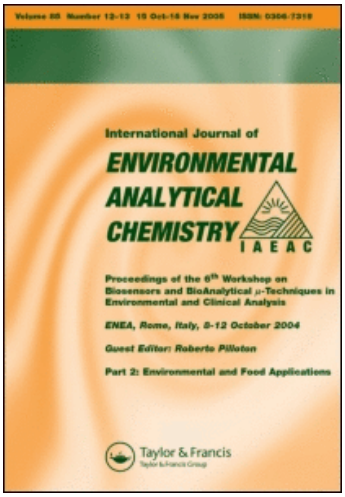

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Development of a Method for the Analysis of Chlorophenoxy Herbicides in Waste Waters and Waste Water Sludges

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A multi-residue method has been developed for the analysis of chlorophenoxy acids in sewage and primary sewage sludge. Several different derivatisation techniques were evaluated using standard herbicide compounds. Replicate blank and fortified samples were extracted, derivatised, cleaned up and analysed for herbicide residues. Reproducilibity and the degree of recovery for the entire method were determined. Both packed and capillary columns were investigated for gas chromatographic analysis with detection by electron capture and mass spectrometry. It is recommended that 2-chloroethylation derivatisation in conjunction with capillary column gas chromatography–mass spectrometry detection and quantitation is employed for the analysis of these materials.

The analytical scheme developed was found to be applicable for six of the chlorophenoxy herbicides (CPH) under investigation. The degree and reproducibility of CPH recovery are acceptable at a concentration of $2.5\,\mu g\,g^{-1}$ (with respect to dry solids) in a 50 ml sewage sludge sample and $5.0\,\mu g\,l^{-1}$ in a 1 l sewage sample, for all of the CPH except 2,4-DB (2,4-dichlorophenoxybutyric acid).

The method has been applied to a large sample of primary sewage sludge, and 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-TP (2,4,5-trichlorophenoxyproprionic acid) have been positively identified and determined at 1.20 and $0.27\,\mu g\,g^{-1}$, respectively.

KEY WORDS: Chemical derivatisation, chlorophenoxy herbicides, gas chromatography, mass spectrometry, sewage.

INTRODUCTION

Esters or amine salts of chlorophenoxy acids have been used for almost 40 years as herbicides in agriculture, forestry and in urban areas. In addition, some chlorophenoxy herbicides (CPH) are recommended for aquatic weed control.[1] The most controversial CPH is 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) which has been used as a defoliant.[2] 2,4,5-T is highly toxic to mammals and aquatic life and is the most persistent of the CPH, though its persistence is less than that of organochlorine insecticides such as aldrin and dieldrin.[3,4]

The toxicity of CPH is complicated by trace quantities of polychlorodibenzo-*p*-dioxin (PCDD) contaminants. For example, 2,4,5-T formulations contain TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). PCDD exhibit greater toxicity than the CPH acids of the formulations in which they occur and therefore the results of toxicology studies on CPH are liable to misinterpretation.[5] It has been suggested that CPH are both teratogenic[6] and carcinogenic.[7] There are indications that relatively pure 2,4,5-T (free of TCDD) can cause embryotoxic effects in laboratory mice.[6] However other workers maintain that 2,4,5-T is teratogenic and fetotoxic principally as a result of TCDD impurities.[8] It has also been suggested that when all the evidence from human exposures to 2,4,5-T is scientifically examined there is no basis for 2,4,5-T teratogenicity in man.[9]

At present, 2,4,5-T use is proscribed in Italy, the Netherlands and West Germany. There have been renewed calls for a ban on 2,4,5-T in the U.K.,[10] following a recent appraisal of its potential for causing soft tissue sarcomas.[7] The EEC has recommended that 2,4,5-T should not be allowed to contaminate food, should be substituted by alternative herbicides wherever possible and should not be used in domestic gardens.[11] Even if CPH formulations were completely free of PCDD it would be necessary to regulate their use since there is evidence that PCDD may be produced from CPH in the environment,[12-15] though this suggestion has been challenged.[16-18]

During the period 1976–1980 approximately 12 tonnes of 2,4,5-T were consumed *per annum* in the U.K. for local authority, industrial and garden use.[19] Herbicides applied in urban areas may enter combined sewerage systems or surface water *via* runoff; indeed, CPH

have been identified in raw and potable water,[20] river water[21] and domestic sewage (where their concentrations may be 1000 times greater than those of organochlorine insecticides).[22] Depending upon their behaviour and degradative properties, CPH may be removed by sewage treatment processes or may enter surface waters in the final effluents. If persistent herbicides associate with sewage sludge and the sludge is used agriculturally, there is a risk of damage to sensitive crop plants.

It is considered that some CPH, such as 2,4-D (2,4-dichlorophenoxyacetic acid); 2,4-DB (2,4-dichlorophenoxybutyric acid); and 2,4,5-TP (2,4,5-trichlorophenoxyproprionic acid) should be degradable by biological sewage treatment provided suitable acclimatisation can be achieved, whilst 2,4,5-T is unlikely to be removed, even after prolonged contact with the biological system.[23] There is some evidence that biological treatment both degrades 2,4-D and converts its acid form into short chain ester forms.[22]

Since indirect re-use of sewage effluent in surface waters accounts for approx. 30% of U.K. water resources, there is concern that organic residues might contaminate potable water supplies, thereby presenting hazards to public health.[24] This may be particularly important in the case of CPH since 2,4-D and its metabolite 2.4-dichlorophenol are inefficiently removed by coagulation during potable water treatment.[25]

Maximum contaminant levels for chlorophenoxy compounds in potable water have been established by the United States Environmental Protection Agency (USEPA),[26,27] World Health Organisation (WHO)[28] and European Economic Community (EEC).[29]

Several analytical methods for CPH in environmental samples have been reported, some specific for natural waters[30,31] and sewage.[22,32] An important factor in these analysis is the production of CPH derivatives which are amenable to gas chromatography (GC). Previous studies have involved esterification by alkylation,[30,32] 2-chloroethylation (2-CE),[31,33] petafluorobenzylation (PFB)[31,34] and 2,2,2-trichloroethylation (TCE).[35] The production of alkyl esters has two main disadvantages in that the use of diazomethane or diazoethane entails the risk of explosions, whilst the use of boron trifluoride/methanol does not result in quantitative alkylation of all CPH acids. In addition, the retention times of alkyl

esters on packed GC columns tend to be relatively short and therefore the determination of these derivatives is subject to interference by extraneous sample contaminants. The relative merits of 2-chloroethylation and pentafluorobenzylation have been evaluated in a multi-residue analytical method for CPH, the conclusion of which was that the former technique was superior in terms of producing acceptable reagent blank values, though the PFB esters exhibited greater sensitivity during electron-capture (EC) GC analysis.[31] Alkyl esterification and TCE derivatisation have been verified for only a small number of CPH.[30, 32, 35]

This study was undertaken in order to develop analytical methodology for the determination of a range of CPH in sewage and sewage sludges. The evaluation of various chemical derivatisation procedures was deemed necessary in order to produce a multi-residue analytical method which could be optimised for a range of CPH compounds. Similarly, the evaluation of both packed and capillary column electron capture-gas chromatography, and capillary column gas chromatography–mass spectrometry was considered to be important for the same reasons.

EXPERIMENTAL

Reagents and standards

All solvents used were of pesticide residue quality, either 'Nanograde' (Mallinckrodt, U.S.A.) or 'Distol' (Fisons, U.K.).

CPH acid standard compounds were obtained from the National Physical Laboratory (Teddington, U.K.). Stock and working solutions of standards were prepared in benzene.

Two 10% w/v boron trichloride/2-chloroethanol reagents were obtained (Alltech Associates, Carnforth, U.K., and Atlas-Bioscan, Canvey Island, U.K.). A third reagent (10% w/v) was prepared by passing BCl_3 gas through 2-chloroethanol (which had previously been purified by extraction ten times with hexane).

A trichloroethylation reagent was prepared immediately prior to use, consisting of a 20% w/v solution of 2,2,2-trichloroethanol in trifluoroacetic anhydride (BDH, Chadwell Heath, U.K.).

Pentafluorobenzyl bromide (Pierce and Warriner, Chester, U.K.) was diluted to 1% solution in acetone immediately before use.

Silica gel, 70–230 mesh ASTM (BDH) was activated at 500°C overnight and stored at 105°C prior to use.

Extraction procedures

Sewage sludges. Initial studies on the extraction and analysis of CPH in sewage sludge were confined to the evaluation of two compounds 2,4-D and 2,4,5-T, using a modification of an extraction procedure originally described by McIntyre *et al.*[36] Four replicates of 200 mg sludge solids were acidified to pH 2 using 25% v/v H_2SO_4 and extracted with 30 ml ethyl acetate by 5 min agitation using a laboratory disperser (Scientific Instrument, London, U.K.). After centrifugation at 2000 rev min^{-1} for 30 min the organic phase was transferred to a 500 ml separating funnel and extracted once with 10 ml 2% $KHCO_3$ solution (shaking for 2 min), then twice with 5 ml aliquots of $KHCO_3$ solution for 1 min each. The $KHCO_3$ fractions were combined and acidified to pH 2. After CO_2 evolution was complete, this aqueous fraction was extracted with 3×10 ml dichloromethane, shaking for 1 min each. The dichloromethane phases were transferred to a clean 100 ml separating funnel and washed with 10 ml water (distilled, de-ionised and extracted with benzene). The dichloromethane layer was removed and evaporated to dryness, initially using a rotary evaporator at 28°C under reduced pressure and subsequently a steam bath. The residue was transferred to a 10 ml stoppered test tube using 5 ml of acetone in several aliquots.

Further sludge replicates were fortified with CPH standard solutions, shaken and allowed to stand for four hours prior to extraction. The extraction efficiency of the method was then determined.

A second extraction procedure for sludge samples was developed, using 4×50 ml sludge replicate samples in 250 ml PTFE centrifuge bottles. The extraction procedure was similar to that described above, although the volumes of extractants were as follows: 150 ml ethyl acetate, 20 ml 2% $KHCO_3$ then 2×10 ml $KHCO_3$, 3×20 ml dichloromethane, 20 ml water.

This sludge extraction method was further modified by extracting the sludge a second time with 100 ml ethyl acetate, then using 1×50 ml 2% $KHCO_3$ and 2×25 ml $KHCO_3$, 3×30 ml

dichloromethane, 20 ml water and back-extracting the water layer with 25 ml dichloromethane. Using this type of procedure eight replicate 50 ml sludge samples (from Hogsmill Sewage Treatment Works, London, U.K.) were extracted with ethyl acetate and extracts combined for the purpose of identification and quantification of CPH in unspiked sludge. This composite extract was then taken through the other extraction steps described in the previous paragraph.

Sewage samples. The extraction procedure for sewage was similar to that for sludges. Four 1 l replicates of sewage were acidified to pH 2 and extracted with 150 ml ethyl acetate. The aqueous layer was transferred to a second separating funnel and re-extracted with 100 ml ethyl acetate. The combined organic extract was centrifuged and extracted with 1×50 ml 2% $KHCO_3$ solution, then 2×25 ml $KHCO_3$. The aqueous fraction was extracted with 3×30 ml dichloromethane. The dichloromethane layers were transferred to a clean separating funnel and washed with 20 ml water. The dichloromethane layer was removed, the aqueous layer back-extracted with 25 ml dichloromethane and the organic layers combined.

Extraction efficiencies for sewage and sewage sludges were determined by fortification of the samples with CPH standards and subsequent analysis, using a series of calibration solutions produced by derivatisation and clean up of CPH standards.

Derivatisation and cleanup procedures

The derivatisation and cleanup procedures are based on the work of Agieman *et al.*,[31] Chau *et al.*[33] (2-chloroethylation); Mierzwa *et al.*[35] (2,2,2-trichloroethylation); and Agieman *et al.*[31,34] (pentafluorobenzylation).

2-Chloroethylation. The final acetone extracts of sludge were evaporated to dryness under a stream of nitrogen. 0.5 ml of derivatising reagent was added and the tubes stoppered, shaken and the contents allowed to react overnight at 60°C in a heating block. The derivatisation mixtures were allowed to cool and 5.0 ml benzene were added to each tube, followed by 5 ml 2% $KHCO_3$ solution. The

tubes were stoppered and shaken for 1 min. The aqueous layer was discarded. The benzene layer was washed with water for 1 min and then dried by addition of anhydrous Na_2SO_4. An accurately measured volume (2–5 ml) was transferred to a clean 10 ml tube and 2 ml 2,2,4-trimethylpentane (TMP) added. The volume of the extract was reduced to 1 ml under a stream of nitrogen. A further 2 ml TMP were added and the volume reduction to 1 ml repeated. The TMP extract with hexane rinsings was then applied to a 5 cm × 0.6 cm i.d. column of 1 g deactivated silica gel (5% w/w H_2O) which had previously been washed with 5 ml hexane. Excess contaminants were eluted with 6 ml benzene/hexane (1:3) and then the CPH derivatives were eluted with 6 ml benzene. This eluate was evaporated to dryness under nitrogen and 1.0 ml TMP added to redissolve the residue prior to GC analysis. (In subsequent analyses the cleanup of 2-chloroethyl esters was undertaken on a similar type of column, except that 2 g of silica gel were used and elution volumes were 10 ml hexane, 10 ml benzene/hexane (1:3) and 10 ml benzene. Furthermore, in later analyses the extracts were prepared for GC by re-dissolving in 100 μl hexane which contained d_{10}-anthracene as an internal standard).

2,2,2-Trichloroethylation. A solution of CPH standards in a 10 ml test tube was evaporated to dryness under a stream of nitrogen, then 10 ml of TCE reagent and 5 μl concentrated H_2SO_4 were added. The test tube was stoppered, shaken and the contents allowed to react overnight at room temperature. The volume was reduced to approx. 0.1 ml and 2 ml TMP added. The tube was shaken and 6 ml 0.5 M NaOH added. The test tube was stoppered and shaken for 1 min. The organic layer was cleaned up on a silica gel column identical to that used for 2-chloroethyl esters, but only 2 ml benzene/hexane (1:3) was used to remove excess contaminants.

Pentafluorobenzyl esterification. To a solution of CPH standards in a test tube was added 0.2 ml 1% w/v PFBBr solution and 30 μl 30% w/v K_2CO_3 solution. The tube was stoppered, shaken and the contents allowed to react overnight at room temperature. The volume of the derivatisation mixture was reduced to 1 ml using TMP and a nitrogen stream as described above. The PFB derivatives were cleaned up on silica gel columns identical to those employed for 2-CE derivatives but using only 4 ml benzene/hexane (1:3) to remove excess contaminants.

Analysis by electron capture-gas chromatography (EC-GC)

EC-GC analyses were performed on a Perkin-Elmer Sigma 2 gas chromatograph equipped with a ^{63}Ni electron capture detector. Two packed columns were used: a glass column (1 m × 3 mm i.d.) packed with 1.5% OV-17 + 1.95% QF-1 on 100/120 mesh Supelcoport (Phase Separations, Queensferry, U.K.) operated at 195°C with a carrier gas (95%/5% argon/methane) flow rate of 50 ml min^{-1}; and a glass column (2 m × 3 mm i.d.) packed with Ultrabond 20 M on 100–120 mesh Chromosorb W (Alltech Associates, Carnforth, U.K.) operated at 200°C with a carrier gas (95%/5% argon/methane) flow rate of 20 ml min^{-1} made up to 50 ml min^{-1} at the detector. Injector and detector temperatures were 300°C. On-column injections of 1–5 μl of standards and sample solutions were made using a 10 μl Hamilton microsyringe (Phase Separations).

Capillary column analyses were performed using a "Grob-type" split-splitless injector in the gas chromatograph. A fused silica capillary column 25 m × 0.24 mm i.d., wall coated with CP Sil 5 liquid phase (similar to OV-101) (Chrompack, London, U.K.) was operated with a (95%/5% argon/methane) carrier gas flow rate of 1.5 ml min^{-1} and the following temperature programme: 55°C for 2 min, 40°C min^{-1} to 150°C, then 8°C min^{-1} to 275°C held for 2 min. Injector and detector temperatures were 300°C. The splitless hot-needle injection technique[37,38] was used for all analyses, with sample volumes of 2 μl. The septum purge flow from the injector was set at 5 ml min^{-1} during analyses and the bottom split valve (50 ml min^{-1}) was opened 40 s after injection. Make-up gas (95%/5% argon/methane) was supplied to the detector at 50 ml min^{-1}.

Analysis by gas chromatography–mass spectrometry (GC–MS)

A Carlo Erba Fractovap 4200 gas chromatograph (Erba Science, Swindon, U.K.) was interfaced to a Jeol JMS-D300 double focussing mass spectrometer with a JMA-2000H data processing system (JEOL, Tokyo, Japan). GC-MS analyses were performed using the capillary column described above, operated with 1.0 ml min^{-1} helium carrier gas flow and the following temperature programme:

55°C for 2 min, 50°C min^{-1} to 150°C, then 8°C min^{-1} to 275°C held for 10 min. The column was connected directly to the ion source of the mass spectrometer in order to optimise resolution and sensitivity. The mass spectrometer was operated under the following conditions.

Accelerating voltage	3.0 KV
Mass range	40–500 m/z
Ionising voltage	70 eV
Ionising current	300 μA
Ion source temperature	250°C
GC-MS transfer line	250°C
Electron multiplier	1.3 KV
Resolution	1000

The mass spectrometer was scanned once a second under data system control and the mass spectra were stored on magnetic disc cartridge. Reconstructed ion chromatograms (RIC), mass spectra of component peaks and mass chromatograms were then retrieved from the stored data. The characteristic ions selected for mass chromatographic quantitation of CPH compounds are shown in Table I.

TABLE I

Ions selected for MS quantitation of CPH derivatives

2-chloroethyl ester derivative	Characteristic ion; m/z value
MCPB	87.0, 149.0
2,4-DB	87.0, 149.0, 231.0
4-CPA	141.0, 248.0
MCPA	141.0, 155.0, 262.0
MCPP	141.0, 169.0, 276.0
2,4-D	175.0, 284.0
2,4,5-TP	196.0, 223.0
2,4,5-T	209.0, 318.0
d_{10} Anthracene	188.0

RESULTS

Derivatisation

The three alternative derivatisation procedures were evaluated by packed column EC-GC, using the mixed phase OV-17/QF-1 column. Five CPH were selected for this study: 4-chloro-2-methylphenoxyproprionic acid (MCPP); 4-chloro-2-methylphenoxyacetic acid (MCPA); 2,4-D; 4-(4-chloro-2-methylphenoxy) butyric acid (MCPB) and 2,4,5-T. The results of this experiment are presented in Table II. For MCPP and MCPA, 2-CE esters displayed the least degree of scatter, whilst for the remaining three compounds (MCPB and 2,4,5-T were not separated by the OV-17/QF-1 GC column) the scatter of TCE esters was marginally superior. PFB esters exhibited a high degree of variation for all five CPH considered. Examination of the gas chromatograms revealed that production of 2-CE esters

TABLE II

Reproducibility of derivatisation procedures for chlorophenoxy herbicides using three different methods.

Compound	Weight derivatised μg	Type of derivative[a]	Retention time (min)	Mean[b] peak height (mm)	Rel. S.D. (%)
MCPP	50.0	2-CE	3.5	6,432	11
	50.0	TCE	4.9	215,815	32
	50.0	PFB	5.8	864,967	17
MCPA	125.0	2CE	4.3	23,115	13
	125.0	TCE	6.2	1,558,510	15
	125.0	PFB	7.8	1,280,968	20
2,4-D	15.0	2-CE	5.9	65,977	12
	15.0	TCE	8.5	144,796	11
	15.0	PFB	10.9	89,102	23
MCPB/ 2,4,5-T	1.25	2-CE	10.2	54,753	14
	+	TCE	14.4	45,017	12
	10.0	PFB	18.0	29,103	25

[a]2-CE = 2-chloroethyl ester.
TCE = 2,2,2-trichloroethyl ester.
PFB = pentafluorobenzyl ester.
[b]Mean of 4 replicates.

gave rise to less interference than did the production of either TCE or PFB esters. This was confirmed by derivatisation and analysis of a series of reagent blanks. Owing to the differing response factors of the five compounds, different concentrations of the determinants were selected to give uniform response at the same detector sensitivity.

The results of an experiment to assess the recovery, efficiency, and reproducibility of the silica gel cleanup procedure for 2-CE derivatives are included in Table III, where replicate standard solutions at two concentrations have been carried through the procedure. Near-complete recoveries, with acceptable reproducibility, were obtained at the higher of the two concentrations, whilst the recoveries and reproducibility were generally poorer at the lower concentration, with the exception of MCPA which exhibited good reproducibility at the lower concentration.

TABLE III

Recovery and reproducibility of silica gel column clean-up procedure for 2-CE herbicide derivatives.

Compound	Weight applied (μg)	Mean[a] recovery (%)	RSD (%)
MCPP	50	101	6
	10	86	5
MCPA	125	94	8
	10	73	11
2,4-D	15	81	6
	10	71	18
MCPB/	1.25/10	85	5
2,4,5-T	10/10	66	22

[a]Mean of 4 replicates.

GC Analysis

After evaluation of the derivatisation and cleanup procedures for 2-CE derivatives of CPH, the range of compounds under consideration was extended to include other common CPH, namely, 4-

chlorophenoxyacetic acid (4-CPA), 2,4,5-TP and 2,4-DB. GC using the mixed phase OV-17/QF-1 packed column did not achieve acceptable separation of the eight selected compounds either from each other or from interfering contaminants. However, capillary column GC resulted in superior resolution of CPH derivatives, although MCPB and 2,4,5-T were still not adequately separated.

An historical problem with capillary columns has been the unreliability of quantiative analyses due to sample introduction techniques and variable quality of the liquid phase coating. However, the results of a calibration experiment for CPH derivatives, presented in Table IV, indicate that quantitative analyses may be performed on this colum for each CPH except 4-CPA and MCPP. These data were obtained using EC detection and the concentration ranges would correspond to CPH concentration in the final condensed extract of a sewage or sludge sample. Subsequent calibration experiments using MS detection routinely yielded regression coefficients of 0.98–1.00 for a 1.0–10.0 $\mu g\,ml^{-1}$ concentration range for each of the eight CPH.

TABLE IV

Calibration data for CPH derivatives from capillary GC analysis

Compound	Retention time (min)	Concentration range ($\mu g\,ml^{-1}$)	Regression coefficient (r)
4-CPA	14.3	0–30.0	0.85
MCPP	15.0	0–30.0	0.85
MCPA	15.3	0–30.0	0.98
2,4-D	16.2	0– 1.0	0.99
2,4,5-TP	17.7	0– 1.0	1.00
2,4,5-T/ MCPB[a]	18.2	0– 1.0	1.00
2,4-DB	19.1	0– 1.0	1.00

[a]Not separated by chromatographic column.

For analysis of sewage samples, procedures involving preparation of 2-chloroethyl esters of CPH and analysis by capillary column GC were therefore selected as being most suitable.

Application of the method to sewage sludges

The results of an experiment to investigate the recovery of 2,4-D and 2,4,5-T from sewage sludges (using 200 mg sludge solids diluted to 200 ml) and fortified sewage sludges are presented in Table V. The extracts from this study were analysed on three different GC columns in order to ascertain the optimum separation and quantitation technique. It is evident that the type of GC column employed significantly influenced the results of analyses. This is probably due to the presence of interfering substances which are co-eluted with the compounds of interest on one particular column and are eluted in a different pattern on each GC column. It is also likely that the peaks measured in gas chromatograms of unspiked sludge extracts are not those due to the presence of 2,4-D and 2,4,5-T since the RSD of the unspiked analyses are particularly high, which is perhaps indicative of the presence of extraneous compounds not quantitatively recovered by the method. This is reinforced by the acceptable percentage recoveries and RSD of the spiked extracts which confirm that both 2,4-D and 2,4,5-T may be determined by this method. Therefore it appears doubtful that the unspiked extracts contained detectable concentrations of these two compounds. The results of the capillary column analyses are considered to be the most reliable because of the superior separation and resolution characteristics of such a system. The unspiked data for 2,4-D agree well with those obtained using the OV-17/QF-1 column, but the results for 2,4,5-T are substantially different.

In an attempt to extend both the sensitivity and scope of the analytical method, larger samples of sewage sludge and consideration of a wider range of CPH were employed. The results of the analysis of the extracts by capillary GC-EC are present in Table VI. Standard solutions were injected with each series of samples in order to obtain a calibration curve. From Table VI it is evident that 2,4-DB was not effectively recovered by this analytical scheme. The recovery efficiencies of MCPB and 2,4,5-T could not be determined by capillary GC-EC owing to co-elution of peaks. Significant interference with the determination of MCPA, MCPP, and 4-CPA was experienced during GC-EC analysis of extracts, with co-elution of determinants and co-extracted material, shown by recoveries for these three CPH of well in excess of 100%. It is likely that the level

TABLE V

Recovery of two chlorophenoxy acid herbicides 2,4-D and 2,4,5-T from sewage sludge analysed as their 2-chloroethyl derivatives on three GC columns[a].

GC column type	Chlorophenoxy acid	Mean[b] concentration (unspiked) ($\mu g\,g^{-1}$)	Mean[b] concentration (spiked at 1.00 $\mu g\,g^{-1}$) ($\mu g\,g^{-1}$)	Extraction efficiency (%)	Unspiked RSD (%)	Spiked RSD (%)
(i)	2,4-D	0.39	1.20	81	15	4
	2,4,5-T	0.92	1.78	86	15	5
(ii)	2,4-D	0.05	1.01	96	20	9
	2,4,5-T	0.74	1.56	82	16	1
(iii)	2,4-D	0.40	1.38	98	36	5
	2,4,5-T	0.01	0.76	75	40	8

[a]GC columns: (i) OV-17/QF-1; (ii) Ultrabond 20M; (iii) Capillary column.

[b]Mean of four replicates, concentration with respect to dry sludge solids.

TABLE VI

Analysis of chlorophenoxy herbicides in sewage sludge by capillary GC-EC

Compound	Mean concentration (unspiked) ($\mu g\,g^{-1}$)	Mean concentration (spiked) ($\mu g\,g^{-1}$)	Extraction efficiency (%)	RSD (%)
MCPP[b]	ND	17.3	173	10
MCPA[b]	ND	30.5	305	6
4-CPA[b]	ND	20.5	205	15
MCPB[b]	ND	—[a]	—	—
2,4-D[c]	0.46	7.7	146	10
2,4-DB[c]	ND	0.7	14	26
2,4,5-T[c]	ND	—[a]	—	—
2,4,5-TP[c]	ND	5.5	110	7

[a]2,4,5-T and MCPB co-elute.
[b]Spiked at 10.0 $\mu g\,g^{-1}$, mean of 4 replicates.
[c]Spiked at 5.0 $\mu g\,g^{-1}$, mean of 4 replicates.

of recovery of these CPH will be highly variable between samples and depend on the type of sample matrix and the number and level of contaminating co-extractants in the sample.

Prior to GC-MS analyses standard solutions of the eight selected CPH were injected into the GC-MS and mass spectra obtained. These were then stored in a library file using the data system 'library create and registration' program, for future comparison with sample mass spectra. The analysis of sewage sludges by GC-MS is summarised in Table VII. Typical mass chromatograms for 4-CPA, MCPP and MCPA and are given in Figure 1. It can be seen that recoveries of CPH are more realistic by GC-MS analysis than by EC-GC. In particular 4-CPA, MCPP and MCPA yield superior results by GC-MS, due to the more selective nature of mass chromatography over electron-capture. Reproducibility at the 5 $\mu g\,g^{-1}$ level of fortification is similar for each mode of detection.

A number of general observations can be made from the GC-MS data. 2,4-DB still exhibits low recovery and poor reproducibility. The CPH recoveries calculated using different m/z values show good agreement though marginally higher recoveries are indicated when ions of lower m/z value are chosen. This may be due to the tendency

TABLE VII

Analysis of sewage sludge sample.

Compound	Retention time (min)	Selected ion fragment (m/z value)	Unspiked sludge[a] concentration (% recovery, RSD)	Spiked sludge[a, b]		
				0.5 $\mu g\,g^{-1}$	2.5 $\mu g\,g^{-1}$	5.0 $\mu g\,g^{-1}$
4-CPA	9.7	141	ND	0.22 (43%, 36%)	3.30 (132%, 7%)	5.95 (119%, 9%)
		248	ND	0.19 (37%, 58%)	3.20 (128%, 9%)	5.60 (112%, 35%)
MCPP	10.3	141	ND	0.26 (52%, 24%)	1.30 (52%, 31%)	5.05 (101%, 16%)
		169	ND	0.32 (63%, 57%)	1.33 (53%, 27%)	5.85 (117%, 7%)
		276	ND	0.41 (82%, 42%)	1.25 (50%, 25%)	5.65 (113%, 10%)
MCPA	10.6	141	ND	0.19 (37%, 56%)	2.00 (80%, 20%)	5.35 (107%, 6%)
		155	ND	0.16 (32%, 48%)	1.90 (76%, 23%)	5.15 (103%, 6%)
		262	ND	0.12 (24%, 53%)	1.70 (68%, 16%)	4.90 (98%, 12%)
2,4-D	11.4	175	ND	0.13 (25%, 39%)	2.88 (115%, 19%)	4.55 (91%, 10%)
		284	ND	0.16 (31%, 49%)	2.08 (83%, 20%)	4.25 (85%, 24%)
2,4-DB	13.9	87	ND	0.02 (4%, 116%)	ND	0.70 (14%, 90%)
		149	ND	0.02 (3%, 116%)	ND	0.85 (17%, 67%)
		231	ND	ND	ND	0.80 (16%, 201%)
2,4,5-T	13.3	209	ND	0.23 (46%, 39%)	ND	3.50 (70%, 22%)
		318	ND	0.05 (10%, 139%)	1.68 (67%, 40%)	3.55 (71%, 25%)
2,4,5-TP	12.8	196	ND	0.21 (41%, 61%)	ND	3.50 (70%, 6%)
		223	ND	0.24 (47%, 9%)	ND	3.45 (69%, 4%)

[a]Mean of four replicates, concentration relative to dry solids. These concentrations correspond to 0.0 μg, 1.0 μg, 5.0 μg and 10.0 μg spikes into 50 ml wet sludge.

[b]The data in brackets refer to percentage recovery and relative standard deviation in that order.

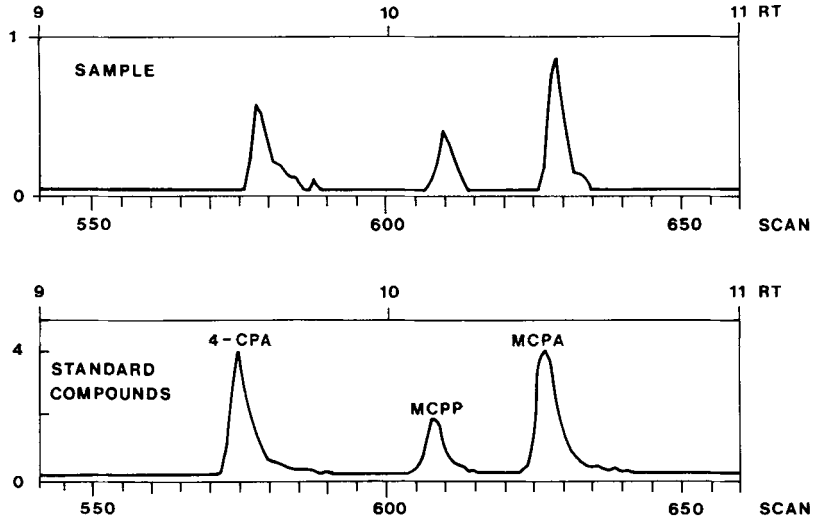

FIGURE 1 Mass chromatograms for 2-CE derivatives of 4-CPA, MCPP and MCPA standard solution and spiked sample extract ($5\,\mu g\,l^{-1}$ in sewage).

of contaminating co-extractants to produce more fragment ions in the lower mass range than the higher. This gives the possibly false impression that the di- and tri-chlorinated CPH are less well recovered than the mono-chlorinated compounds, whereas this may be merely a function of the choice of fragment ions of relatively high m/z value.

CPH (except 2,4-DB) showed good recovery at $5.0\,\mu g\,g^{-1}$ level in sludge. The reproducibility and level of recovery at the $2.5\,\mu g\,g^{-1}$ level was acceptable for 4-CPA, MCPA, 2,4-D and 2,4,5-T. Contamination did not allow the quantitation of 2,4,5-TP. MCPP recovery efficiency was lower than at $5.0\,\mu g\,g^{-1}$. 2,4-DB was not recovered at a concentration of $2.5\,\mu g\,g^{-1}$. Extractions of sludge spiked at $0.5\,\mu g\,g^{-1}$ yielded unacceptable results for level and efficiency of recovery for all the CPH under investigation.

The results of an analysis of a composite extract of 400 ml sewage sludge are presented in Table VIII. Two of the eight CPH under investigation were identified and quantified by GC-MS. The concentration of 2,4-D shows good agreement between the two m/z values chosen, whereas the 2,4,5-TP concentration calculated using

TABLE VIII

Analysis of large composite sewage sludge sample.

Compound	Similarity index[a]	Selected ion fragment (m/z value)	Concentration ($\mu g\,g^{-1}$)
2,4-D	0.74	175	1.30
		284	1.11
2,4,5-TP	0.73	196	0.21
		223	0.34

[a]Similarity index = a measure of the 'fit' of the spectrum of CPH derivative suspect peak with the corresponding spectrum from the computer library.

m/z 223 is significantly greater than when m/z 196 is used for mass chromatographic purposes. Evidently there is contamination by coextractants at the higher of the m/z values. This discrepancy was not observed when spiked sludge was analysed, although higher concentrations of CPH were involved. The similarity indices are also shown for 2,4-D and 2,4,5-TP between the composite samples and the library. Figure 2 shows the spectra for library and sample 2,4-D.

The results of analysis of sewage samples by GC-MS are presented in Table IX, which shows that CPH (except 2,4-DB) are adequately recovered with good reproducibility at the $5.0\,\mu g\,l^{-1}$ and $10.0\,\mu g\,l^{-1}$ levels. The relative standard deviations and levels of recovery at the $1.0\,\mu g\,l^{-1}$ concentration were unacceptably high. None of the CPH were detected in the unspiked 11 sewage samples.

DISCUSSION

The assessment of derivatisation procedures for CPH analysis by EC-GC has revealed that 2-chloroethylation is most suitable in terms of low background response and good reproducibility (Table II). These 2-CE derivatives have the advantage of intermediate retention times on packed columns—shorter than the PFB esters and longer than methyl esters. However the chromatographic resolution of 2-CE esters on packed columns shows no improvement over the other types of derivative.

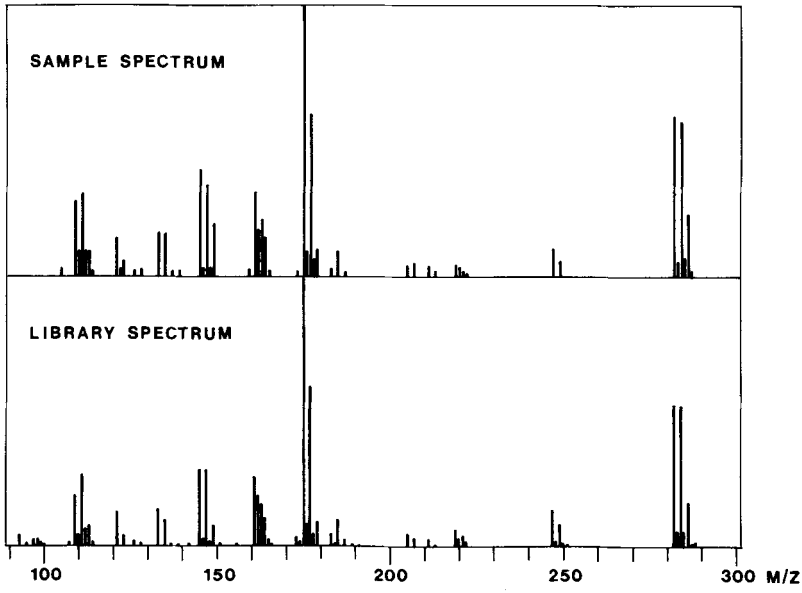

FIGURE 2. Mass spectra of 2-CE derivative of 2,4-D from GC-MS computer library and sewage sludge extract.

The use of capillary column GC enabled improved separation of the CPH derivatives, although using argon/methane carrier gas and the conditions described earlier, there were still two CPH (2,4,5-T and MCPB) which co-eluted from the capillary column. The experimental work demonstrated that capillary column GC could be used for quantitative CPH analysis, since acceptable calibration curves were obtained (see Table IV). However, when EC-GC using a capillary column was applied to the analysis of extracts from sewage sludge it was found that the degree of variation in contamination from interfering co-extractants prohibited reliable quantiative analysis of these sample types (Table VI).

The problem of selectivity has been addressed by GC-MS using mass chromatographic quantitation. This approach has been described previously for the determination of 2,4-D in air samples.[39] Selected ion monitoring GC-MS has also been applied previously to the analysis of ester forms of 2,4-D in sewage samples.[32] The results in Tables VII and IX demonstrate that GC-MS analysis of

TABLE IX

Analysis of sewage sample.

Compound	Selected ion fragment (m/z value)	Unspiked sewage[a] concentration (% recovery, RSD)	Spiked sewage[a, b]		
			1.0 $\mu g l^{-1}$	5.0 $\mu g l^{-1}$	10.0 $\mu g l^{-1}$
4-CPA	141	ND	2.06 (206%, 20%)	5.00 (100%, 9%)	8.60 (86%, 12%)
	248	ND	1.87 (187%, 31%)	4.95 (99%, 6%)	7.00 (70%, 12%)
MCPP	141	ND	1.25 (125%, 21%)	4.30 (86%, 10%)	8.90 (89%, 12%)
	169	ND	1.25 (125%, 13%)	3.65 (73%, 4%)	6.80 (68%, 14%)
	276	ND	0.90 (90%, 35%)	3.25 (65%, 2%)	5.30 (53%, 9%)
MCPA	141	ND	1.06 (106%, 18%)	4.25 (85%, 6%)	8.80 (88%, 7%)
	155	ND	1.35 (135%, 14%)	3.80 (76%, 3%)	7.70 (77%, 10%)
	262	ND	1.20 (120%, 17%)	3.35 (67%, 3%)	5.40 (54%, 10%)
2,4-D	175	ND	1.97 (197%, 21%)	5.50 (110%, 9%)	11.70 (117%, 14%)
	284	ND	1.55 (155%, 145%)	4.30 (86%, 4%)	7.20 (72%, 10%)
2,4-DB	87	ND	1.30 (130%, 152%)	ND	3.50 (35%, 10%)
	149	ND	1.53 (153%, 116%)	ND	3.40 (34%, 8%)
	231	ND	ND	ND	ND
2,4,5-T	209	ND	2.32 (232%, 22%)	5.30 (106%, 22%)	11.40 (114%, 10%)
	318	ND	2.07 (207%, 46%)	4.85 (97%, 12%)	8.10 (81%, 9%)
2,4,5-TP	196	ND	1.07 (107%, 6%)	3.65 (73%, 4%)	8.10 (81%, 15%)
	223	ND	1.37 (137%, 5%)	3.95 (79%, 1%)	7.60 (76%, 7%)

[a]Mean of four replicates. These concentrations to 0.0 μg, 5.0 μg and 10.0 μg spikes into 1 l sewage.

[b]The data in brackets refer to percentage recovery and relative standard deviation in that order.

2-chloroethyl CPH derivatives was satisfactory for $5.0\,\mu g\,l^{-1}$ and $2.5\,\mu g\,g^{-1}$ concentrations in sewage and sludges respectively, for six of the seven CPH investigated.

Agieman *et al.*[34] have reported that MCPA and MCPB can be determined at concentrations as low as $0.1\,\mu g\,l^{-1}$ and $0.2\,\mu g\,l^{-1}$ in 1 l fortified distilled water using a dichloromethane extraction, PFB derivatisation and EC-GC. The level of recovery at this concentration was approx. 80% and the relative standard deviation approx. 15%. This method was unsuitable for other CPH because of interfering peaks from the derivatisation reagent.

More recently, Mierzwa *et al.*[35] have analysed 2,4-D and MCPA in natural waters. Extraction was by sorption on Amberlite XAD-4 resin, benzene elution, 2,2,2-TCE derivatisation and EC-GC detection. The recoveries at the $0.4\,\mu g\,l^{-1}$ level were $92.1 \pm 3.4\%$ (2,4-D) and $87.8 \pm 2.1\%$ (MCPA) for a 1 l sample, suggesting that this method is superior than that of Agieman *et al.*,[34] at least for MCPA in natural waters. However, Mierzwa *et al.*[35] have verified their techniques only for 2,4-D and MCPA. Saleh[32] assessed the reliability of analysis of 2,4-D methyl, ethyl, isopropyl and isobutyl esters. The analytical procedure involved benzene and 15% ethyl ether/hexane extractions, Florisil column clean-up and EC-GC. At a concentration of $0.03\,\mu g\,l^{-1}$ in 3 l of distilled water these esters were recovered with an efficiency of 98–100%. No details of relative standard deviations were given. A multi-residue analytical procedure for seven CPH in natural waters has been described by Agieman *et al.*,[31] using 2-chloroethylation and electron capture detection. Water samples of 1 l were fortified at the folowing levels: MCPA ($0.25\,\mu g\,l^{-1}$), 2,4-dichlorophenoxyproprionic acid 2,4-DP ($0.02\,\mu g\,l^{-1}$), 2,4-D ($0.03\,\mu g\,l^{-1}$), 2,4,5-TP ($0.01\,\mu g\,l^{-1}$), MCPB ($2.5\,\mu g\,l^{-1}$), 2,4,5-T ($0.02\,\mu g\,l^{-1}$) and 2,4-DB ($0.1\,\mu g\,l^{-1}$). The recoveries were 78–109% (except MCPA at 64%) and relative standard deviations 7–17%.

Although each of the methods discussed above reports lower CPH concentrations (than in this paper) at which recovery of CPH is reproducible and near-complete, the results are not directly comparable, since the sample type involved was generally distilled water, which contains far fewer organic compounds and virtually no solids compared to raw sewage.

The herbicide 2,4-DB cannot be quantiatively analysed by the method described here since both electron capture and mass

spectrometric detection revealed low levels of recovery. This suggests that detection of 2,4-DB is acceptable but solvent extraction is inefficient. However, a multi-residue method, which has been described previously for surface waters, successfully recovered 2,4-DB.[31] A possible explanation could be that 2,4-DB decomposed in the sewage or sludge during the 4-h equilibration period between foritification and extraction.

Although the capillary column used for GC-MS was similar to that in the EC-GC instrument the former was able to separate all of the eight CPH. Presumably this was because helium carrier gas was employed for GC-MS work.

Generally the results of CPH concentration by mass chromatography compared well between different m/z values for the same CPH compound. Therefore any of the fragment ions could be recommended for CPH mass chromatographic quantitation, but the use of higher m/z values carries less risk of interference from background contaminants. If two fragment ions are chosen per herbicide the similarity of concentration obtained for each serves to confirm that the GC peak is the suspected CPH.

The analytical method in this paper is able to determine only the acid form of CPH. This group of herbicides may also exist in various ester forms which are not extracted by this procedure. In previous analytical methods the initial step has been to reflux the sample at pH 3 for 4 h.[32] This converts the esters to acids, so 'total' CPH are then determined. Alternatively, individual ester forms of CPH may be analysed by a method which obviously does not require a derivatisation step.[32]

Therefore, the concentrations of 2,4-D and 2,4,5-TP reported from the analysis of unspiked sewage sludge (Table VIII) may be underestimates of the actual CPH content. The sewage treatment works from which this ample was obtained has a partially separate sewerage system and treats an influent of low average industrial waste content (2.6% of dry weather flow) with no CPH manufacturers in the vicinity. The area served is largely residential, with little runoff or effluent from farming. Therefore the likely source of the CPH identified is runoff from herbicice treated areas. In areas where agriculture, forestry or herbicide production takes place the level of CPH in runoff and waste water may be greater.

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