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Alternative Tissue Analysis Method Developed for Organochlorine Contaminants in Aquatic Organisms

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The exposure of aquatic life to organochlorine contaminants has been investigated during the past two decades because of human and ecosystem health concerns related to the bioaccumulation of hazardous, lipophilic substances. The toxic effects of polychlorodibenzo-p-dioxins and polychlorinated biphenyls (PCBs) are well known, and recent evidence also suggests that low level exposure to lipophilic organochlorines may interfere with normal development during sensitive early life history stages (Hileman 1993). As the use of lipophilic organochlorines, such as DDT, in third world countries continues and with the purported global cycling (Bidleman and Olney 1974; Tanabe et al. 1983) and food chain accumulation (Thomann and Connoly 1984; Thomann 1989) of persistent organochlorines, the occurrence of these compounds in aquatic organisms is a critical global environmental issue. An understanding of the fate of organochlorines in the environment clearly remains an extremely important subject related to water quality.

The U.S. Geological Survey (USGS) has recently gained congressional approval in the United States to track nation wide trends in water quality through the establishment of the National Water Quality Assessment Program (NAWQA). Among the goals defined by NAWQA, aquatic organisms, including fish, shellfish, and plants, collected from major drainage basins will be analyzed for, along with other contaminants, polychlorinated biphenyls (PCBs), organochlorine insecticides, and chlorobenzenes (Crawford and Luoma 1992). The purpose of this report is to present quality assurance data obtained from the development of a PCB, chlorobenzene, and organochlorine insecticide tissue analysis method in support of NAWQA and other large-scale water quality programs conducted through our laboratory.

MATERIALS AND METHODS

Individual organochlorine insecticides, chlorobenzenes, and Aroclors 1242, 1254, and 1260 were obtained from the U.S. EPA Pesticides and Industrial Chemicals

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Repository (Research Triangle Park, NC). The Aroclors were mixed together in a 1:1:1 weight ratio to prepare tissue spike and GC calibration standards. Silica gel (60-200 mesh), neutral alumina (80-200 mesh) and granular anhydrous sodium sulfate were acquired from Fisher Scientific (Fair Lawn, NJ). Bio-Beads SX-3 (200-400 mesh) were purchased from Bio-Rad Laboratory (Richmond, CA). All solvents used were of high purity (Burdick and Jackson, Muskegon, MI).

The tissue matrices were derived from shucked, whole-body soft-shell clams (Mya arenaria) and submerged aquatic plants (Hydrilla verticillata). Samples of Hydrilla were lyophilized (VirTis Unitrap II, VirTis Company, Gardiner, NY) prior to extraction to enable a large enough mass of plant tissue to be analyzed, while Mya samples were only surface dried prior to the onset of sample preparation. A 6 to 10 g sample of clam tissue or 4 g sample of plant tissue was homogenized in a Waring blender and subsequently mixed with either 60 g (clam tissue) or 12 g (plant tissue) of granular anhydrous sodium sulfate in a mortar and pestle. The dry tissue mixture was Soxhlet extracted with 200 mL of dichloromethane (DCM) for 20 hr. The DCM extract was reduced to a volume of ca. 2.5 mL using rotary flash evaporation, and a 0.5-mL aliquot was removed and air dried to measure the total extractable lipid content gravimetrically.

Gel permeation chromatography (GPC) was accomplished using a lab-built system consisting of a metering pump (Model RP-SY-1CKC, Fluid Metering Inc., Oyster Bay, NY), Rheodyne Model R6031 SV six-way injection valve (Valco Instruments Co. Inc., Houston, TX) with 5-mL sample loop, a 2.5-cm (id) X 50-cm glass chromatography column (Pharmacia Fine Chemicals, Uppsala, Sweden), and Schoeffel variable-wavelength UV detector (Model SF 770 Spectroflow, Schoeffel Instrument Corp., Westwood, NJ). The GPC column was packed with a 37-cm bed height of Bio-Beads. DCM was used as the mobile phase at a flow rate of ca. 2 mL/min. The UV detector monitored the GPC eluents at a fixed wavelength of 254 nm. The eluents collected from GPC were concentrated by using rotary flash evaporation and nitrogen gas blowdown to a final volume of ca. 1 mL and subjected to alumina/silica fractionation.

Fractionation was performed in 25-mL polypropylene solid-phase extraction (SPE) cartridges (Varian, Harbor City, CA) that were fitted with 25-mm ANOTOP PLUS filters (0.2 μm pore size, Alltech Assoc., Deerfield, IL) to retain the sorbents. Attached to the filters were PTFE Teflon on/off valves (Burdick and Jackson) to regulate solvent flow. The fractionation cartridges were loaded with 2 g of granular anhydrous sodium sulfate, 3 g of silica gel, previously activated at 130 °C for 12 hr, 6 g of neutral alumina (2.0% wt/wt water deactivated), previously activated at 500 °C for 12 hr, and 4 g of granular anhydrous sodium sulfate. The SPE cartridges were eluted under nitrogen gas head pressure at a rate of ca. 15 mL/min with 45 mL of n-hexane followed by 45 mL of DCM providing two fractions. Each fraction was volume-reduced as described previously, and the DCM fraction was solvent exchanged with hexane prior to analysis by using high-resolution gas chromatography.

A Hewlett-Packard (HP) Model 5890 Series II gas chromatograph (GC) equipped with a ^{63}Ni electron capture detector and a split-splitless injector inlet was used in the analysis. The GC was fitted with a 0.25-mm (id) X 30-mm (0.25-μm film thickness) Rt_x-5 (Restek, Bellefonte, PA) fused-silica capillary column. Two GC temperature-programmed separations were used in the analysis. The GC oven in the first separation (S_1) was programmed from 85 °C (1 min hold) to 260 °C at 2 °C/min with a final hold of 0.25 min at 260 °C, then from 260 °C to 285 °C at 10 °C/min and a final hold at 285 °C for 2.5 min (94 min program). In the second separation (S_2), the GC oven was programmed 85 °C (1 min hold) to 285 °C at 4 °C/min with a final hold of 10 min at 285 °C (61 min program). In all separations, the injector and detector temperatures were maintained at 250 °C and 300 °C, respectively. Helium was used as the GC carrier gas, and the detector make-up gas consisted of a mixture of 95% argon and 5% methane.

Internal injection standards were used for analyte quantitation. PCB congener number 204 -- PCB congener designations in this report conform to the classification scheme of Ballschmitter and Zell (1982) -- was used for the quantitation of PCBs and chlorobenzenes, and isodrin was used for the quantitation of the organochlorine insecticides (Note: isodrin elutes completely with n-hexane during fractionation and native isodrin does not interfere with quantitation.) Internal injection standards were added to the sample extracts immediately prior to GC analysis. An HP Model 3396A integrator was used for recording chromatograms, and the report files generated from integration were uploaded via HP 3396A file server software (v1.2) to an HP Vectra QS/20 computer. GC report files on the Vectra computer were imported into Quattro Pro (v.2) spreadsheets (Borland, Scotts Valley, CA) to calculate sample concentrations.

Quality assurance evaluation of the tissue analysis method was accomplished by spiking the dry tissue mixture with a DCM solution containing the analytes for both clam and plant preparations. The spike concentrations were 0.5 mg/kg (high spike) and 0.2 mg/kg (low spike) for total PCBs, and 0.1 mg/kg (high) and 0.02 mg/kg (low) for the individual organochlorine insecticides and chlorobenzenes. Plant tissues were spiked at just one concentration of chlorobenzenes, PCBs and organochlorine insecticides (low spike), and only a subset of the organochlorine insecticides amended to the clam tissues were spiked in the plant tissues. The recoveries of the target analytes were determined by subtracting from the measured amount the level of each analyte present in non-spiked samples. Tissue blanks were analyzed by adding 1.0 mL of DCM in place of the spiking standard followed by the procedures used for standard tissue analysis.

RESULTS AND DISCUSSION

The relative retention times of individual analytes, calculated from the retention time of the analyte divided by the retention time of the isodrin or PCB-204 internal standard, were used to identify analyte peaks in the chromatograms for

quantitative analysis. PCBs, chlorobenzenes, aldrin, heptachlor, 4,4'-DDE, and mirex were analyzed by using the S_1 GC program, and the remaining analytes were analyzed with the S_2 GC program.

Pentachlorobenzene, hexachlorobenzene, heptachlor, aldrin, 4,4'-DDE, mirex, and all of the PCB congeners eluted quantitatively in the n-hexane fraction, while the remaining organochlorine insecticides eluted in the DCM fraction. trans-Nonachlor eluted partially (30%) in the n-hexane fraction, and 4,4'-DDE eluted partially in the DCM fraction (20%); these two pesticides were the only examples of incomplete fractionation. Co-chromatography in GC analysis existed for mirex and PCB congener 170 and 4,4'-DDE and PCB congener 85, as well as for several of the PCB congeners. Co-elution of PCB congeners has been previously reported by Shulz et al. (1989) and Eganhouse et al. (1989) from comprehensive chromatographic characterizations of Aroclor mixtures. Identical co-elution patterns for PCBs were observed in this study. In the case of co-elution, individual PCB congener recoveries could not be determined accurately and a total mass recovery for all of the congeners in a single peak was determined. No co-eluting compounds were present in the DCM fraction.

Recoveries and percent relative standard deviations (%RSD) of the organochlorine pesticides, chlorobenzenes, and PCBs from spiked tissues are listed in Table 1. Only the n-hexane fraction of the low spike in clam tissues was analyzed. The low recoveries of trans-nonachlor and 4,4'-DDT in the high spike (Table 1) can be attributed in part to the partial elution of these two pesticides in fractionation as mentioned previously, and, in addition, it was difficult to detect low levels of these pesticides in either the n-hexane or DCM fractions. The low recovery of DDT is likely due its instability in the GC after injection. It has been observed that DDT response factors relative to isodrin varied from 0.0755 to 0.2467 from calibrations conducted throughout this study. The area of isodrin peaks remained relatively constant through time in both the calibration standards and sample extracts; therefore, this type of behavior indicates that DDT can be subject to extensive breakdown during GC analysis. Recoveries were quite similar between the two matrices for five of the six organochlorine insecticides, while dieldrin recoveries were markedly higher in plant tissues (Table 1). Total-PCB recoveries were higher in clam relative to plant tissues.

Individual PCB congener recoveries from spiked clam and plant tissues are shown in Fig. 1. Because PCB recoveries from clam tissues at both spike levels were nearly identical, results from both spike levels were averaged for the congener data shown in Fig. 1A. Peak designations shown in Fig. 1 are listed in Table 2 for both matrices. The majority of PCB congeners were recovered above the 80% level in clam tissues and all of the 65 congeners were detected (Fig. 1A). Only PCB-22/51, -37/42/59, and -170/190 were recovered with less than 70% efficiency from spiked clam tissues (Fig. 1A), and %RSDs in most cases were $<10\%$ except for PCB-16/32, -74, -99, -196, and -201. Recoveries of PCB congeners from spiked plant tissue (Fig. 1B) were generally less precise with

Table 1. Recoveries of organochlorines from spiked tissues.

Analyte	Soft-Shell Clams		Plants
No. of replicates	6	4	3
Spike levels, mg/kg[1]	0.5/0.1	0.2/0.02	0.2/0.02
	%Rec (%RSD)[2]		
Chlorobenzenes			
Pentachlorobenzene	72 (6)	89 (2)	na
Hexachlorobenzene	75 (6)	50 (4)	na
Insecticides & PCBs			
α-HCH[3]	73 (5)	na	na
β-HCH	110 (4)	na	na
γ-HCH (Lindane)	77 (7)	na	na
Heptachlor	71 (5)	70 (2)	na
Aldrin	100 (4)	108 (7)	105 (2)
Oxychlordane	82 (11)	na	84 (22)
γ-Chlordane	78 (10)	na	68 (18)
α-Chlordane	69 (12)	na	64 (27)
trans-Nonachlor	44 (8)	na	na
4,4′-DDE	46 (4)	67 (8)	na
Dieldrin	87 (7)	na	106 (11)
Endrin	88 (7)	na	na
4,4′-DDD	99 (11)	na	na
4,4′-DDT	49 (5)	na	35 (18)
Methoxychlor	90 (7)	na	na
Mirex	65 (9)	62 (5)	na
ΣPCBs[4]	88 (3)	82 (8)	71 (15)

[1]Spike levels listed as PCBs/(chlorobenzenes & pesticides); [2]%Rec is the percent recovery of spiked analyte and %RSD is percent relative standard deviation; [3]HCH=hexachlorocyclohexane; [4]ΣPCBs is the sum of 65 congeners in clam tissue analysis and 112 congeners in plant tissue analysis; na=not analyzed.

%RSDs >15% in many cases, although more congeners were analyzed in these samples. The minor congeners had the largest %RSDs in recoveries from plant tissues. PCB-4/10, -40, -67, -63, -119, -134, and -114/122/131 were not detected in spiked plant tissues, but all of these represented minor constituents in the PCB mixture. Negative recoveries were observed for PCB-99 and -85 due to the subtraction of spiked levels from relatively high concentrations of native PCBs and 4,4′-DDE in plant tissues (Fig. 1B). Greater variability in PCB congener recoveries was observed for the low molecular weight PCBs in plant tissues, while the higher molecular weight congeners showed recoveries converging near 70% in this matrix.

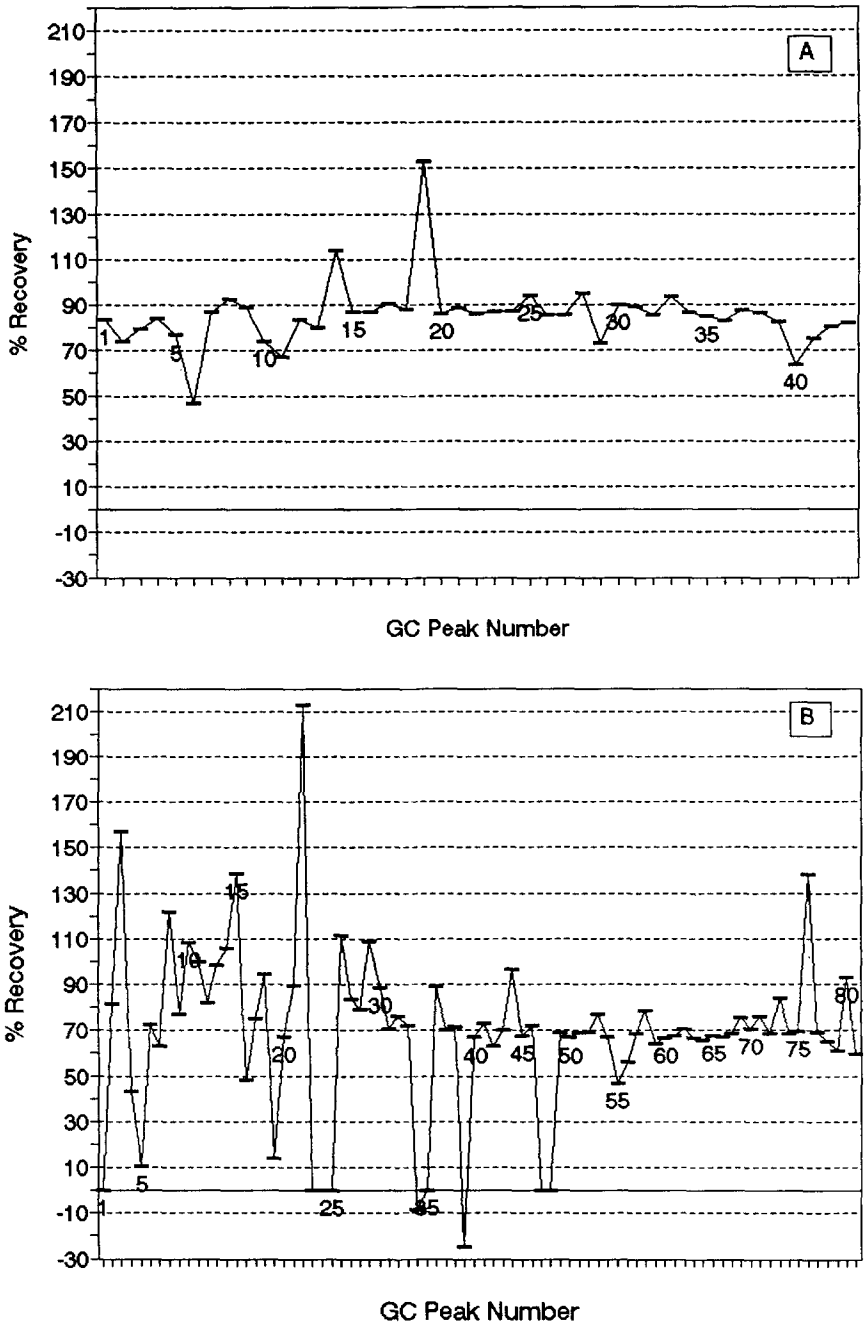

Figure 1. Percent recoveries of PCB congeners from spiked tissues: (A) average of high and low spikes in clam tissues; (B) average of low spikes in plant tissues. Congeners are denoted by GC peak numbers (every fifth peak) listed in Table 2.

Table 2. Correlation table for PCB congener recoveries shown in Fig. 1. GC peak numbers (GC) are listed with corresponding congener designations.

	Matrix			Matrix			Matrix
GC	Clam	Plant	GC	Clam	Plant	GC	Plant
	Congeners			Congeners			Congeners
1	18	4,10	31	138	84	61	128
2	15,17	7,9	32	178	90	62	185
3	16,32	6	33	187	101	63	174
4	28,31	5,8	34	183	99	64	177
5	33,53	19	35	128	119	65	156,171
6	22,51	18	36	185	83	66	172
7	52	15,17	37	174	97	67	180
8	49	27	38	177	87,115	68	193
9	47,75	16,32	39	180	85	69	191
10	44	26	40	170,190	136	70	199
11	37,42,59	25	41	198	77,110	71	170,190
12	41,64	28,31	42	196,203	82,151	72	198
13	40	33,53	43	194	135	73	201
14	74	22	44	end	107	74	196,203
15	70	45	45		149	75	189
16	66,95	46	46		118	76	195,208
17	56,60	52	47		134	77	207
18	101	49	48		114,122,131	78	194
19	99	47,75	49		146	79	205
20	97	44	50		153,132	80	206
21	87,115	37,42,59	51		105		end
22	136	41,64	52		141,179		
23	77,110	40	53		176		
24	82,151	67	54		137		
25	135	63	55		138		
26	149	74	56		158		
27	118	70	57		178		
28	146	66,95	58		175		
29	153,132	91	59		187		
30	141,179	56,60	60		183		

The tissue method developed in this study is similar to previously reported methods for organic contaminants (Pellezarri 1985; Birkholz et al. 1988; Eganhouse et al. 1989; Draper and Kozdin 1991) and is suitable for the analysis of PCB congeners, organochlorine insecticides, and chlorobenzenes (i.e., penta- and hexachlorobenzene). A modification relative to other procedures has been made by including custom SPE cartridges with elution under nitrogen gas head pressure to achieve rapid analyte fractionation.

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