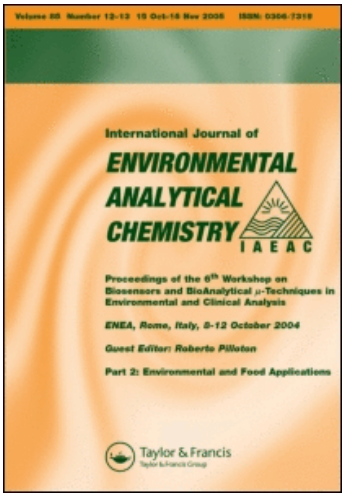

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B. G. Oliver[a]; K. D. Bothen[a]
[a] Organic Properties Section, Environmental Contaminants Division, National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ontario, Canada

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Extraction and Clean-Up Procedures for Measuring Chlorobenzenes in Sediments and Fish by Capillary Gas Chromatography

B. G. OLIVER and K. D. BOTHEN

Organic Properties Section, Environmental Contaminants Division, National Water Research Institute, Canada Centre for Inland Waters, P.O. Box 5050, Burlington, Ontario, Canada L7R 4A6

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A soxhlet extraction technique for recovering chlorobenzenes from sediments and fish is described. The extract is concentrated by evaporation, cleaned up with florisil (sediments) or a combination of alumina, silica gel, florisil and acidified silica gel (fish) before quantitation by capillary gas chromatography with an electron capture detector. Recoveries for all chlorobenzenes are greater than 80%. Detection limits of the technique are ~0.05 ng/g (ppb) for penta- and hexachlorobenzene, ~0.2 ppb for the tetrachlorobenzenes, ~0.4 ppb for the trichlorobenzenes, ~5 ppb for the dichlorobenzenes, and ~1500 ppb for monochlorobenzene.

INTRODUCTION

We have recently developed a simple capillary gas chromatographic method for determining chlorobenzenes, CB's, in water samples.[1] Our recent observations of CB concentrations in water samples in the Great Lakes Basin[2] showed that CB concentrations, except near point sources, were quite low (in most cases < 1 ng/l) and highly variable. We found that to properly assess environmental significance and persistence of these compounds we required information on CB concentrations in other compartments of the aquatic ecosystem. This paper reports extraction and clean-up procedures that can be used with the capillary gas chromatographic procedure developed in Reference 1 to quantify these widespread environmental contaminants[3,4,5,6] in sediments and fish.

Experimental

Reagents: High purity (99%) chlorobenzenes (Ultra Scientific Inc., Hope, R.I.), glass-distilled hexane, cyclohexane, and acetone (Caledon Ltd., Georgetown, Ontario) and Celite 545 (Fisher Scientific Co.) were used as received. The following materials were heat treated prior to use: Na_2SO_4 (BDH Analar), 650°C for 24 h; Fisher florisil (60–100 mesh), 650°C for 24 h; then deactivated with 6% H_2O; Fisher neutral alumina (80–200 mesh Brockman Activity 1), 800°C for 4 h then deactivated with 5% H_2O; and Fisher silica gel (60–200 mesh), 600°C for 24 h then deactivated with 5% H_2O. Sulfuric acid (40%) on silica gel, H_2SO_4/silica gel, as described by Lamparski *et al.*[7] was prepared by shaking 40 g of high purity sulfuric acid (BDH, Aristar) with 60 g of the heat treated silica gel until no clumping was observed.

Gas chromotographic procedure

A Varian 3700 gas chromatograph with a ^{63}Ni electron capture detector was used for this research. Three wall-coated open tubular (WCOT) glass capillary columns, 30 m in length, 1 mm o.d., 0.25 mm i.d., were used—Carbowax 20 M and SP2100 (Canadian Capillary Co.) and SP1000 (Supelco). Nitrogen flow rate through the columns was 1.3 ml/min. A spitless injection of 2 μl of sample in hexane (reproducibility ± 5%) was employed. The following gas chromatographic conditions were used: injector 250°C, detector 320°C, oven program—initial temperature 50°C, program rate 5°C/min, final temperature 190°C. The data was analyzed using a Spectra-Physics 4000 chromatograph data system.

Sediment extraction and clean-up

Thirty grams of wet sediment (10–15 g dry weight), in a glass thimble over 10 g of preextracted celite, was soxhlet extracted for 24 h with 300 ml of 41% hexane/59% acetone. The extract was back extracted with 1 l of distilled water to remove the acetone. The hexane extract is then filtered through 25 mm of Na_2SO_4 and evaporated to 10 ml using a round bottom flask equipped with a three stage Snyder condenser. Cleanup of this extract is accomplished by eluting 1 ml of extract with hexane from a column of 10 mm Na_2SO_4 + 40 mm deactivated florisil and collecting the first 1.6 ml in a calibrated vial. To remove sulfur interference the sample is then agitated with a few drops of triple distilled mercury with a vortex mixer. This procedure is repeated until the mercury remains shiny.

Fish extraction and clean-up

Fifteen grams of homogenized whole fish was ground with Na_2SO_4 until freeflowing and soxhlet extracted like the sediments. This extract was back extracted with water, dried with Na_2SO_4, then evaporated to 25–30 ml as described for the sediments. To remove most of the lipids, the extract was then eluted with hexane through a 20 mm o.d. column containing from top to bottom: 25 mm Na_2SO_4 (~12 g), 75 mm deactivated alumina (~30 g), 75 mm deactivated silica gel (~20 g) and 75 mm deactivated florisil (~12 g). The first 70 ml of eluate containing the CB's was collected from this column and this was again concentrated by evaporated to 10 ml as described earlier. (The next 80 ml hexane fraction from this column contains most of the polychlorinated biphenyls, PCB's.) Further clean-up of the sample from the remaining interfering lipids is accomplished by eluting 1 ml of the 10 ml 1st fraction extract concentrate with hexane from a column of 4 cm of H_2SO_4/silica gel in a disposable pasteur pipette and collecting 1.6 ml in a calibrated vial.

RESULTS AND DISCUSSION

After unsuccessful attempts to consistently recover chlorobenzenes from sediments using the ultrasonic extraction technique of Chau and Babjak,[8] we decided to employ the more exhaustive soxhlet extraction method. A mixture of the twelve chlorobenzenes was spiked into a clean Lake Superior sediment sample and the mixture was extracted and cleaned up

TABLE I

Recoveries of chlorobenzenes spiked into uncontaminated sediment ($n=4$)

Chlorobenzene	Amount added (ppb)	Mean recovery (%)	% C.V.
CB	24,000	80	10
1,3-DCB	130	92	8
1,4-DCB	160	97	8
1,2-DCB	120	98	9
1,3,5-TCB	12	93	8
1,2,4-TCB	15	94	7
1,2,3-TCB	13	94	9
1,2,3,5-TeCB	2.3	99	8
1,2,4,5-TeCB	16	95	9
1,2,3,4-TeCB	5.8	94	9
QCB	3.1	94	10
HCB	2.7	97	8

as described in the experimental section. Table I shows that 90% of all the CB's except monochlorobenzene were recovered by this method and that the percent coefficient of variation, % C.V., was better than 10%. Next a naturally contaminated sediment from Lake Ontario was soxhlet extracted for 72 h with solvent which was changed every 24 h. No observable increase in chlorobenzenes recovery was obtained by extending the extraction period beyond 24 h.

A large sample of wet surficial sediment from three Great Lake locations was subsampled in triplicate and extracted by the above procedure. Table II shows that the reproducibility of our analytical procedure is better than $\pm 10\%$ except when chlorobenzene concentrations in the sediment are near the detection limit. (Monochlorobenzene was below detection limits for these samples.)

A similar soxhlet extraction procedure was used for extraction of the chlorobenzenes from homogenized fish samples. Previous authors have employed florisil column chromatography,[9] silica gel and alumina column chromatography,[10] or alkaline hydrolysis[6] to clean-up extracts for chlorobenzene determinations on packed columns. We have adopted the H_2SO_4/silica gel reagent[7] for the removal of lipids from the chlorobenzene fraction and combined various portions of previous procedures to obtain an extract which will allow quantitation of chlorobenzenes by capillary column gas chromatography with electron capture detection. The change in lipid content of the extract was followed by gel permeation chromatography (GPC) using a variable wavelength ultraviolet detector

TABLE II

Mean chlorobenzene concentrations (ppb) and percent coefficient of variation of three naturally contaminated lake sediments ($n=3$)

Chlorobenzene Chlorobenzene	Sample #1		Sample #2		Sample # 3	
	M	%C.V.	M	%C.V.	M	%C.V.
1,3-DCB	175	10	14	7	2	23
1,4-DCB	141	5	100	9	21	10
1,2-DCB	19	10	45	5	4	14
1,3,5-TCB	257	5	3.5	7	0.8	30
1,2,4-TCB	124	4	26	10	8.4	7
1,2,3-TCB	17	5	1.3	9	0.4	21
1,2,3,5-TeCB	13	6	0.5	10	0.6	10
1,2,4,5-TeCB	113	10	9.4	6	2.1	9
1,2,3,4-TeCB	31	7	8.1	5	2.2	8
QCB	60	4	2.6	6	1.2	10
HCB	190	6	3.4	3	2.0	9

set at 230 nm. The column packing was BioBead S-X2[11] (swelled overnight in cyclohexane) and the elution solvent was cyclohexane (see Figure 1).

Our large Na_2SO_4, alumina, silica gel, florisil column reduces the lipid content of the lake trout fish extract ($\sim$100 mg lipid/ml hexane) by 99% (based on ultraviolet absorbance, Figure 1C). Color changes of the materials in the column seem to indicate that each material absorbs different lipid components. The alumina became light brown to orange in color, the silica gel became bright yellow and the florisil became pale blue. At this

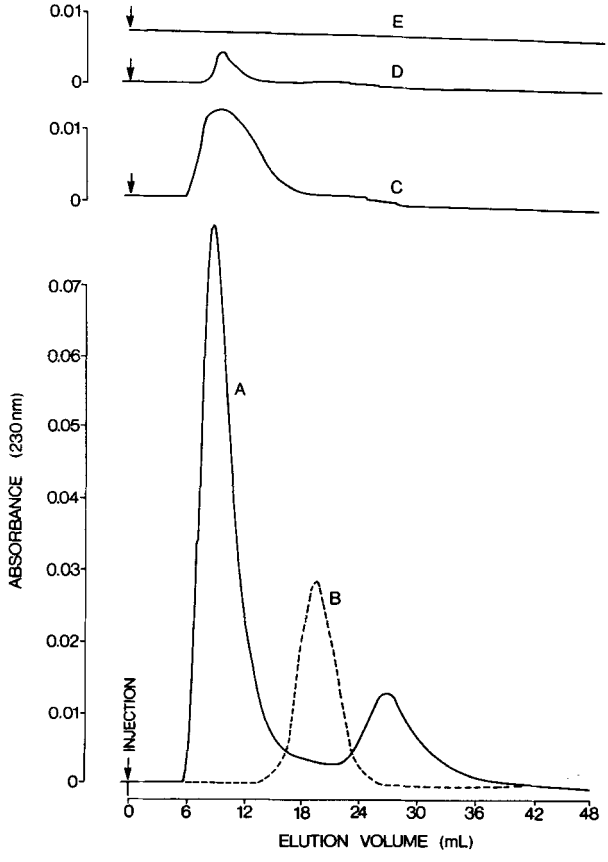

FIGURE 1 GPC CHROMATOGRAM ON Biobeads S-X2. Column: 250 mm long, 10 mm i.d., Detector: 230 nm; Solvent: cyclohexane, Flow rate: 1.5 ml/min; Injection volume: 100 μl. A: Lake trout hexane extract (1/40 dilution). B: 5 ppm 1,3 DCB + 5 ppm HCB in hexane. C: Lake trout extract after 4-stage cleanup column (C.C. extract). D: C.C. extract after alkaline hydrolysis. E. C.C. extract after H_2SO_4/silica gel column.

stage the column cleaned (C.C.) extract was not sufficiently clean for capillary gas chromatography.

Alkaline hydrolysis of 1 ml of the C.C. extract with 1 ml of 2% ethanolic KOH for 2 h at 95°C[6] reduced the lipid content by a further 90% (Figure 1D). Even after this treatment there was still sufficient lipid in the extract to severely affect the quantitation of the chlorobenzenes by capillary gas chromatography. The alkaline hydrolysis step was then replaced by the H_2SO_4/silica gel column step (experimental) to polish the C.C. extract. Figure 1E shows that this step essentially removed all the remaining ultraviolet absorbing materials in the C.C. extract and subsequent injections of this extract on the capillary system showed a nearly clean chromatogram except for the chlorobenzenes and related halogenated compounds.

A homogenized lake trout (10.5% lipid) from a fish hatchery was spiked with chlorobenzenes and extracted in triplicate together with an unspiked sample to determine the recovery efficiency of the method. The results of this experiment are shown in Table III. The recoveries for all the chlorobenzenes (corrected for background) are at least 80% with a % C.V. of better than 10%.

Four lake trout samples from diverse locations in the Great Lakes Basin were analyzed in duplicate by the method. The reproducibility of the analyses for each sample was ±10%.

TABLE III

Mean recovery of chlorobenzenes added to homogenized lake trout sample ($n=3$)

Chlorobenzene	Added (ppb)	Mean recovery (%)	% C.V.
CB	7500	80	8
1,3-DCB	85	82	6
1,4-DCB	139	82	4
1,2-DCB	90	80	5
1,3,5-TCB	15	87	5
1,2,4-TCB	12	80	5
1,2,3-TCB	8	80	4
1,2,3,5-TeCB	7	88	4
1,2,4,5-TeCB	9	92	3
1,2,3,4-TeCB	7	82	2
QCB	2	83	4
HCB	1	89	9

Chromatograms for a typical sediment and fish extract on a SP1000 column are shown in Figures 2A and 2B, respectively. The chromatograms are seen to be clear of any major interfering components. All samples are run on three columns to confirm peak identities and areas. This is required since the 1,2,4,5-TeCB and 1,2,3,5-TeCB coelute on the SP2100 column, and 1,3,5-TCB and hexachlorobutadiene (present in many of the samples) coelute on the Carbowax 20 M column.

We have already reported the gas chromatographic response factors for the chlorobenzenes.[1] Technique detection limits for sediments and fish (15 g sample) based on the least sensitive chlorobenzene containing the same number of chlorines are: monochlorobenzene, 1500 ng/g (ppb);

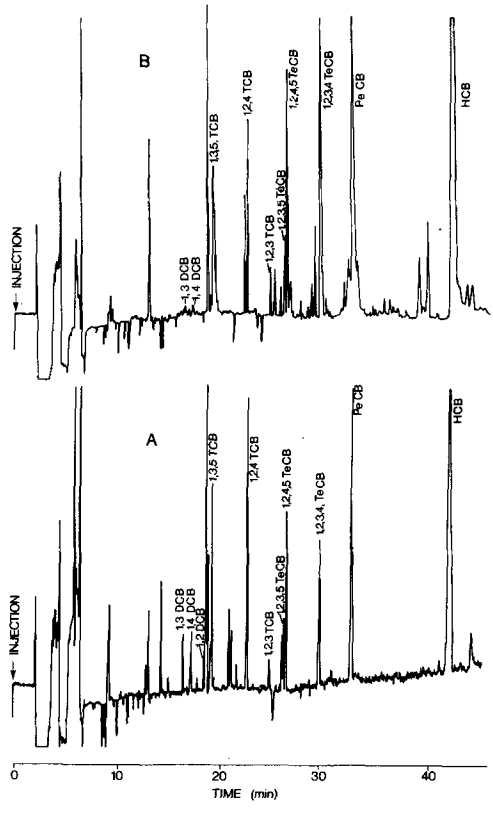

FIGURE 2 A: Chromatogram of a typical sediment extract on SP1000 capillary column (G.C. conditions in experimental). B: Chromatogram of a typical fish extract on SP1000 capillary column (G.C. conditions in experimental).

dichlorobenzenes, 5 ppb; trichlorobenzenes, 0.4 ppb; tetrachlorobenzenes, 0.2 ppb; pentachlorobenzene, 0.06 ppb; hexachlorobenzene, 0.05 ppb. Due to the poor response of the electron capture detector to monochlorobenzene the technique is not sensitive enough to measure the concentration of this compound in environmental samples. This compound is recovered as well as the other chlorobenzenes by the methods described, but a gas chromatographic detector more sensitive to this compound (possibly the photoionization detector)[12] should be used for quantitations in this case.

If samples contain low concentrations of chlorobenzenes or if only a limited quantity of sample is available, the detection limits can be reduced by at least a factor of 10 with minimal evaporative losses ($< 10\%$). This is accomplished by reducing the extract volume (prior to final clean-up) to 1 ml or less instead of 10 ml by the technique previously described.[1]

To date we analyzed over 100 sediment samples from diverse locations and have not encountered any difficulties with the method. Of the ~ 75 fish we have analyzed so far, we have encountered some problems in samples containing high concentrations of PCB's and/or organochlorinated pesticides. These compounds elute from the GLC column after the chlorobenzenes and, therefore, can interfere with subsequent analyses. This problem can be minimized by programming the capillary column to higher temperatures and waiting until these compounds are eluted.

Another approach which works well is to separate the chlorobenzenes from most of the common organochlorinated pesticides using 10% $AgNO_3$ on silica as described by Needham *et al.*[13] After this clean-up the extract still contains some the PCB's and most of the mirex in the original sample. For samples containing high concentrations of PCB's we now routinely collect the first 150 ml fraction off the lipid removal column, concentrate this extract to an appropriate volume. Clean up the extract on H_2SO_4/silica gel + $AgNO_3$/silica gel, then run a complete chlorobenzene, PCB, and mirex gas chromatographic scan.

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