibration curves (peak height *vs.* sample weight) for the respective compounds. Calibration curves were constructed

prior to each batch of analyses. Every fourth injection was of a standard from the mean of the calibration range. Typical within-batch variation was of the order of 5%—response increasing with repeated injections. Identification of components was by co-injection of standards.

CS–GC–MS quantitation was by comparison of peak heights for samples and standards. The validity of this procedure is discussed below. Identification of column eluates was by mass spectroscopy.

RESULTS AND DISCUSSION

The concentrations of DDT, DDD and DDE for pre-application and post-application soils extracted by steam (Tables I and II), and by solvents (Tables III and IV); and determined by GLC–ECD and CS–GC–MS (Table V), are tabulated below. All values are quoted in ppm dry weight. Recoveries for the various compounds through the two extraction procedures are given in Table VI. All samples were dried *after* extraction. While this presents no difficulty for steam-extracted samples the use of very wet or fatty samples complicates the solvent extraction procedure.

TABLE I

DDT AND RELATED COMPOUNDS IN PRE-APPLICATION SOIL EXTRACTED BY STEAM AND DETERMINED BY GLC–ECD

Compound	*Concentration ($\mu g\ g^{-1}$, dry weight)* *Site No.*				
	1	*2*	*3*	*4*	*5*
DDE	0.33	0.21	0.19	0.26	0.22
DDD	0.01	0.01	0.01	0.01	0.01
o,p'-DDT	0.04	0.04	0.05	0.05	0.04
p,p'-DDT	0.11	0.11	0.12	0.13	0.13
ΣDDT*		0.44			

* ΣDDT = [DDT] + [DDD] + [DDE].

TABLE II

DDT AND RELATED COMPOUNDS IN POST-APPLICATION SOIL EXTRACTED BY STEAM AND DETERMINED BY GLC–ECD

Compound	*Concentration ($\mu g\ g^{-1}$, dry weight)* *Site No.*				
	1	*2*	*3*	*4*	*5*
DDE	0.44	0.30	0.26	0.25	0.29
DDD	0.02	0.02	0.02	0.01	0.02
o,p'-DDT	0.08	0.08	0.09	0.08	0.11
p,p'-DDT	0.20	0.25	0.29	0.18	0.26
ΣDDT		0.68			

TABLE III

DDT AND RELATED COMPOUNDS IN PRE-APPLICATION SOIL EXTRACTED BY SOLVENT AND DETERMINED BY GLC–ECD

Compound	*Concentration ($\mu g\ g^{-1}$, dry weight) Site No.*				
	1	*2*	*3*	*4*	*5*
DDE	0.21	0.22	0.14	0.18	0.15
DDD	0.00	0.01	0.00	0.00	0.00
o,p'-DDT	0.05	0.06	0.07	0.06	0.06
p,p'-DDT	0.06	0.10	0.09	0.08	0.07
ΣDDT		0.36			

TABLE IV

DDT AND RELATED COMPOUNDS IN POST-APPLICATION SOIL EXTRACTED BY SOLVENT AND DETERMINED BY GLC–ECD

Compound	*Concentration ($\mu g\ g^{-1}$, dry weight) Site No.*				
	1	*2*	*3*	*4*	*5*
DDE	0.27	0.17	0.19	0.22	0.21
DDD	0.02	0.02	0.03	0.01	0.01
o,p'-DDT	0.09	0.08	0.13	0.07	0.09
p,p'-DDT	0.18	0.15	0.26	0.16	0.20
ΣDDT		0.50			

TABLE V

MEAN ΣDDT VALUES DETERMINED BY GLC–ECD AND CS–GC–MS

	ΣDDT values ($\mu g\ g^{-1}$, dry weight)		
	GLC–ECD	*CS–GC–MS*	
Pre-application soil	0.36	0.29	Solvent extraction
Post-application soil	0.50	0.42	Solvent extraction
Pre-application soil	0.44	0.42	Steam extraction
Post-application soil	0.68	0.63	Steam extraction

TABLE VI

COMPARISON OF RECOVERY EFFICIENCY FOR STEAM EXTRACTION AND SOLVENT EXTRACTION PROCEDURES

Compound	*Recovery (%)*	
	*Steam extraction ± S.D.**	*Solvent extraction ± S.D.**
DDE	89 ± 4.9	56 ± 4.8
DDD	88 ± 3.9	80 ± 4.2
o,p'-DDT	88 ± 1.8	67 ± 7.0
p,p'-DDT	89 ± 5.0	89 ± 6.6

* $n = 10$.

Recovery of compounds

The recoveries of the various compounds through the two extraction procedures used are given in Table VI. Although comparable recoveries were found for *p,p'*-DDT and DDD use of the solvent extraction procedure results in some loss of *o,p'*-DDT and considerable loss of DDE. The chromatogram displayed in Fig. 1 was obtained from the discarded hexane solvent layer in the solvent extraction procedure and confirms a significant quantity of DDE is discarded rather than carried through.

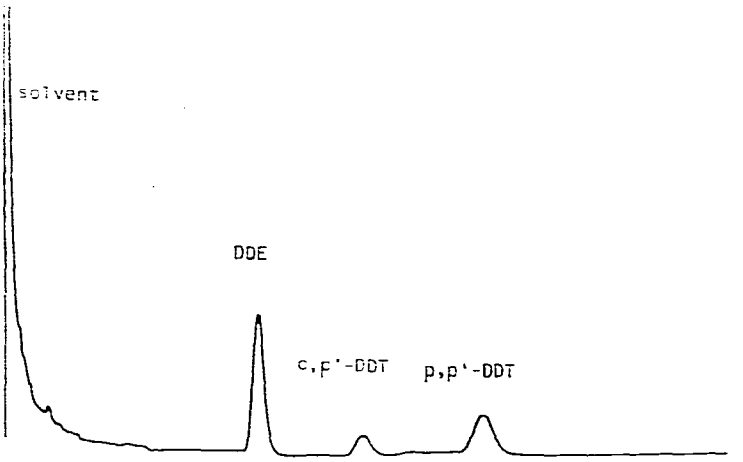

Fig. 1. GLC–ECD chromatogram of hexane solvent discarded from solvent extraction procedure illustrating the loss of DDE, *o,p'*-DDT and some *p,p'*-DDT at this stage.

The steam extraction of samples has considerable advantages over the more conventional solvent extraction procedure quite apart from the improved recoveries. The latter process requires considerable volumes of two solvents each of which must be sufficiently free of contaminants to permit a large reduction of volume, *i.e.* a concentration step, without giving rise to contaminants on the chromatogram. By comparison the steam-extraction procedure requires approximately 10–15 ml of singly distilled hexane and no volume reduction is necessarily involved.

Duration of analysis

The approximate time taken to extract the organochlorine species from the soil by steam extraction and to prepare the sample for injection onto the gas chromatograph was 2.5 h. In contrast, sample preparation to the same point by the solvent extraction procedure took two days. This included fractionation of the extract by liquid chromatography to remove potential contaminants. Such a cleanup procedure was not necessary for the steam extracts which were suitable, (after drying over anhydrous sodium sulphate for 20 min), for injection into the gas chromatograph.

Clearly, steam extraction of organochlorine residues from environmental samples offers the triple advantages of speed, simplicity and efficient recovery.

Quantitation

Quantitation was by peak height correlation with a calibration curve. For GLC–ECD, calibrations were made for the four constituent components of ΣDDT. These were *p,p'*-DDT, *o,p*-DDT, *p,p'*-DDD and *p,p'*-DDE. For CS–GC–MS, the calibration curve was obtained by plotting peak height (mean of three injections, 4-μl volumes) against the weight of diphenylethane generated by injection of DDE. It should be noted however, that the species monitored was the m^+/e 167 ion of diphenylethane corresponding to $M^+ - CH_3$. The use of this ion is recommended because it is of greater intensity than the parent ion, m^+/e 182, thus giving a much increased sensitivity. Conversion of DDE to diphenylethane was quantitative under the conditions used (3% Pd, 300°). The regression line for this curve was $y = 8.186\,x + 0.957$ with a correlation coefficient of 0.9961.

Quantitation of an unknown sample is as follows. The peak height corresponding to the concentration of the m^+/e 167 ion in the sample is converted to a concentration of diphenylethane from the calibration graph. Conversion to the equivalent amount of DDT is achieved by multiplying by 1.94, this being the ratios of the molecular weights of DDT and diphenylethane. The procedure is summarized in eqn. 1.

$$\left.\begin{matrix} p,p'\text{-DDT} \\ o,p'\text{-DDT} \\ \text{DDD} \\ \text{DDE} \end{matrix}\right\} \xrightarrow{\text{catalysis}} m^+/e\ 167 \xrightarrow[\text{curve}]{\text{calibration}} \text{concn. diphenylethane} \xrightarrow{\times 1.94} \Sigma\text{DDT} \qquad (1)$$

The precision of quantitation by MS was evaluated. A 1 ng μl^{-1} solution of DDE in hexane was used together with a 3% palladium catalyst and 2% RbCl column. Monitoring was at m^+/e 167. At least ten injections of 5 μl, 4 μl, 3 μl, 2 μl and 1 μl of this solution were made. Amplification was adjusted so that output was approximately 70% f.s.d. for all injections, in an attempt to minimise the error inherent in measurement of peak height. The injection technique used was as follows: Using a 10-μl syringe, the barrel was filled with more than the required volume. Adjustment to the required volume was followed by withdrawal of that portion of the sample contained in the needle into the syringe barrel. Injection then followed normal practice. This procedure was designed to reduce the evaporation at the high injection temperature used (300°) of that portion of the sample which normally remains unmeasured in the needle of the syringe. The precision of quantitation by GC–MS in this manner is reflected in the standard deviations obtained for the various volumes (Table VII). Somewhat surprisingly, there is little evidence of increasing error ac-

TABLE VII

VARIATION OF PRECISION WITH VOLUME OF SAMPLE INJECTED FOR CS-GC-MS DETERMINATION OF ΣDDT

Volume (μl)	5	4	3	2	1
No. of injections	10	11	11	10	11
Mean peak height (mm)	84.8	74.8	73.2	64.0	60.9
Standard Deviation	3.88	6.22	4.94	3.23	5.34
Relative Standard Deviation	4.58	8.31	6.75	5.01	8.76
Variance	15.1	38.8	24.4	10.4	28.6

companying the measurement of smaller volumes. Use of large (*ca.* 5 μl) sample volumes to improve precision as recently recommended by the Environmental Protection Agency does not, therefore, appear necessary. Thus, duplicate or triplicate injections for each sample should be sufficient to illustrate any spurious response.

Interferences

No interference was encountered either with GLC–ECD or CS–GC–MS determination of the ΣDDT content of these samples. Thus, other organochlorine compounds such as PCBs, PCNs, and other steam extractable species such as polycyclic aromatic hydrocarbons and alkanes were not present[7]. It should be noted, however, that had a PCB residue been present, then this would have seriously interfered with determination by GLC–ECD and would have necessitated a prior cleanup of the extract. No such interference problem arises via CS–GC–MS determination because of the selectivity of the mass spectrometer as a detector. Under CS–GC–MS conditions a PCB emerges from the gas chromatograph as biphenyl (m^+/e 154)[8].

The values of ΣDDT

The pre-application soils (Tables I and III) display significant residual levels of DDE and *p,p'*-DDT. The relative ratio of DDE to *p,p'*-DDT was approximately 3:1. The recovery of DDT and related compounds by steam extraction is more efficient than by solvent extraction largely due to losses of DDE by the latter procedure (Table VI; Fig. 1). Thus, the values in Table III obtained by solvent extraction of pre-application soil are consistently lower than those of Table I.

For the post-application soils several points should be noted. Values obtained by steam extraction are higher than those obtianed by the solvent method. The ratio of DDE to *p,p'*-DDT changed to approximately 1:1 indicating that some of the *p,p'*-DDT applied to the trees had translocated to the soil as the absolute concentration of DDE remained approximately the same. Likewise, the levels of DDD and *o,p'*-DDT have increased slightly. (Commercial grade DDT contains small amounts of these compounds).

The values of ΣDDT were obtained by addition of the concentration of the various components after adjustment to the equivalent weight of DDT according to eqn. 2.

$$\Sigma\text{DDT} = \text{concn. } p,p'\text{-DDT} + \text{concn. } o.p'\text{-DDT} + \frac{354}{320} \times \text{concn. DDD} + \frac{354}{318}\text{concn. DDE} \quad (2)$$

This summation is carried out on the column by CS–GC–MS as all components are converted to diphenylethane. Hence for CS–GC–MS eqn. 3 applies

$$\Sigma\text{DDT} = 1.94 \times \text{concn. diphenylethane (DPE)} \quad (3)$$

where 1.94 = 354/182 = mol.wt. DDT/mol.wt. DPE.

Clearly, the values obtained by steam extraction followed by CS–GC–MS are comparable with those obtained by the more conventional but lengthy, expensive and

inefficient solvent extraction–GLC–ECD procedure. The two quantitative procedures of CS–GC–MS and GLC–ECD subsequent to steam extraction gave results in excellent agreement. A combination of steam extraction and GLC–ECD determination may thus be used for samples where no contaminants are co-extracted.

CONCLUSIONS

Steam extraction of the residues of DDT from agricultural soils was more efficient, more rapid and less costly than the conventional solvent extraction–partition procedure. Quantitation of the extract by GLC–ECD and CS–GC–MS gave results in excellent agreement. However, the selectivity of the detector involved in the CS–GC–MS technique renders it superior when contaminants such as PCBs, PNAs or hydrocarbons are present[7]. Consideration should therefore be given to the use of steam extraction rather than solvent extraction in laboratories where speed and cost are important criteria. Also, CS–GC–MS is preferable to GLC–ECD for determinations where multi-component extracts containing such species as PCBs, PCNs, polychlorinated terphenyls and PCAs are under investigation. Although the concentrations of organochlorine species present in the soils studied necessitated the use of a mass spectrometer as a detector, it is suggested that where higher levels of these species are encountered (for example in liver extracts of various birds of prey) the carbon skeleton technique allied to a flame ionisation detector may well prove capable of detection and quantitation.

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