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Automated HPLC Fractionation of PCDDs and PCDFs and Planar and Nonplanar PCBs on C_{18}-Dispersed PX-21 Carbon

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The separation of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and a number of specific di-, mono-, and non-*ortho,-ortho'*-chlorine (*o,o'*-Cl) substituted chlorobiphenyl congeners (PCBs) of toxicological significance from bulk PCBs and other interfering residues is critical for accurate quantitation of these compounds. Activated carbon has long been the most commonly used adsorbent for separation of these compounds into fractions suitable for analysis by capillary GC/ECD and by GC/MS. We have developed a procedure that uses HPLC columns containing PX-21 activated carbon dispersed by C_{18} (octadecylsilane) sorbent in combination with an automated, quaternary HPLC autoinjector-fraction collector apparatus to attain these separative objectives. Our system produces four discrete fractions containing bulk PCBs (di- to tetra-*o,o'*-chlorosubstituted); mono-*o,o'*-Cl PCBs; non-*o,o'*-Cl PCBs; and PCDDs/PCDFs. The fractionations are achieved with reduced requirements for manpower, and our system provides flexibility toward easy customization to meet specific analytical needs.

Introduction

The increased concern about the environmental consequences of widespread PCDD/PCDF and PCB contamination has focused attention on the expedient separation and subsequent enrichment of these compounds from biological matrices and co-contaminants. Special attention has been focused on a few select PCB congeners that are stereochemically similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and that also have toxicological properties similar to 2,3,7,8-TCDD (*1*). These congeners are potent inducers of liver microsomal aryl hydrocarbon hydroxylase (AHH) and are thus referred to as AHH-active or dioxin-like PCBs (*2–4*). The AHH-active PCB congeners are further classified as to the number of chlorines in the *ortho,ortho'*-position (*5*): (a) non-*o,o'*-Cl (IUPAC Nos. 81, 77, 126, and 169); (b) mono-*o,o'*-Cl (IUPAC Nos. 123, 118, 114, 105, 167, 156, 157, and 189); and (c) di-*o,o'*-Cl (IUPAC Nos. 138, 158, 166, 128, and 170). Collectively, the PCDDs, PCDFs, and these dioxin-like PCBs, which elicit their toxic affects through a common receptor-mediated mode of action, are known as planar halogenated hydrocarbons (PHHs) (*6*).

Isolation and enrichment of PHHs from animal tissue is complicated by the presence of co-extracted, halogenated compounds that can interfere with gas chromatographic analysis by electron capture detection (GC/ECD) or by mass spectrometry (GC/MS). The interferents are typically present in greater amounts than the PHHs (*7–9*). Historically, activated carbon has been the primary adsorbent used to fractionate and enrich PHHs (*10*). Certain activated carbons are well suited for separation of PHHs on the bases of their planarities and degrees of chlorination (*11*). Jensen and Sundström first reported the use of activated carbon to separate PCBs on the basis of the degree of *o,o'*-chlorination (*12*). Smith *et al.* (*7*), Smith (*13*), and Stalling *et al.* (*14*) reported refinements of carbon chromatography for isolation of PHHs, and their methods became the standard for many laboratories (*15–17*). Recent articles describe carbon dispersed on silica gel (*18, 19*) or glass fibers (*20, 21*) in closed columns that allow further conservation of solvent by reversal of the column flow to collect the more strongly adsorbed PHHs.

Three recent developments for fractionating PHHs are (a) porous graphitic carbon (PGC) (*22*); (b) 2-(1-pyrenyl)-ethyldimethylsilylated silica gel (PYE) (*23*); and (c) C_{60}/C_{70} fullerenes bonded to polystyrene divinylbenzene ($C_{60/70}$-PSDVB) (*24*). The three adsorbents, PGC, PYE, and $C_{60/70}$-PSDVB, have similar elution characteristics for PCBs and PCDDs/PCDFs to activated carbon and require less solvent volume and strength, but they are costly, have lower sample capacities, or require samples to be almost completely devoid of lipids and co-extractants (*23, 25, 26*).

Our goal was to develop and characterize a comprehensive automated method for the fractionation and enrichment of PHHs from environmental samples that did not suffer from the described disadvantages. To achieve this, we chose to exploit the demonstrated propensity of PHHs to adsorb to activated carbon in proportion to their planarities (and generally in proportion to their toxicities)

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by further refining the carbon fractionation concept (*1*–*5*, *7*). This fractionation procedure assists not only analytical analysis but may be employed in H4IIE bioassay studies (*6*). The specific objectives were to develop a carbon chromatography system having the following advantages: (1) produces virtually complete resolution of bulk PCBs, mono-*o,o'*-Cl PCBs, non-*o,o'*-Cl PCBs, and PCDDs/PCDFs into four discrete fractions; (2) has a carbon column adaptable to automation with existing high-performance liquid chromatography (HPLC) equipment; and (3) has a column that is inexpensive, is easily packed, and can be tailored to the desired sample loading. The approach taken in these studies was to use sieved, activated carbon (Amoco PX-21) dispersed with C_{18}. [AX-21 (Anderson Development Co.) is more widely available than PX-21 and has been shown to have similar chromatographic properties (*27*, *28*).] Octadecylsilane is a sorbent whose properties lend itself to use as a dispersant due to its rapid equilibration to new solvent conditions, rigid particles that withstand HPLC back-pressure, and essential nonselectivity and nonadsorptivity with the solvents used for elution of PCBs and PCDDs/PCDFs from carbon.

Materials and Instrumentation

Materials. (a) Na_2SO_4: sodium sulfate, calcined and anhydrous. (b) SG: silica gel 60 (70–230 mesh, EM Science). (c) SA/SG: 40/60 (w/w) sulfuric acid/silica gel 60. (d) Coarse SA/SG: 30/70 (w/w) sulfuric acid/Davisil grade 635 (60–100 mesh, Fisher Scientific). (e) KS: potassium silicate prepared with methanolic KOH and silica gel 60 (*7*). (f) Amoco PX-21: activated carbon (2–10 μm) dispersed on Lichroprep RP-18 (15–25 μm, EM Science). (g) Basic Alumina: activity grade I, (60–325 mesh, Fisher Scientific) activated at 200 °C.

Instrumentation. For those fractions analyzed by GC/ECD, we used a Hewlett-Packard 5890A Series II GC equipped with a ^{63}Ni electron capture detector and a HP 7673 autosampler. The detector temperature was 330 °C, and the injector temperature was set to follow the oven (Oven Track). Carbon fractions were introduced by automated cool on-column injection onto a 1 m × 0.53 mm (i.d.) deactivated fused silica retention gap (J&W Scientific Inc.). Bulk PCB fractions were analyzed on a 60 m × 0.25 mm (i.d.) fused silica capillary column with a 0.25-μm film thickness DB-5 bonded phase (J&W Scientific Inc.), with H_2 carrier gas pressure maintained at 25 psi. The oven temperature program was 40 °C, 3 °C/min to 120 °C, 1 °C/min to 260 °C (2 min), and then 10 °C/min to 320 °C (1 min). Mono-*o,o'*-Cl PCB fractions were analyzed on a 30 m × 0.25 mm (i.d.) fused silica capillary column with a 0.25-μm film thickness DB-1 bonded phase, with H_2 carrier gas pressure maintained at 12 psi. The oven temperature program was 60 °C, 10 °C/min to 140 °C, 2 °C/min to 240 °C, and then 10 °C/min to 320 °C (2 min).

GC/high-resolution MS (GC/HRMS) (HP 5890A/VG 70-250S) was used to analyze the fractions containing non-*o,o'*-Cl PCBs; the fractions containing Cl_{4-8} PCDFs and PCDDs were analyzed by either GC/HRMS or GC/quadrupole MS (HP 5890 Series II/Finnigan 4023). An HP 7673 autosampler injected the final enriched extract onto a 2.5 m × 0.53 mm deactivated fused silica retention gap via cool on-column injection or direct injection into a heated (285 °C) Restek cyclouniliner. A DB-1 column (30 m × 0.25 mm i.d.) of 0.25-μm film thickness was used to chromatograph non-*o,o'*-Cl PCBs using a temperature program of 120 °C (1 min), 3 °C/min to 250 °C, and then 6 °C/min to 300 °C (5 min). PCDDs/PCDFs were chromatographed on DB-5 columns (40 or 60 m × 0.25 mm i.d.) of 0.25-μm film thickness heated from 120 °C (1 min), 20 °C/min to 210 °C, and then 3 °C/min to 300 °C (15 min). Helium carrier gas flowed through the columns at a linear velocity of 25–35 cm/s. The columns were connected to the MS by a 1–2 m × 0.25 mm (i.d.) deactivated fused silica transfer line maintained at 300 °C.

The VG 70-250S GC/HRMS was operated at 10 000 resolution with perfluorodecalin lock masses for selected ion monitoring (SIM) of non-*o,o'*-Cl PCBs in two groups and with perfluorotetradecahydrophenanthrene lock masses for SIM of PCDFs and PCDDs in five groups. Two or three isotopic molecular ions were monitored for each analyte. Additional ions were used to monitor for potential interferences, e.g., halogenated diphenyl ethers in PCDF and PCDD analyses and co-eluting nonplanar PCB congeners in non-*o,o'*-Cl PCB analyses. Quantitation was performed using wide-range calibration response curves of native vs ^{13}C-labeled forms of all the targeted compounds except for PCB congener 81. The determined concentrations were inherently self-corrected for lack of full recovery through use of the ^{13}C-labeled surrogates that had been fortified into the samples just prior to extraction.

Method

Sample Preparation and Extraction. Portions of homogenized tissue (50 g wet weight) or sediment (50 g air dried) were blended with four times their weights of Na_2SO_4. Samples were spiked at this point with selected ^{13}C-labeled PCB and PCDD/PCDF congeners (see below), then samples were extracted in 4-cm (i.d.) glass chromatography columns with 700 mL of CH_2Cl_2 (Figure 1, step A). Extracts were concentrated, and lipid percentages for tissue extracts were determined gravimetrically with small aliquots from each extract.

Reactive Cleanup. Two reactive columns were used in sequence to remove biogenic materials from tissue extracts; only the second of the two columns was used for sediments (Figure 1, steps B and C). The initial reactive column (4 cm i.d.) contained the following materials, from bottom to top: a 1-cm segment of Na_2SO_4; 25 mL of KS; 25 mL of SA/SG; a 1-cm segment of Na_2SO_4; and finally, 50 mL of coarse SA/SG. All adsorbents were stored at room temperature prior to use, with the exception of the KS, which was stored at 130 °C. The adsorbents were measured in graduated cylinders, poured into the columns, and settled by gentle tapping to achieve level top surfaces. The adsorbents were then prewashed with 100 mL of CH_2Cl_2. The tissue extracts were applied to the columns, and the adsorbents were washed with 150 mL of CH_2Cl_2. (The initial reactive column can only accommodate 5 g of total lipid; extracts exceeding this value were split between multiple columns.) The eluates were concentrated by rotary evaporation and transferred to isooctane.

The second reactive column (1 cm i.d.) contained the following materials, from bottom to top: a 1-cm segment of Na_2SO_4; 10 mL of SG; 3 mL of KS; 5 mL of SA/SG; and finally, a 1-cm segment of Na_2SO_4. The adsorbents were prewashed with 25 mL of 3% (v/v) CH_2Cl_2 in hexane. The eluates from the first reactive cleanup were applied to the second columns, and the analytes were eluted with 70 mL

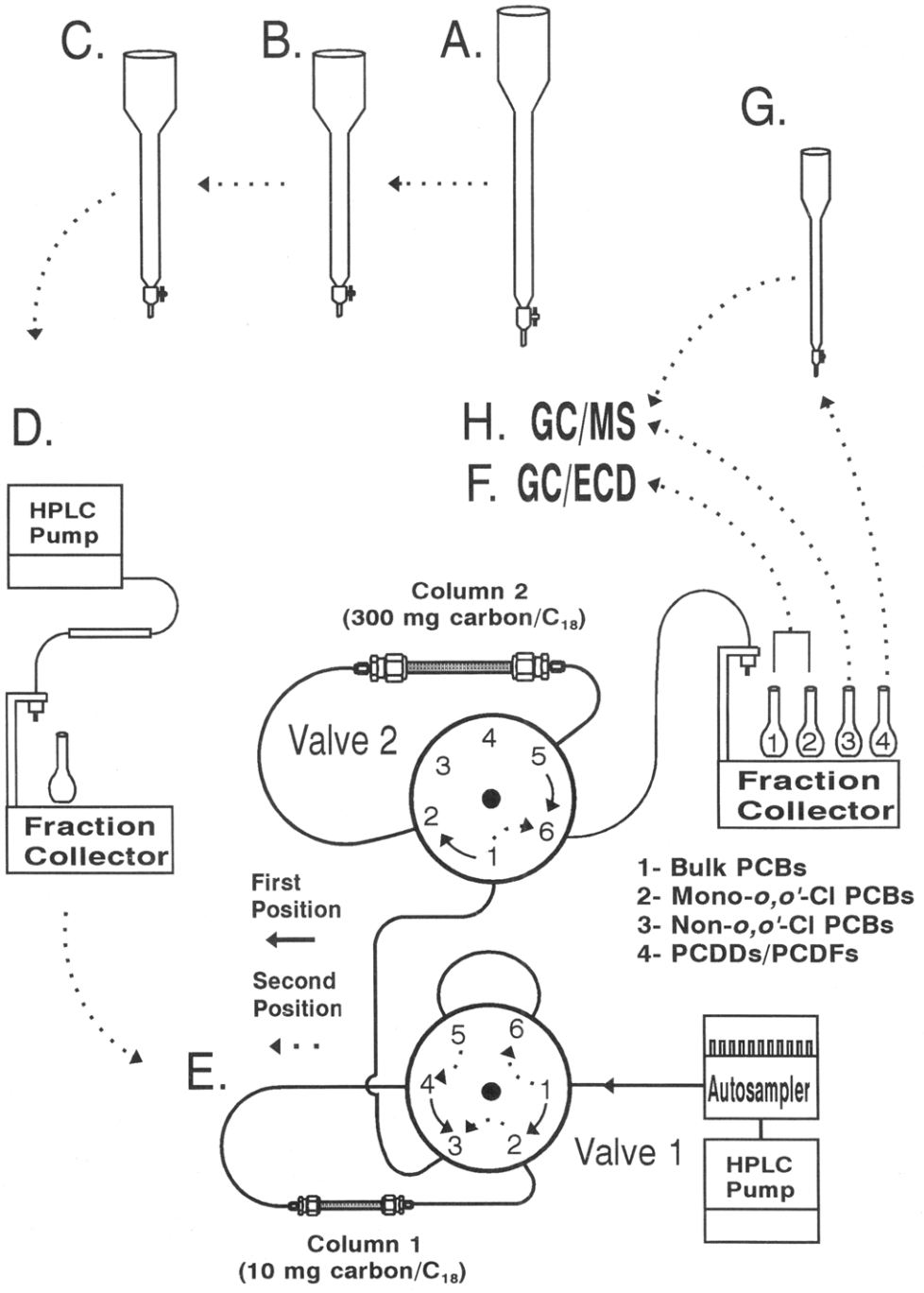

FIGURE 1. Representation of the enrichment, fractionation, and analytical scheme. (A) Extraction of tissue/Na_2SO_4 with CH_2Cl_2; (B) first reactive cleanup using KS and SA/SG; (C) Second reactive cleanup using SG, KS, and SA/SG; (D) automated SEC; (E) valve configuration and positioning for collection of the four PCB fractions collected from carbon/C_{18} column(s); (F) bulk PCBs and mono-*o,o'*-Cl PCB fractions are analyzed by GC/ECD; (G) PCDD/PCDF fraction cleaned up on alumina; (H) analysis of non-*o,o'*-Cl PCB and PCDD/PCDF fractions on GC/MS.

of 3% (v/v) CH_2Cl_2 in hexane. The eluates were concentrated after the addition of isooctane.

Size Exclusion Chromatography (SEC). This automated cleanup step is represented as step D in Figure 1. Sample solutions were injected by a Perkin-Elmer ISS 100 autosampler onto the SEC column (Phenogel; 25 cm × 22.5 mm i.d.; 10-μm particle size; 100-Å pore size; Phenomenex, Inc., Torrance, CA). The mobile phase, 20% (v/v) CH_2Cl_2 in hexane, was pumped at 4.0 mL/min with an Isco 2350 HPLC pump. The fractions eluting from 20 to 35 min were collected by an Isco Foxy 200 fraction collector.

PCB Prequantitative Analysis. After SEC, a portion (typically 2−5%) of each extract was sacrificed for quantitative evaluation by GC/ECD to determine an approximate total PCB concentration. Knowledge of the total amount of PCBs present in a sample was useful to avoid overloading the carbon fractionation systems (see below).

Carbon/C_{18} Sample Fractionation. Two configurations, having single and dual stainless steel carbon columns, were developed and used in this work. The simple configuration utilized a single column (designated column 2; 0.95 × 25 cm) containing 300 mg of PX-21 dispersed with 9.6 g of C_{18} and featured a valve that allowed reversal of the column flow. The more elaborate of the two systems used a column 1 (0.46 × 4.0 cm; 10 mg of PX-21 dispersed on 0.45 g of C_{18}) in tandem with column 2. An additional switching valve

was incorporated with the dual column system to enable the switching of column 2 off-line. Figure 1 (step E) illustrates the instrument arrangement and collected fractions. Each system uses a different solvent program. The PCB concentration encountered in an environmental sample was the criterion by which the appropriate fractionation system was chosen. For samples having relatively low total quantities of PCBs, the dual column system was used, thereby saving time and solvent. The single column system, as used with its particular solvent program, has a larger effective PCB capacity; samples having relatively high total PCB amounts were relegated to this system. Regardless of which system is in use, the two switching valves remain in place for both systems. All that is required to switch from the dual to the single column system is to replace column 1 with column 2 on valve 1 and turn valve 2 to the second position.

Samples were introduced by an autosampler (Perkin-Elmer ISS 100), and the solvent was delivered by a (Perkin-Elmer Series 410) quaternary pump. The solvent programs for the single and dual column systems are outlined in Figures 2 and 3, respectively. For each system, four fractions were collected by the fraction collector (Isco Foxy 200) and contained the PHHs distributed according to their degrees of planarity: bulk PCBs; mono-*o,o'*-Cl PCBs; non-*o,o'*-Cl PCB congeners; and PCDDs/PCDFs. After concentration, bulk and mono-*o,o'*-Cl PCB fractions were analyzed by GC/ECD (Figure 1, step F), and the non-*o,o'*-Cl PCB fraction was analyzed by GC/MS (Figure 1, step H). The PCDD/PCDF fractions were concentrated, and the solvent was exchanged to nonane in preparation for alumina cleanup (see below).

Carbon/C_{18} System Operation. Key features of these automated systems are the six-port, air-actuated switching valves (Vici Valco, Houston, TX). In the single column system, the valve reversed the column flow for collection of the PCDD/PCDF fraction. The other fractions had been collected previously by a forward flow gradient (Figure 2).

For the dual column system, valve 1 reversed the solvent flow through column 1, and valve 2 switched column 2 on- and off-line, as illustrated in Figure 1, step E. The dual column system is designed to allow the focusing of non-*o,o'*-Cl PCBs and PCDDs/PCDFs on column 1, while using column 2 to separate the bulk and mono-*o,o'*-Cl PCBs. Isolation of the most highly retained PHHs on column 1 reduces the time and solvent required to collect separate fractions, in comparison with system 1 (a single large column). This fractionation is accomplished by using a mobile phase just strong enough to elute the PCB congeners with one *o,o'*-Cl directly through column 1 onto the larger column 2, and those PCB congeners with two or more *o,o'*-chlorines directly through both columns (resulting in the bulk PCB fraction). Column 2 is then switched off-line by valve 2, isolating the PCDDs/PCDFs and non-*o,o'*-Cl PCBs on column 1. Thus, the first collection from column 2 is, predominately, PCBs with more than one *o,o'*-Cl (Figure 3, segments 1 and 2). Column 1 is then washed with an increasingly aromatic mobile phase to elute the non-*o,o'*-Cl PCBs (Figure 3, segments 3 and 4). The flow is then reversed by valve 1, and toluene is used to elute the PCDDs/PCDFs from column 1 (Figure 3, segment 5). These valve positions are continued while column 1 is washed in preparation for the next sample (Figure 3, segment 6). Valve 2 is actuated to switch column 2 on-line to elute the mono-*o,o'*-Cl PCBs with toluene (Figure 3, segment 7). This also serves to wash column 2. Valve 1 is actuated to return column 1 to forward flow for re-equilibration of both columns with the initial mobile phase (Figure 3, segment 8).

Alumina. Columns were prepared as follows. Serological pipets (5 mL) with small plugs of glass fiber filter at the tips were dry-packed with 3.6 g of basic alumina. The alumina was prewashed with 20 mL of 5% (v/v) CH_2Cl_2 in hexane, the concentrated PCDD/PCDF fractions (in nonane) were applied, and the columns were washed with 12 mL of the same solvent. All eluate collected to this point was discarded. PCDD/PCDF congeners were then eluted with 15 mL of CH_2Cl_2 and concentrated prior to GC/MS analysis (Figure 1, step H).

Test of Procedures with Environmental Matrices. A number of environmental and laboratory control sample matrices were spiked with ^{13}C-labeled procedural internal standards and native PCB congeners to check recoveries through the two analytical schemes. Which carbon system was used constituted the primary difference between the schemes.

Extracts of several types of environmental samples (usually 50-g portions) were processed through the dual column system. These included three replicate portions of a Saginaw River, MI, common carp and three replicate portions of negative control grass carp (fortified at 50 ng/g concentrations of selected mono-*o,o'*-Cl and non-*o,o'*-Cl PCBs). Two additional environmental matrices were processed through the dual column scheme, with deviations from the delineated method noted as follows. For 10 individual peregrine falcon eggs, the SEC step was performed after carbon. Extracts of three replicate portions of a Saginaw River, MI, sediment had been cleaned up on Florisil and treated with acid-activated copper before dual column carbon fractionation.

The single carbon column system was used to process four negative control chicken eggs (20-g portions) and the following environmental matrices: 11 fish (50-g portions of common carp and squawfish) from the Columbia River, OR; five bald eagle eggs (50-g portions of five individual eggs) from northwest Washington; and 17 peregrine falcon eggs (20-g portions of 17 individual eggs). The samples for both carbon systems were spiked with 600 pg (PCDDs/PCDFs) and 5000 pg (non-*o,o'*-Cl PCBs) of the ^{13}C-labeled surrogates listed in Tables 1 and 2. One exception was a portion of negative control grass carp (included with the Columbia River fish samples) spiked with native and not ^{13}C-labeled non-*o,o'*-Cl PCBs to concentrations of 100 ppb.

Results and Discussion

Recovery. The average percent recoveries for each sample matrix are summarized in Tables 1 and 2. Good recoveries of most PCDDs, PCDFs, and non-*o,o'*-Cl PCB congeners were attained with either carbon system and were generally between 60 and 100%. For the dual column system, recoveries of [^{13}C]-2,3,7,8−TCDF and PCB congener no. 77 were sometimes lower due to their partial elution into the previous fraction. Variations among recoveries of surrogates were small; coefficients of variation (CV) generally ranged from 5 to 15%. For grass carp negative control samples (Table 1) and procedural blanks (not shown), recoveries of [^{13}C]PCDD/PCDFs were lower (50−60%) and the variation among samples was greater (≈25% CV) than

A. **Solvent Program**

Segment	Volume(mL)	Time(min)	%A	%B	%C	Segment Description	Fractions
I	0-112	0-45	100	0	0	Isocratic	Bulk PCBs: 0 - 120 mL
II	112-212	45-85	100→75	0→25	0	Linear Gradient	Mono-*o*-PCBs: 120 - 200 mL
III	212-275	85-110	75→0	25→100	0	Linear Gradient	
IV	275-295	110-118	0	100	0	Isocratic	Non-*o*-PCBs: 200 - 300 mL
V	295-520	118-208	0	100	0	Isocratic: Reverse flow	PCDDs/PCDFs: 300 - 520 mL
VI	520-1020	208-408	0	100	0	Isocratic ← Column wash	
VII	1020-1040	408-416	0	0	100	Isocratic ←	
VIII	1040-1115	416-446	100	0	0	Isocratic: Column re-equilibration	

Flow Rate 2.5 mL/min

A - 10% CH_2Cl_2 / 90% hexane B - toluene C - 10% CH_2Cl_2 / 90% methanol

B.

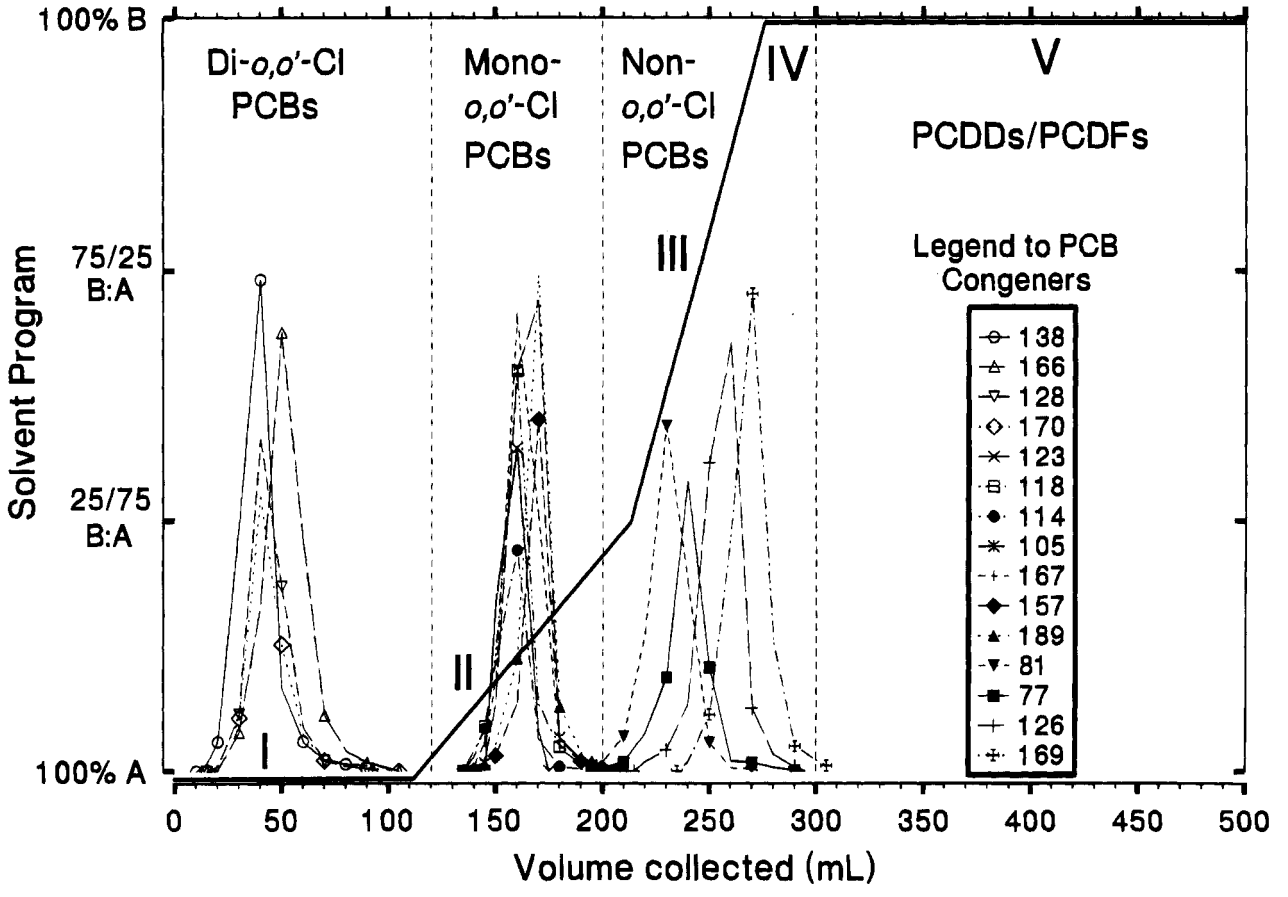

FIGURE 2. (A) Outline of solvent program and fraction collection volumes. (B) Representation of elution of PHHs superimposed over the solvent program for collected fractions from the 300 mg of carbon/C_{18} column. The dotted lines denote the collected fraction boundaries.

with environmental samples. A plausible explanation for this phenomenon is that lower concentrations of native co-contaminants were available to bind with any reactive sites on glassware or adsorbents.

A.

Solvent Program

Segment	Volume (mL)	Time (min)	%A	%B	%C	%D	Segment Description	Valve Status	Fractions
I	0-20	0-10	75	25	0	0	Isocratic	Col 1&2 in-line	Bulk PCBs: 0 - 56 mL
II	20-70	10-35	75→0	25→100	0	0	Linear Gradient	Col 2 off-line at 22 minutes	
III	70-94	35-47	0	100→75	0→25	0	Linear Gradient		Non-*o*-PCBs: 56 - 130 mL
IV	94-130	47-65	0	75	25	0	Isocratic	Col 1 reverse flow at 65 min	
V	130-202	65-101	0	0	0	100	Isocratic		PCDDs/PCDFs: 130 - 202 mL
VI	202-274	101-137	0	0	0	100	Isocratic	Col 1 wash	
VII	274-334	137-167	0	0	0	100	Isocratic	Col 2 in-line at 137 minutes	Mono-*o*-PCBs: 274 - 334 mL
VIII	334-374	167-187	75	25	0	0	Isocratic	Col 1 forward flow	

Flow Rate 2.0 mL/min

A - hexane B - 80%/15%/5% CH_2Cl_2:hexane:methanol C - benzene D - toluene

B.

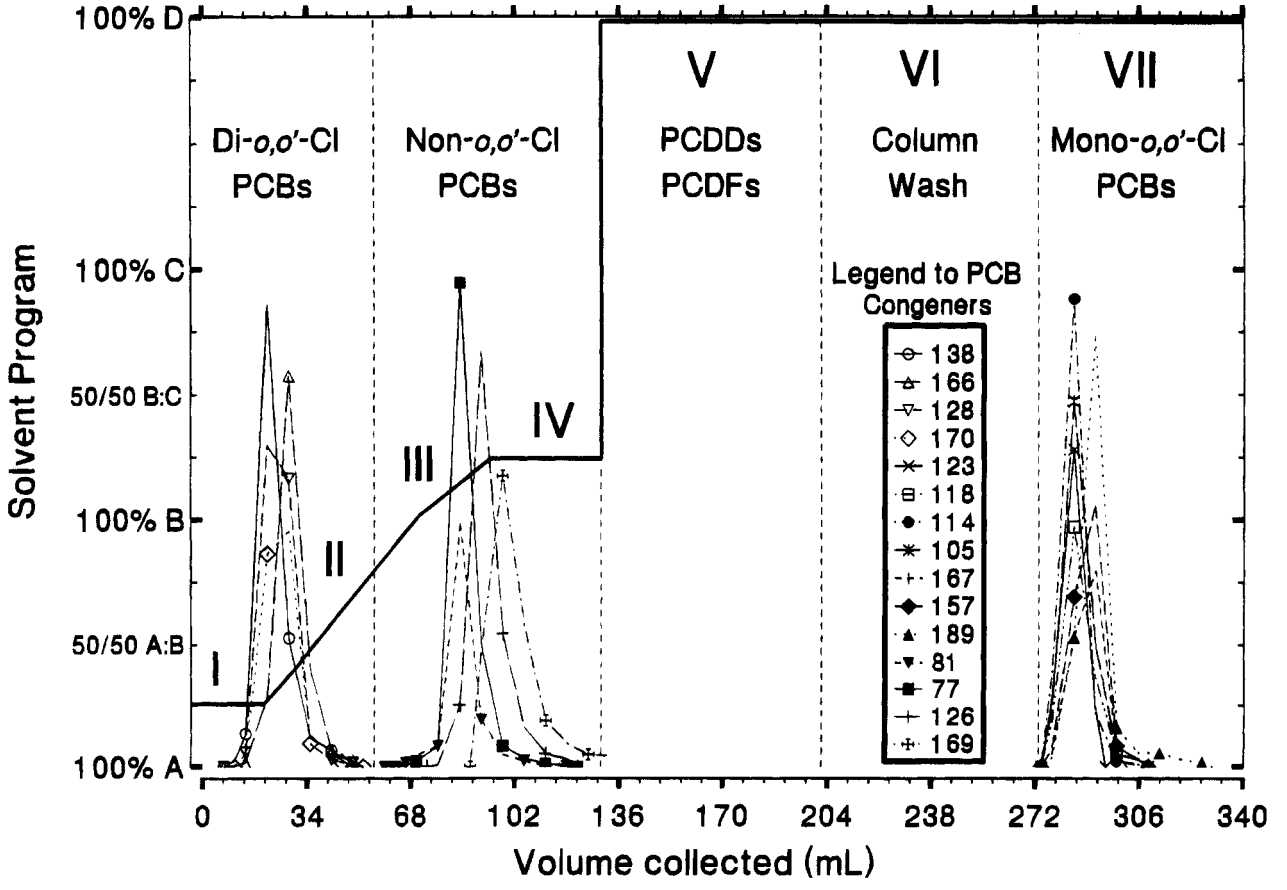

FIGURE 3. (A) Outline of solvent program and fraction collection volumes. (B) Representation of elution of PHHs superimposed over the solvent program for collected fractions from the 10 and 300 mg of carbon/C_{18} columns. The dotted lines denote the collected fraction boundaries.

TABLE 1

Recovery of ^{13}C PCDDs/PCDFs/PCBs and Native PCBs from 10- and 300-mg Carbon/C_{18} Columns[a]

	Saginaw River sediment ($n = 3$)	Saginaw River carp ($n = 3$)	peregrine falcon eggs ($n = 10$)	grass carp spikes ($n = 3$)
		GC/MS Analysis		
[^{13}C]PCDD/PCDFs				
2,3,7,8-TCDD	65 (5)	67 (18)	71 (5)	62 (12)
1,2,3,7,8-PeCDD	85 (12)	75 (13)	83 (6)	65 (13)
1,2,3,4,7,8-HxCDD	94 (13)	67 (10)	76 (9)	59 (15)
1,2,3,6,7,8-HxCDD	80 (6)	71 (17)	84 (9)	65 (14)
1,2,3,7,8,9-HxCDD	88 (13)	75 (9)	80 (10)	69 (14)
1,2,3,4,6,7,8-HpCDD	96 (12)	69 (12)	80 (11)	61 (15)
OCDD	104 (10)	58 (5)	68 (12)	52 (13)
2,3,7,8-TCDF	41 (4)	43 (9)	61 (5)	40 (9)
1,2,3,7,8-PeCDF	83 (9)	71 (10)	76 (6)	64 (14)
2,3,4,7,8-PeCDF	84 (8)	76 (9)	77 (7)	64 (14)
1,2,3,4,7,8-HxCDF	89 (10)	70 (7)	75 (9)	63 (14)
1,2,3,6,7,8-HxCDF	84 (15)	67 (11)	81 (8)	65 (14)
1,2,3,7,8,9-HxCDF	92 (8)	81 (12)	91 (8)	66 (14)
1,2,3,4,6,7,8-HpCDF	101 (6)	79 (11)	83 (9)	62 (14)
1,2,3,4,7,8,9-HpCDF	86 (16)	71 (8)	75 (11)	55 (14)
[^{13}C]non-*o,o'*-Cl PCBs				
3,3'4,4'-TCB (no. 77)	22 (2)[b]	96 (9)	92 (8)	32 (3)
3,3',4,4'5-PeCB (no. 126)	47 (9)[b]	92 (8)	105 (12)	85 (8)
3,3',4,4',5,5'-HxCB (no. 169)	45 (3)[b]	88 (9)	95 (8)	88 (10)
		GC/ECD Analysis		
native non-*o,o'*-Cl PCBs				
3,4,4',5-TCB (no. 81)				85 (8)
3,3'4,4'-TCB (no. 77)				82 (6)
3,3',4,4'5-PeCB (no. 126)				88 (8)
3,3',4,4',5,5'-HxCB (no. 169)				88 (12)
native mono-*o,o'*-Cl PCBs				
2',3,4,4',5-PeCB (no. 123)				83 (5)
2,3',4,4',5-PeCB (no. 118)				86 (7)
2,3,4,4',5-PeCB (no. 114)				93 (4)
2,3,3',4,4'-PeCB (no. 105)				85 (6)
2,3',4,4',5,5'-HxCB (no. 167)				89 (5)
2,3,3',4,4',5-HxCB (no. 156)				91 (4)
2,3,3',4,4',5'-HxCB (no. 157)				89 (5)
2,3,3',4,4',5,5'-HxCB (no. 189)				91 (5)

[a] Standard deviation is given in parentheses. [b] Low recoveries due to mechanical malfunction.

Class Separation. Early uses of carbon were to separate chlorinated pesticides from PCBs or PCBs from PCDDs/PCDFs. Jensen and Sundström (*12*) reported the use of activated carbon for class separation of PCBs on the basis of planarity, and Stalling *et al.* (*29*) used polyurethane foam-dispersed carbon to fractionate PCBs according to degree of *o,o'*-Cl substitution. Automation and refinement of this stepwise elution approach to separation of PCBs on carbon is illustrated in Figures 2 and 3. Both of our carbon/C_{18} systems provide enhanced class resolution and convenience in comparison to the previous methods from which they are evolved. Figures 2 and 3 represent the discrete PHH class resolution attainable with either system. The separated analytes are, conveniently, also distributed among the fractions in descending order of concentration, facilitating quantitation. For example, the concentrations of the PCB congeners with one or more *o,o'*-chlorines in Aroclor mixtures and environmental samples are 10−>100 times those of the non-*o,o'*-Cl PCBs. The calibration range of a GC/ECD is commonly within a 20-fold range of concentrations, which precludes analyzing sample components with large concentration differences in a single injection. Separation of the mono-*o,o'*-Cl PCB congeners (with greater concentration) from the smaller quantities of non-*o,o'*-Cl PCB congeners avoids the complication of solvent evaporation to enable analysis for non-*o,o'*-Cl PCBs followed by dilution to enable analysis for mono-*o,o'*-Cl PCBs. Also, with GC/ECD or GC/MS, the greater degree of discretion achieved with fractionation on carbon significantly minimizes co-eluting interferences.

Loading. The effective loading capacities of the carbon/C_{18} systems were determined by using a radiochromatographic detector to monitor the elution of ^{14}C-labeled PCB congeners 153 and 77. Fixed quantities of radiolabeled PCB congeners were co-injected with increasing quantities of a 1:1:1:1 mixture of Aroclors 1242/1248/1254/1260. The loading tests revealed that the two carbon systems do not have the same effective capacities when used with their individual solvent and valve switching programs. The effective capacity of the dual column system is limited as to the total quantity (μg) of planar compounds (PCDFs/PCDDs and non-*o,o'*-Cl PCBs) that can be applied to the initial (10 mg) column before breakthrough occurs. The effective capacity of this system was determined to be approximately 1.5 μg of total planar compounds. Since an Aroclor mixture contains ≈6.5 ng/μg of total non-*o,o'*-Cl PCBs (*20*), the maximum loading would be approximately 230 μg of the Aroclor mixture. Overloading column 1 of this dual column system with planar compounds shifts the early eluting compounds within a fraction into the previous (more weakly retained) fraction. As a consequence, PCB congeners 81 and 77 would partially elute with the mono-*o,o'*-Cl PCBs (fraction 4), and 2,3,7,8-TCDF will partially appear in the non-*o,o'*-Cl collection (fraction 2). We do

TABLE 2

Recovery of [^{13}C]PCDDs/PCDFs/PCBs and Native PCBs from a 300-mg Carbon/C_{18} Column[a]

	Columbia River fish (n = 11)	Washington eagle eggs (n = 5)	peregrine falcon eggs (n = 17)	chicken egg spikes (n = 4)
		GC/MS Analysis		
[^{13}C]PCDD/PCDFs				
2,3,7,8-TCDD	91 (10)	81 (13)	67 (18)	74 (4)
1,2,3,7,8-PeCDD	92 (11)	85 (11)	68 (14)	72 (11)
1,2,3,4,7,8-HxCDD	91 (16)	85 (10)	73 (17)	90 (10)
1,2,3,6,7,8-HxCDD	85 (19)	87 (10)	62 (12)	80 (8)
1,2,3,7,8,9-HxCDD	85 (10)	103 (14)	72 (17)	88 (8)
1,2,3,4,6,7,8-HpCDD	82 (14)	84 (16)	62 (16)	71 (6)
OCDD	78 (15)	70 (7)	57 (16)	77 (7)
2,3,7,8-TCDF	89 (7)	79 (10)	68 (13)	75 (6)
1,2,3,7,8-PeCDF	91 (9)	81 (10)	73 (14)	79 (3)
2,3,4,7,8-PeCDF	90 (9)	88 (10)	75 (15)	83 (8)
1,2,3,4,7,8-HxCDF	88 (11)	84 (11)	70 (15)	81 (8)
1,2,3,6,7,8-HxCDF	79 (19)	84 (10)	64 (13)	80 (6)
1,2,3,7,8,9-HxCDF	84 (11)	95 (13)	69 (15)	87 (11)
1,2,3,4,6,7,8-HpCDF	80 (13)	82 (9)	64 (14)	86 (15)
1,2,3,4,7,8,9-HpCDF	82 (14)	85 (9)	67 (15)	92 (26)
[^{13}C]non-o,o'-Cl PCBs				
3,3'4,4'-TCB (no. 77)		96 (9)	57 (8)	54 (2)
3,3',4,4'5-PeCB (no. 126)		92 (8)	71 (9)	65 (4)
3,3',4,4',5,5'-HxCB (no. 169)		88 (9)	62 (6)	57 (3)
		GC/ECD Analysis		
native non-o,o'-Cl PCBs				
3,4,4',5-TCB (no. 81)	92[b]			
3,3'4,4'-TCB (no. 77)	92[b]			
3,3',4,4'5-PeCB (no. 126)	98[b]			
3,3',4,4',5,5'-HxCB (no. 169)	100[b]			

[a] Standard deviation is given in parentheses. [b] n = 1.

not apply more than 200 μg of total PCBs from environmental samples because of potentially greater planar compound-to-total PCB ratios than are present in Aroclors. In contrast, the effective capacity of the single column system is limited by the amount of total (bulk) PCBs rather than the amount of total planar compounds. Overloading of the system with total PCBs deteriorates the resolution between fraction 1 (bulk PCBs) and fraction 2 (mono-o,o'-Cl PCBs). The practical loading limit was found to be approximately 650 μg of the Aroclor mixture or about three times as much as for the dual column system. By the above criteria, 50 g of many environmental samples would effectively overload the carbon systems; in such situations multiple injections of the sample are required.

The advantage of having both a single and dual column system is the versatility they provide. Using the information obtained by prequantitation, we can choose the carbon system that will most expeditiously and economically fractionate a given group of samples.

Interferences. The identification and quantitation of PCDDs/PCDFs in sediment or biota at low ppt levels and of planar PCBs at high ppt to low ppb levels requires a preparative procedure that will effectively remove the many potential interferences to GC/ECD or GC/MS analyses. Chromatographic interferences on GC/ECD can be a major obstacle to the accurate quantitation of certain PHHs and to the avoidance of positive biases. The class separation provided by the method presented herein allows GC/ECD quantitation and detection of the bulk PCB and mono-o,o'-Cl PCB congeners without major interferences. If not fractionated, the toxic non-o,o'-Cl PCB congeners (PCBs 81, 77, and 126) would elute near other relatively nontoxic PCB congeners that have ≥2 o,o'-chlorines. Our fractionation scheme removes >99.5% of these PCB interferences

TABLE 3

Residual Interfering PCB Congeners in Non-o,o'-Cl Fraction of Eagle Egg Samples through Carbon/C_{18} Column(s)

PCB congener	column type	concn range of PCB congener in samples (ng/g)	% residual in non-o,o'-Cl fraction	
			average	% SD
87	single column	63–1273	0.03 (n = 23)	2.2
	dual columns	4–648	0.16 (n = 42)	38.0
136	single column	34–224	0.01 (n = 14)	0.7
	dual columns	4–69	0.04 (n = 35)	2.1
110	single column	97–1649	0.05 (n = 23)	2.6
	dual columns	3–616	0.34 (n = 42)	95.0
129	single column	70–573	0.01 (n = 23)	0.4
	dual columns	3–345	0.04 (n = 42)	5.4

from the non-o,o'-Cl PCB fraction. Remaining interference problems (congeners 87 with 81; 110/136 with 77; and 129 with 126) in the non-o,o'-Cl fraction from carbon/C_{18} are minimal (Table 3), even with total PCB quantities per sample ranging from 25 to 3100 μg. Halogenated pesticides are almost totally removed by earlier cleanup steps, but their particular fate is beyond the scope of this method. Though interferences are not totally removed, they are at manageable levels for GC/MS analysis.

PCDD/PCDF analyses on GC/MS require that samples be virtually devoid of certain interfering compounds such as PCBs, halogenated diphenyl ethers (HDPEs), and polychlorinated naphthalenes (PCNs). Fractionation on carbon/C_{18} results in the elution of the HDPEs in the mono-o,o'-Cl fraction and most of the PCNs in the non-o,o'-Cl fraction. Shifting the bulk of the PCNs into the non-o,o'-Cl fraction introduces the potential of interference to [^{13}C]PCB congener 126 by hexachloronaphthalenes, but this problem

can be avoided with analysis by GC/HRMS. Alumina cleanup of the PCDD/PCDF fractions aids in removal of residual PCNs, HDPEs, and PCBs from that fraction.

Comparison with Other Methods. Other methods for PHH fractionation and analysis do not include as comprehensive a one-step sample fractionation as described here. The selective isolation of particular groups of PHHs at the expense of others can be achieved by several methods, most of which employ carbon or materials that have carbon-like characteristics. A number of methods use carbon to isolate only one fraction—the PCDDs/PCDFs (*8, 9, 14, 16*). Bicking and Wilson (*30*) used high-performance size exclusion chromatography to isolate PCDDs/PCDFs in fat, oil, and sediment from other co-extractants (PCBs). Lamparski and Nestrick (*31*) isolated PCDDs/PCDFs with HPLC using reverse-phase (ODS) and silica gel HPLC techniques. Some methods isolate a single fraction containing either PCDDs/PCDFs and non-*o,o'*-Cl PCBs (*7, 13, 17, 18, 22*) or non-*o,o'*-Cl PCBs only (*11, 32, 33*). Others have used carbon chromatography to isolate two PCB fractions: (a) PCBs with >1 *o,o'*-Cl and (b) PCBs with ≤1 *o,o'*-Cl (*19, 20*), or (a) PCBs with ≥1 *o,o'*-Cl and (b) non-*o,o'*-Cl PCBs (*25, 34*). Some have used carbon chromatography to produce three PCB fractions—PCBs with >1 *o,o'*-Cl, mono-*o,o'*-Cl PCBs, and non-*o,o'*-Cl PCBs (*21, 26, 35*); Haglund *et al.* (*36*) produced similar fractions using a PYE column. Our fractionation and analytical method goes beyond these earlier works in that four discrete, analytically ready fractions are generated.

Lundgren *et al.* (*37*), using an automated HPLC gradient method with a 100-mg carbon/C_{18} column provided by our laboratory, developed a fractionation scheme similar to that of Haglund *et al.*, except the third fraction was extended to include additional planar compounds (PCDDs/PCDFs and PCD-thiophenes) (*38*). Their fractionation and GC/MS analysis of selected PCB congeners (^{12}C and ^{13}C), a Clophen A50 standard, and an interlaboratory positive control herring resulted in excellent recoveries and agreement with accepted values. Their work demonstrates the performance of the carbon/C_{18} column and reinforces the utility of obtaining discrete fractions that can be selectively analyzed by GC/ECD or GC/MS.

Compared with some methods, the sample fractionation on carbon presented in this work has greater solvent requirements, which can be attributed to the extensive wash of the carbon column before reuse and to the deliberately gradual increases of elutropic strength that provide good separation between fractions for large PCB loadings. Replacement of the 10-mg carbon column with a PGC column (before the 300-mg carbon/C_{18} column) might simultaneously provide larger sample capacity and reductions in time and solvent requirements and eliminate the need for benzene.

The lower recovery for ^{13}C-labeled internal standards in blanks compared with samples may be due, in part, to the use of sulfuric acid silica gel. Kannan *et al.* (*39*) demonstrated that recovery of PCB congeners is reduced in samples containing low amounts of co-extracted biological compounds that are treated with acid or alkali.

Conclusions

The goal of this work was to achieve four discrete fractions of PHHs. This aids analysis of the non-*o,o'*-Cl and PCDD/PCDF fractions as evidenced by the low residual interfering PCBs in the non-*o,o'*-Cl fraction and the shifting of PCBs, HDPEs, and PCNs away from the PCDD/PCDF fraction. The results demonstrate the capability of our carbon/C_{18} systems to accomplish this goal. An established history of carbon use for fractionation of PHHs provides a familiarity with its assets (low sensitivity to lipids; low cost; good loading capacity) and its limitations (broad elution profile with strong aromatic solvents for PCDD/PCDF fraction) as compared to more recently developed materials. No previous method accomplished as extensive a one-step fractionation of PCBs plus a PCDD/PCDF fraction as that described here.

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