Efficient One-Step Method for the Extraction of Cyclodiene Pesticides from Aqueous Media and the Analysis of Their Metabolites

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An efficient method for the extraction of cyclodiene organochlorines from small samples of aqueous microbial growth media is described. Metabolites of endosulfan and aldrin, some of which are current environmental pollutants, can be detected on the electron capture detector (ECD) gas chromatograph with separation on a packed column. This has been done with their underivatized forms with prior column conditioning, with the exception of dihydrochlordenedicarboxylic acid (DHCDA). Separation of endosulfan and all of its metabolites has been achieved on a single packed column, and a comparison has been made of the relative sensitivity of the ECD to these compounds, aldrin, and its major metabolites. The limits of detection and the extent of linearity of the response of the ECD toward these compounds are reported. The importance of adequate column conditioning and likely problems encountered with the analysis of these cyclodiene compounds are described.

INTRODUCTION

The cyclodiene pesticides, endosulfan, aldrin, and their metabolites, are pollutants often detected in the natural environment. The presence of the two isomers of endosulfan and their biologically active oxidation product, endosulfan sulfate, in waterways (Otton, 1991) is of particular concern, as they are extremely toxic to fish (Goebel et al., 1982). In the environment, aldrin is converted to its epoxide, dieldrin, and both aldrin and dieldrin may undergo various transformations at slow rates (Scheunert, 1989) to compounds that may be more or less toxic than these parent compounds. In determining the environmental fate of cyclodienes, it is therefore necessary to detect all metabolites, taking into account their toxicity, their ability to bioaccumulate in animal tissue (in the case of dieldrin and endosulfan sulfate) (Noble, 1991; Albertson et al., 1990; Mitchell, 1990), and their ability to move from the places of initial formation to other parts of the environment (Day, 1991).

In laboratory studies it is desirable to have an extraction system that is effective in extracting pesticides in a fast and reproducible way. There are many methods currently available for extracting pesticides from liquid media. The most popular procedures include use of the separating funnel and various liquid/liquid partitioning systems; these methods have been reviewed by Beroza et al. (1969). Other methods for pesticide extraction include solid-phase minicleanup columns, which are applications of the principles of reversed-phase chromatography. In a more recent development, Cias and Shimoni (1981a,b) described a novel liquid/liquid partitioning system (Mixxor), which utilized a new concept in mass transfer, and applied the system to extractions and separations in immunoassays. In the current study, the Mixxor liquid/liquid partitioning device was applied to the extraction of endosulfan, aldrin, and their major metabolites from small samples of aqueous media, as is commonly required in studies of the microbial degradation of pesticides. The optimal gas chromatographic conditions for the determination of the metabolites of these cyclodiene pesticides using packed columns and an ECD are also described.

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MATERIALS AND METHODS

Chemicals and Glassware. The parent compounds endosulfan I (99.8%) and endosulfan II (99.8%) and the metabolites, endosulfan diol (98%), endosulfan sulfate (98%), endosulfan ether (98%), endosulfan lactone (98%), and endosulfan hydroxy ether (98%), were kindly provided by Dr. Klaus Stumpf, Hoechst AG, Frankfurt am Main, FRG, and Hoechst Limited Australia. Aldrin metabolites were a gift from Dr. David H. Hutson, Shell Research, Sittingbourne, U.K., and Shell Chemicals Australia. A dechlorinated cyclodiene derivative, *syn*-monodechlorodieldrin, was kindly provided by Dr. Andrew Maule, Porton Down Laboratories, Salisbury, U.K. All parent compounds and metabolites were prepared as 1000 and 100 ppm stocks in methanol, which were used as spiking solutions. Dilutions were made into hexane to prepare gas chromatography standards. The standard solutions were stored at −10 °C. All solvents used in the extraction and analysis were of Nanograde (Mallinckrodt). Glassware used was unsilanized borosilicate glass, washed twice in concentrated Pyroneg detergent (Diversey Australia Pty. Ltd) and rinsed with distilled water. The glassware was rinsed once with AR grade acetone (Pronalysis) prior to use. Anhydrous sodium sulfate was of AR grade (BDH Chemicals). Boron trifluoride/methanol (12–14%) was purchased from BDH Chemicals. Acetic anhydride was of laboratory grade, purchased from May and Baker. Pyridine was of analytical grade, purchased from Univar. The pyridine was dried over KOH and redistilled. The distillation fraction of 114.5–115 °C was stored over KOH.

Extraction Procedure. Pesticides were added at 1–5 $\mu g\ mL^{-1}$ to 500–1000 μL of aqueous medium and this was extracted with a total solvent volume of 10 mL in hexane/acetone/methanol/medium (15:5:2:2) in the Mixxor reservoirs (Figure 1). The level of added pesticide exceeded the limits of the solid-phase solubility for these compounds in water. The piston of the Mixxor was moved 60 times in its reservoir to partition the pesticides into the solvent phase. After the phases were allowed to separate (approximately 1 min), the solvent layer was decanted off the aqueous phase directly from the Mixxors into volumetric flasks, and the total volumes were made to either 10.0 or 25.0 mL with hexane only. A subsample was dried with anhydrous sodium sulfate to remove all traces of water prior to gas chromatography. The liquid/liquid partitioning devices (tradename Mixxors, manufactured by Genex Corp., Gaithersburg MD 20877, and distributed by Alltech Australia Pty. Ltd.) of 10-mL capacity (cat. no. 69104) were used in the routine extractions, all carried out in duplicate.

Gas Chromatographic Analysis. Extracts were analyzed on a Shimadzu GC 8A ^{63}Ni ECD gas chromatograph. Columns used were an OV-210 (5%):SE-30 (5%) (7:3) on Chromosorb W-HP, 80/100 mesh, and an SE-30 (5%):DC-200 (5%) (3:1) on

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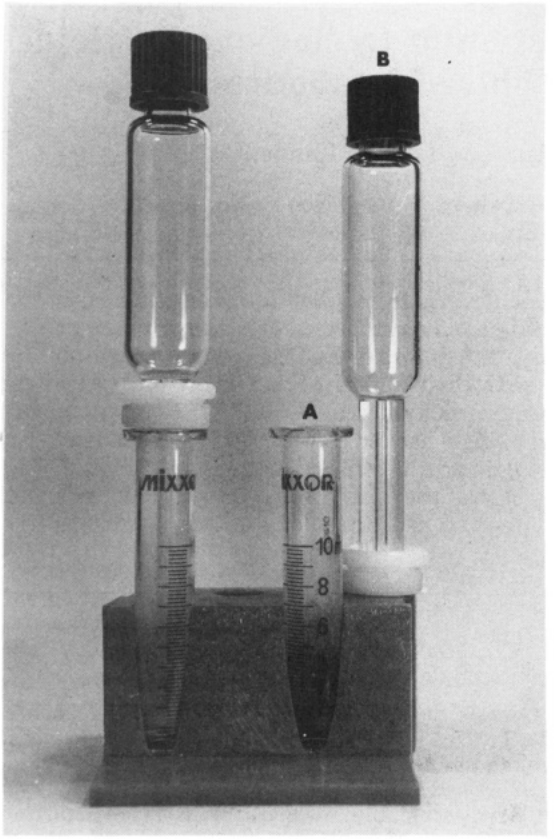

Figure 1. Mixxor liquid/liquid partitioning device. Sample to be extracted and solvent are added to the glass mixing reservoir (A). The mixer–separator (B) is inserted into the mixing reservoir and moved up and down up in a piston-like fashion to extract the pesticides.

Gaschrom Q, 80/100 mesh, packed into 3 m × 2.0 mm custom-made unsilanized borosilicate glass columns. The N_2 gas flow rate on all columns was adjusted to 25 mL min^{-1}. The column and injector/detector temperatures were set at 210 and 290 °C, respectively. The columns were prepared for routine use by standard procedures followed by conditioning in the gas chromatograph at 250 °C for 48–60 h (Zweig and Sherma, 1972). Before routine use, the liquid phases were equilibrated by injecting four or five times 1–5 μL of 1–10 ppm of pesticides and metabolites in hexane, with the end of the column disconnected from the detector. When the parent compounds and metabolite standards showed consistent sensitivity and retention time behavior, the column was considered ready for use. Columns were maintained by using extra glass wool packing as a pseudo-precolumn and replacing it when it became discolored after many injections. Peak area integration was performed using a Shimadzu Chromatopac CR-3A integrator set at 32 mV full-scale deflection (FSD) sensitivity. The external standard method was used for calculating residue values.

Determination of Limits of Detection. These were determined for each of the compounds analyzed by calculating the size of the smallest peak able to be distinguished from baseline noise under the conditions described under Gas Chromatographic Analysis. This was defined as being a peak 3 times the mean baseline noise at 32-mV FSD.

Derivatization Procedure. Derivatization of endosulfan diol was achieved by mixing 1 mL of extract, 2 mL of pyridine (treated as described), and 1 mL of acetic anhydride and incubating for 15 min at 37 °C. After 15 min, 5 mL of water was added and the solution was extracted with 5 mL of hexane. The hexane layer was washed in sequence with 5 mL of H_2SO_4 (1 M), 5 mL of $NaHCO_3$ (1 M), and 5 mL of water. The sample was dried with anhydrous Na_2SO_4 and made to volume for gas chromatographic analysis.

RESULTS AND DISCUSSION

Comparison of the Efficiency of Extraction. Extraction efficiencies of aldrin, dieldrin, and endosulfans I and II from growth medium and pure water were equal to, or above, 83% when the Mixxor was used without subsampling, i.e., extracting the entire vessel and its contents (Table I). The average coefficient of variation for all of the compounds using the Mixxor without subsampling was 5% at 1.0 ppm level of spiking. The value for endosulfan sulfate over all of the treatments was slightly higher than this. The liquid/liquid partitioners gave comparable recoveries to the separating funnel for equal amounts of time spent partitioning. However, use of the Mixxors was more convenient, allowing multiple samples (three or four) to be processed at once. Another benefit of the Mixxor was that consistent phase separation was achieved. An alternative method for liquid extraction method was used preliminary in this study. This approach involved extraction of the media directly from vials by adding the extraction solvent and vortexing for 30 s. The extraction efficiency was relatively low (70%) and variable. In addition, phase separation was erratic and low-speed centrifugation was often required to bring this about.

Subsampling from the total bulk of the growth medium, at the 1.0 ppm spiking level, had the effect of reducing the extraction efficiency to approximately a third of that obtained without subsampling (Table I). The average coefficient of variation when the extract was subsampled increased to 8.4%. This indicates that if cyclodiene residues are to be determined in an aqueous sample, and their residues are likely to be above the limits of their solubility, it is necessary that the entire vessel be extracted to ensure that material absorbed at the interfaces is recovered. For example, if a water sample is collected in a glass vessel, it is recommended that the vessel should be rinsed with the extracting solvent rather than subsampling from the total volume and extracting this subsample. This is also important where metabolites are sought for, for example, endosulfan sulfate and endosulfan diol, even though these compounds are slightly more water soluble than either of the parent isomers.

Effect of Media Composition and Level of Spiking. There was little difference between recoveries from different media types, but there were significantly higher recoveries of all compounds from pure water as compared with that from microbial growth medium (Table II). The mean coefficients of variation for each of the media types were close to 5%. Optimal extraction efficiency from the phosphate-buffered soil extract yeast mannitol broth (SEYMB) was achieved when the compounds were present at between 0.1 and 1.0 μg mL^{-1} (Table II). Recoveries significantly decreased at 10 μg mL^{-1}, although the coefficient of variation was considerably reduced.

Optimization of Extraction Conditions. An optimum extraction was considered to be reached after 60 strokes of the partitioner piston in its reservoir. Where further extractions of a sample are desired, the Mixxor reservoir may be emptied into a quick-fit flask for rotary evaporation and then refilled with the extraction solvent and the sample re-extracted. There was no significant improvement in extraction efficiency when the duration of extraction was increased to 120 or 180 strokes; therefore, 60 strokes was used routinely throughout the study to minimize unnecessary wear of the glass surfaces.

Optimization of Gas Chromatographic Conditions. The analysis of a wide range of chlorinated cyclodiene compounds using gas chromatography is common practice. There are, however, no descriptions in the literature of

Table I. Comparison of Efficiency of Extraction of Pesticides from Various Sterile Aqueous Media Using a Separating Funnel and a Mixxor Extractor[a]

extraction method and media	extraction efficiency, %					CV,[d] %
	aldrin	dieldrin	endosulfan I	endosulfan II	endosulfan sulfate	
separating funnel (250 mL)[c]	84.5 ± 5.9	87.5 ± 4.5	83.5 ± 3.5	87.0 ± 5.4	95.6 ± 7.3	6.1
Mixxor[c]	85.4 ± 2.9	88.5 ± 4.1	89.4 ± 4.1	82.7 ± 3.4	83.9 ± 6.6	4.9
Mixxor subsampling[c]	29.8 ± 2.2	27.8 ± 2.5	20.0 ● 1.4	27.1 ± 2.5	35.4 ± 3.2	8.4
Mixxor anaerobic medium[d]	84.3 ± 3.2	85.0 ± 5.0	88.6 ± 3.5	83.1 ± 3.7	82.5 ± 6.5	4.4
Mixxor pure water	92.7 ± 2.9	91.5 ± 2.6	91.3 ± 2.6	82.7 ± 1.8	72.6 ± 3.0	3.0

[a] Pesticide concentration in the medium was 1 ppm for aldrin, dieldrin, endosulfan I, and endsofulan II and 5 ppm for endosulfan sulfate. There was no subsampling from larger total volumes unless indicated. [b] CV, coefficient of variation across each treatment. [c] Soil extract yeast mannitol medium (SEYMB) contained soil extract (10%), yeast extract (2.0 g/L), mannitol (5.0 g/L), sucrose (2.0 g/L), and trace elements in 100 mM phosphate buffer (Allen, 1957). [d] Anaerobic medium was described by Balch et al. (1982) and modified by Maule et al. (1987) with the addition of formate (2.0 g/L).

Table II. Effect of Level of Spiking on the Efficiency of Pesticide Extraction from Growth Medium Using the Mixxor Liquid/Liquid Partitioning Devices

spike,[a] ppm	extraction efficiency, % ± SD					CV,[b] %
	aldrin	dieldrin	endosulfan I	endosulfan II	endosulfate	
0.1	88.5 ± 1.3	83.4 ± 2.0	91.9 ± 2.5	78.0 ± 4.0	88.7 ± 7.6	4.0
1.0	86.8 ± 3.2	86.3 ± 3.7	83.7 ± 4.5	79.1 ± 3.5	86.0 ± 7.1	5.1
10.0	62.7 ± 1.4	61.2 ● 1.6	72.1 ± 1.9	72.1 ± 1.8	65.1 ± 3.4	3.0

[a] Endosulfan sulfate was spiked at 5 times the amount of the other cyclodienes, i.e., 0.5, 5.0, and 50.0 ppm into SEYMB (described in Table I). There was no subsampling in this experiment. [b] CV, coefficient of variation across each spiking treatment.

the relative sensitivity and linearity of the electron capture detector toward the various cyclodiene metabolites in their underivatized forms or on their limits of detection using the gas chromatographic technique. An attempt was made to characterize optimal conditions for the analysis of cyclodiene metabolites using ECD gas chromatography with packed columns.

It was apparent that the chromatography columns required a period of equilibrating with the compounds to be analyzed prior to use. This was demonstrated by the very low relative sensitivities of the compounds, in particular the diols, prior to equilibrium. In fact, the sensitivity of the detection of endosulfan diol, *cis*- and *trans*-aldrin diol, increased 3-fold after the equilibration period. This period was kept to a minimum when 1–10 ppm solutions in hexane were injected with the detector end of the column disconnected. This effect is likely to be due to strong interactions of the analytes at sites within the packed column.

Although both columns tested were able to chromatograph the parent compounds and metabolites in the study, only one of the columns successfully separated all of the endosulfan compounds when they were present in a mixture (Figure 2). This was the column containing a mixture of the liquid phases SE-30 (5%):DC-200 (5%) in a 3-to-1 ratio.

In studies where XE-60, QF-1, DC-200, SE-52, OV-225, and XE-60:SE-52 were used as liquid phases at 2% in packed columns, useful separation was only achieved with XE-60 (Goebel et al., 1982, and references cited therein). The major difficulty in separating the endosulfan metabolites, on the column configurations previously reported, arises from the similarity of retention times of endosulfan diol and endosulfan hydroxy ether. When both are present in a mixture, they tend to elute with the same retention time, although when analyzed individually they have slightly different retention times. Therefore, to be able to quantify all of the endosulfan compounds in a study, it has been necessary to use two columns routinely. The choice of the SE-30:DC-200 liquid-phase mix in the current study has overcome this inconvenience, so that two columns need only be used for metabolite confirmation (Table III). DC-200 can separate endosulfan diol and endosulfan hydroxy ether very well but not the other

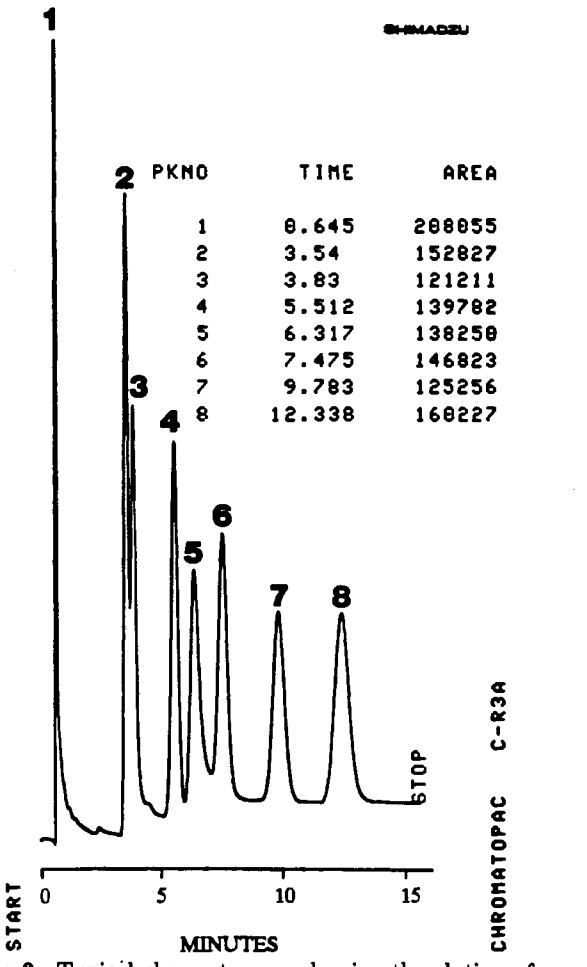

Figure 2. Typical chromatogram showing the elution of endosulfan compounds on the packed column containing a 3:1 mixture of SE-30 (5%):DC-200 (5%) liquid phases on Gaschrom Q 80–100 mesh. Injection, column, and detector temperatures were 290, 210, and 290 °C, respectively. The compounds in order of elution are solvent (1), endosulfan ether (2), endosulfan hydroxy ether (3), endosulfan lactone (4), endosulfan diol (5), endosulfan I (6), endosulfan II (7), and endosulfan sulfate (8).

endosulfan compounds (Goebel et al., 1982, and references cited therein), while SE-30 can separate all of the other

Table III. Analysis of Endosulfan and Its Metabolites Using Two Chromatographic Columns on an Electron Capture Gas Chromatograph[a]

parent compound or metabolite	column I[b] retention time, min	column I[b] relative sensitivity[d]	column II[c] retention time, min	column II[c] relative sensitivity
endosulfan I	7.88	1.00	8.67	1.00
endosulfan II	10.43	0.79	11.34	1.11
endosulfan sulfate	13.26	0.08	21.87	0.67
endosulfan diol[e]	6.67	0.01	4.73	0.01
endosulfan ether	3.62	1.28	3.35	1.04
endosulfan lactone	5.78	0.98	7.23	0.98
endosulfan hydroxy ether	3.95	1.25	5.08	1.17

[a] Conditions described under Materials and Methods. [b] SE-30 (5%):DC-200 (5%) (3:1) liquid phase mix. [c] OV-210 (5%):SE-30 (5%) (7:3) liquid phase mix. [d] Relative to endosulfan I. [e] The acetylated derivative of the diol eluted at 11.6 min and showed a 60-fold increase in sensitivity compared to endosulfan I.

Table IV. Chromatographic Characteristics of Aldrin and Related Compounds Using Two Chromatographic Columns on an Electron Capture Gas Chromatograph[a]

parent compound or metabolite	column I[b] retention time, min	column I[b] relative sensitivity[d]	column II[c] retention time, min	column II[c] relative sensitivity
aldrin	5.23	1.00	4.08	1.00
dieldrin	8.82	1.08	8.56	0.85
syn-dechlorodieldrin	7.63	0.54	9.05	0.42
pentachloroketo-dieldrin	11.18	0.80	19.28	0.23
dieldrin ketone	8.48	0.16	10.43	0.30
dihydrochlordene-dicarboxylic acid dimethyl ester	15.12	0.78	15.09	0.26
photoaldrin	11.09	0.04	11.95	0.14
photodieldrin	19.69	0.05	28.10	0.02
cis-aldrin diol	16.37	0.02	12.05	0.02
trans-aldrin diol	12.53	0.004	15.0	0.001

[a] Conditions described under Materials and Methods. [b] SE-30 (5%):DC-200 (5%) (3:1). [c] SE-30 (50%):OV-210 (5%) (1:1) liquid phase mix. [d] Relative to aldrin.

endosulfan compounds, except endosulfan diol and endosulfan hydroxy ether. The second column used in the study, containing the OV-210:SE-30 liquid-phase mix, gave good separation of all the endosulfan compounds except for endosulfan diol and endosulfan hydroxy ether and therefore was not used routinely in the analysis of endosulfan (Table III).

Only one potential metabolite of aldrin, dihydrochlordenedicarboxylic acid, could not be chromatographed without derivatization, even when injecting concentrations of up to 10 ppm were injected. The dimethyl ester standard (provided by Shell Chemicals), however, could be detected under these chromatographic conditions. For analyzing this compound in environmental samples it is therefore necessary to form the dimethyl ester derivative prior to its analysis. A simple and efficient method using BF_3/methanol, modified from that of Hallas (1965), has been developed by N. Ahmad (1989, Fox Anamet Laboratory, Sydney, personal communication). The methyl ester derivative is considerably more volatile and less water soluble than the diacid, and this has allowed it to be detected in the gas chromatograph under the conditions described. All other potential metabolites of aldrin were successfully chromatographed on both of the columns used (Table IV).

Linearity of Detector Response. The linearity of the response of the ECD to the endosulfan compounds was determined. Both parent compounds and metabolites showed good linearity over the working concentration range. The indicator of linearity used was the regression coefficient from the mass of compound injected vs peak area plots (Table V). Endosulfans I and II and hydroxy ether data points showed the closest fits on the linearity plots. Endosulfan sulfate, although linear over the entire concentration range examined, gave the lowest regression coefficient of all the endosulfan compounds, i.e., compared with that of endosulfan I. Similarly, the ECD response for aldrin and related compounds was determined. All of the aldrin-related compounds gave very good linearity response curves over the concentration ranges analyzed, except for *trans*-aldrin diol (Table VI). This response for the *trans*-aldrin diol is correlated with a low sensitivity and therefore a high limit of detection. The response curves demonstrate that after the column is initially equilibrated with parent compounds and metabolites, there is a proportional response with amount of pesticide injected (Figures 3 and 4). Since the response curves for all of the compounds, including the diols, pass through the origin, any losses of compounds occurring in the chromatography system are proportional to the amount injected.

Table V. Detection Parameters for Endosulfan and Its Metabolites (Underivatized) on the Electron Capture Gas Chromatograph[a]

compound	LOD,[b] pg	mass range analyzed, pg	linearity of detector response[c]
endosulfan I	1.01	0–1600	0.997
endosulfan II	1.36	0–1600	0.992
endosulfan sulfate	7.41	0–1000	0.965
endosulfan diol	42.0	0–1000	0.989
endosulfan ether	1.1	0–200	0.974
endosulfan lactone	1.20	0–200	0.974
endosulfan hydroxy ether	1.23	0–200	0.995

[a] Using SE-30:DC-200 column under the conditions described under Materials and Methods. [b] Limit of detection is defined as being the quantity of compound which gives a peak 3 times the baseline noise at 32 mV FSD. [c] Regression coefficients determined from linear plot of mass of compound analyzed vs peak area.

Table VI. Detection Parameters for Aldrin and Its Metabolites on the Electron Capture Gas Chromatograph[a]

compound	LOD,[b] pg	concn range, pg	linearity of detector response[c]
aldrin	1.03	0–4000	1.000
dieldrin	1.11	0–4000	0.998
syn-dechlorodieldrin	1.79	0–4000	1.000
pentachloroketodieldrin	1.22	0–4000	0.999
dieldrin ketone	167	0–4000	0.999
dihydrochlordenedicarboxylic acid dimethyl ester	1.24	0–4000	0.998
photoaldrin	23.8	0–1500	0.990
photodieldrin	18.2	0–1500	0.996
cis-aldrin diol	66.2	0–5000	0.985
trans-aldrin diol	250	0–5000	0.956

[a] Using SE-30 (5%):DC-200 (5%) (3:1) column under the conditions described under Materials and Methods. [b] Limit of detection is defined as being the quantity of compound which gives a peak 3 times the baseline noise at 32 mV FSD. [c] Regression coefficients determined from linear plot of mass of compound analyzed vs peak area.

Sensitivity of Detector toward Metabolites. After many hours of routine use of the OV-210:SE-30 column, the apparent sensitivity of the ECD toward endosulfan hydroxy ether decreased. Full sensitivity was restored by replacing the glass wool packing located at the injection zone, which had become discolored with use. This effect was not observed with any of the other endosulfan or aldrin

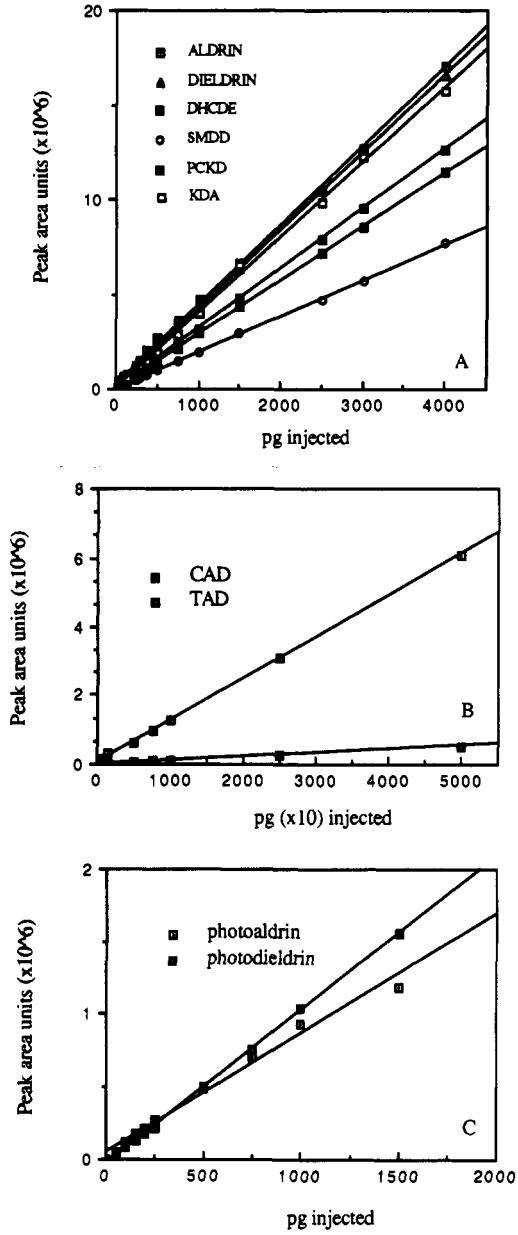

Figure 3. Comparison of the ECD response toward (A) aldrin, dieldrin, their pentachloro, ketone, and methylated acid derivatives, (B) aldrin diols, and (C) photoisomers of aldrin and dieldrin.

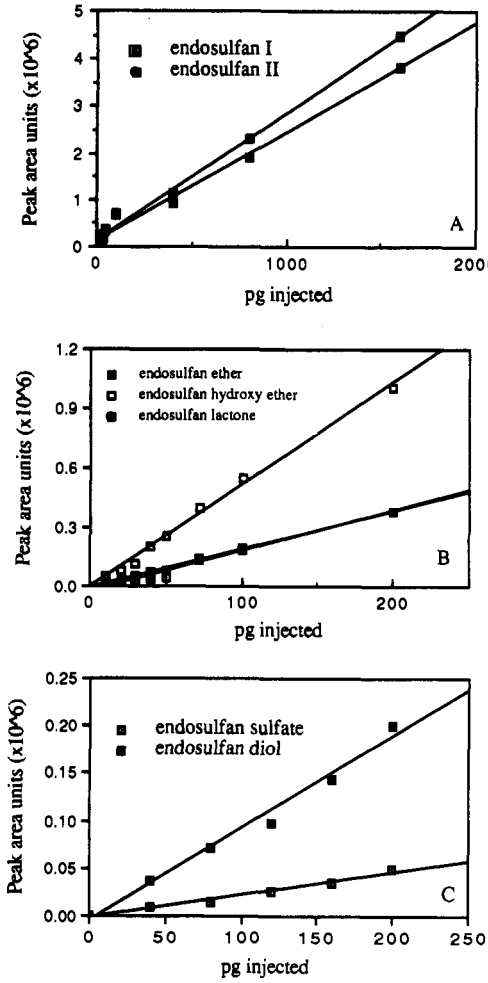

Figure 4. Comparison of the ECD response toward (A) the endosulfan isomers, (B) endosulfan hydroxy ether, ether, and lactone, and (C) endosulfan diol and sulfate.

compounds. This observation indicates that endosulfan hydroxy ether is particularly sensitive to degradation in the injection zone once the glass wool packing has aged. This problem will be avoided in routine analysis if the glass wool packing is replaced frequently, i.e., after approximately 200 injections.

The mean coefficients of variation from each of the linearity plots were determined as follows: endosulfan I, 5.1%; endosulfan II, 6.6%; endosulfan sulfate, 14%; endosulfan diol, 7%; endosulfan ether, 6.4%; endosulfan hydroxy ether, 6.9%; and endosulfan lactone, 3.5%. The greater variability in the detection of endosulfan sulfate means that when the sulfate is being analyzed, at least duplicate or preferably triplicate injections of the same sample should be made. The variation does indicate that during analysis endosulfan sulfate may show erratic stability. The limits of detection of all compounds studied were determined (Tables V and VI). The parent compounds showed very low limits of detection. *trans*-Aldrin diol and endosulfan diol, as well as endosulfan sulfate, demonstrated relatively high limits of detection, indicating a possible need to concentrate the solvent phase of extracts of these samples when these degradation products are analyzed in environmental samples.

When endosulfan diol was derivatized with acetic anhydride, the sensitivity of the detector response was increased. On column I (Table III), the acetylated diol derivative eluted at 11.6 min, which was equivalent to a relative retention time of 1.5 when compared with endosulfan I. This increase in sensitivity was 60-fold over that of the underivatized diol. Although it is not possible to claim this result alone means there are on-column losses of the underivatized form, we have found using the mass selective detector (Hewlett-Packard HP 5971 series) that a similar loss also occurs, indicating that the loss of diol occurs before detection (unpublished data).

Given that all of the cyclodiene residues could be detected with the chromatographic conditions described, except for the very polar diacid derivative of aldrin, it is apparent that lengthy derivatization procedures can be kept to a minimum in analyzing for environmental residues of the metabolites of aldrin and endosulfan. The limitation, however, of not derivatizing the diol compounds is that their limits of detection are greatly increased using the detection system described. The low sensitivity of the ECD detector to the other oxygenated aldrin compounds and the photoisomers could be further improved if necessary by derivatization of these compounds using silanizing or halogenating reagents.

Relevance to Microbiological and Aqueous Residue Analysis Studies. A consequence of the higher water solubility of many of the cyclodiene metabolites is that they can be more difficult to detect, in their underivatized forms, by the gas chromatographic technique. These metabolites generally have additional oxygen atoms, increasing their polarity and solubility in water and so reducing their volatility in the gas chromatograph and increasing interaction within the column system. These studies demonstrated that the liquid phase of a column requires an equilibration period with the metabolite to achieve consistent chromatography. Therefore, in microbial degradation studies where the parent cyclodienes are being detected, potential metabolites in their underivatized form may escape detection. Although likely to be detected after several injections, the metabolite would probably pass undetected if the column were not previously equilibrated with the compound. The appropriate standards therefore are necessary prior to any degradation study so that the chromatographic system can be properly equilibrated prior to any routine analyses.

CONCLUSION

The current study describes an efficient one-step method for extracting organochlorine compounds and their metabolites from small volumes of aqueous media. Although the extraction procedure described has been applied to the removal of cyclodiene organochlorines from aqueous phases, it may be equally applied to the extraction of other water-insoluble compounds. The extraction procedure may be coupled to a Pasteur pipet cleanup column method (Ahmad et al., 1988) to provide a simple and efficient system for the extraction of pesticides and their cleanup from relatively small aqueous samples prior to analysis.

The gas chromatographic configuration described allows the routine detection of endosulfan, aldrin, and their metabolites. Electron capture detection was linear over the working ranges of the dimethyl ester derivative of dihydrochlordenedicarboxylic acid, as well as all of the other underivatized compounds, indicating that they can be routinely analyzed, on a single packed column, without the need for capillary column technology, with minimal derivatization procedures.

A range of aqueous extraction procedures for removing chlorinated pesticides from microbial growth media have been developed for aldrin and endosulfan (Goebel et al., 1982; Schuenert, 1989, and references cited therein). These procedures, while effective, are time-consuming, often involving the use of a separating funnel with a range of different solvents with a series of evaporation and redissolving steps. The method described in the current study provides an attractive alternative in routine laboratory studies where small volumes of media are to be analyzed, as the recoveries are relatively high and the time required for each extraction is minimal (approximately 2 min). Solvent use was also kept to a minimum. Under the conditions described, the Mixxor was shown to be more reproducible, more convenient for multiple extractions, and therefore more suitable for routine use.

An advantage of the Mixxor was that effective phase separation was rapidly achieved for all of the extractions performed. This is a major improvement over the direct extraction of pesticides in the incubation vessels using a vortex device, where both phases may be difficult to separate when using a close ratio of solvent-to-extract volume. Such direct extraction methods, used in preliminary trials in the current study, did not warrant further investigation because of the relatively low extraction efficiency for the compounds tested and the erratic phase separation demonstrated after extraction.

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