1998

Loading capacity and chromatographic behavior of a porous graphitic carbon column for polychlorinated biphenyls

Kathy R. Echols
Environmental and Contaminants Research Center

Robert W. Gale
Environmental and Contaminants Research Center

Kevin Feltz
Environmental and Contaminants Research Center

Jerome O'Laughlin
University of Missouri-Columbia

Donald E. Tillitt
Environmental and Contaminants Research Center

See next page for additional authors

ELSEVIER

Journal of Chromatography A, 811 (1998) 135–144

JOURNAL OF CHROMATOGRAPHY A

Loading capacity and chromatographic behavior of a porous graphitic carbon column for polychlorinated biphenyls

Kathy R. Echols[a,*], Robert W. Gale[a], Kevin Feltz[a], Jerome O'Laughlin[b], Donald E. Tillitt[a], Ted R. Schwartz[a]

[a]*Environmental and Contaminants Research Center, (USGS-BRD), 4200 New Haven Road, Columbia, MO 65201, USA*
[b]*Department of Chemistry, University of Missouri-Columbia, Columbia, MO 65211, USA*

Received 24 October 1997; received in revised form 23 March 1998; accepted 23 March 1998

Abstract

A porous graphitic carbon column (Hypercarb) was used for the fractionation of polychlorinated biphenyls (PCBs) into classes of 2-4 *ortho* chlorines, 1 *ortho* chlorine and 0 *ortho* chlorine congeners. A method was developed that combined the fractionation of PCBs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans in a variety of biotic environmental samples. Many of these samples have high concentrations of PCBs which cause fractionation problems as adsorption sites on the graphitic surface are occupied. The loading capacity of the column for PCBs was determined by injecting up to 1 mg of total PCBs and monitoring changes in chromatographic behavior of tetra-/di-*ortho*, mono-*ortho* and non-*ortho* substituted PCBs. Effective loading capacities were 1 mg for tetra-/di-*ortho* PCBs, but only 3–5 μg for non-*ortho* PCBs and about 2 μg for mono-*ortho* PCBs. Loading capacity of the PGC column for environmental fish and avian egg samples was determined to depend on the mono-*ortho* and non-*ortho* PCB levels found in these samples.

Keywords: Porous graphitized carbon; Environmental analysis; Food analysis; Column loading capacity; Polychlorinated biphenyls; Polychlorinated dibenzodioxins; Polychlorinated dibenzofurans

1. Introduction

The determination and quantification of the non-*ortho* substituted polychlorinated biphenyls (PCBs), 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs) and 2,3,7,8-substituted polychlorinated dibenzofurans (PCDFs) is difficult because they are at such low concentrations, typically 10^{-9}–10^{-6} times lower, relative to the amount of PCBs and other compounds in biotic environmental samples. Enrichment of selected, toxic, non-*ortho* PCB congeners (IUPAC numbers 81, 77, 126, 169) from other, more prevalent PCBs at ng g^{-1} to μg g^{-1} levels is necessary to detect them at pg g^{-1} to ng g^{-1} levels, when using capillary gas chromatography–high-resolution mass spectrometry [1–4]. Enrichment of PCDDs and PCDFs at toxicologically important, pg g^{-1} levels is also required [2,3]. Therefore, choosing an appropriate sample size has a large effect on the ability to detect and quantify the non-*ortho* PCBs and 2378-substituted PCDDs/PCDFs.

Many types of activated carbons have been used in

*Corresponding author.

0021-9673/98/$19.00
PII: S0021-9673(98)00241-6

fractionation methods for PCBs, PCDDs and PCDFs [3,4,6,8–13]. These activated carbons have large surface areas and a large number and variety of polar functional groups [14]. Interactions of polar solutes can be strong on these activated carbons. In addition, there are bonded phases, such as the 2-(1-pyrenyl)ethyldimethylsilylated (PYE) silica column which are used to fractionate PCBs [15]. Separation on the PYE column is due to the formation of electron donor–acceptor complexes [15,16]. Separation of PCBs on the graphitic surface of the porous graphitic carbon is through π–π interactions – dispersion interactions – of the biphenyl rings [10,14,16]. The strength of the interaction is dependent upon the planarity of the two rings. PCDDs and PCDFs are more strongly bound because they are rigidly planar compounds that have π–π interactions plus lone pair oxygen electron-donor interactions with the graphitic surface [16]. For PCBs the strength of the interaction is dependent upon the ease of achieving a planar configuration which is dependent upon *ortho* substitution and the total number of chlorines on the biphenyl rings, zero *ortho* chlorines having the strongest interaction and four *ortho* chlorines having the weakest.

The PGC column is capable of separating the tetra-/di-*ortho* PCBs, mono-*ortho* PCBs, non-*ortho* PCBs, and PCDD/PCDFs into distinct fractions (Table 1) [5]. In order to achieve the necessary $pg\,g^{-1}$ detection limits for PCDDs and PCDFs, sample sizes from 10 to 50 g of tissue are extracted [1–6]. In the course of applying environmental samples of fish and avian eggs to the PGC column (and to a PX-21 column), we noticed recovery problems for mono-*ortho* PCBs. Upon closer examination we found 30–50% of the mono-*ortho* PCBs in many samples eluting in the tetra- through di-*ortho* PCB fraction (Table 1). This demonstrated that the carbon fractionation step in the clean-up was sensitive to sample size, probably due to PCB overloading. According to Snyder [7], the linear capacity of an adsorbent for a compound is that sample size sufficient to cause a 10% decrease in retention time of that compound. PCB loading studies were done in order to determine the extent of the total PCB and planar PCB loading capacities of the PGC column. It is important to determine the capacity of the column for the PCB/PCDD/PCDF fractionation procedure because of the need for precisely defined cutoffs, for collection of the bulk (tetra-/di-), mono-, and non-*ortho* PCB fractions.

The loading capacity of the PGC column (1 g of packing) was estimated by Knox to be between 5–50 μg of solute per gram of adsorbent based upon the capacities determined for other types of graphitized carbons [17]. For PX-21 columns of 300 mg, the maximum PCB loading capacity was 650 μg [6]. Loading capacity of the PYE column was determined to be up to 250 μg of Chlophen A50 [15]. Activated carbons, such as AMOCO PX-21 (or AX-21), have a greater surface area, 2000 $m^2\,g^{-1}$, and therefore greater capacity, than the porous graphitic carbon

Table 1
HPLC solvent program for fractionation of PCBs, PCDDs and PCDFs on the porous graphitic carbon column

Step	Time (min)	Solvents	Flow direction and gradient	Collected fractions (min)
0	–	Hexane	F; equilibration	
1	0–4	Hexane	F	F1; 0–4.5 (2-4-*ortho*)
2	4–12	To hexane–toluene (60:40)	F; linear	F2; 4.5–12 (1-*ortho*)
3	12–20	Hexane–toluene (60:40)	F	F3; 12–20 (0-*ortho*)
4	20–50	100% Toluene	R	F4; 20–50 (D/Fs)
5	50–110	Wash and re-equilibration		dump

Note: F=forward flow; R=reverse flow. flow-rate: 2.5 $ml\,min^{-1}$. Fractions: F1–F4.

with a surface area of 150–200 $m^2 g^{-1}$ [5,8,9,18]. The objective of this paper is to summarize the results of loading capacity studies of the PGC column, using Aroclor mixtures, individual PCB congeners, PCDDs/PCDFs and various environmental samples.

2. Experimental

2.1. Materials

Two ^{14}C-radiolabeled PCB congeners, 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) and 77 (3,3′,4,4′-tetrachlorobiphenyl), 2,3,7,8-tetrachlorodibenzofuran and octachlorodibenzodioxin; unlabeled PCB congeners, 153, 77, 157 (2,3,3′,4,4′,5′-hexachlorobiphenyl), 105 (2,3,3′,4,4′-pentachlorobiphenyl), and 180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl); a PCB window mix for the carbon column containing-54 (2,2′,6,6′-tetrachlorobiphenyl), 170 (2,2′,3,3′,4,4′,5-heptachlorobiphenyl), 118 (2,3′,4,4′,5-pentachlorobiphenyl), 189 (2,3,3′,4,4′,5,5′-heptachlorobiphenyl), 81 (3,4,4′,5-tetrachlorobiphenyl), and 169 (3,3′,4,4′,5,5′-hexachlorobiphenyl); and a mixed standard of Aroclors 1242, 1248, 1254, 1260 (1:1:1:1 w/w) were used in loading experiments. Radiolabeled standards were 98% or better pure (Pathfinder Labs., MO, USA; NIEHS, NC, USA; ChemSyn Labs., KS, USA). Solutions of unlabeled PCBs were made from 99% pure neat compounds (Ultra Scientific, RI, USA). Standards of mixed Aroclors were made from neat 1242, 1248, 1254, and 1260 (Monsanto, MO, USA). HPLC-grade hexane, toluene, methanol and methylene chloride (Fisher Optima, NJ, USA) were used for HPLC.

2.2. HPLC equipment

Three 100 mm×4.6 mm Hypercarb columns with 5 μm particle size (Keystone Scientific, Bellefonte, PA, USA) were used in this study. The HPLC system included a Perkin–Elmer Series 410 LC pump (Perkin–Elmer, Norwalk, CT, USA) with either a Rheodyne 7125 manual injection valve or a Perkin–Elmer ISS-100 Autosampler; a Valco N60 6-port switching valve (VICI, Houston, TX, USA) for forward or reverse flow; and a FLO-ONE\Beta A-140 Radio–Chromatography Detector (Radiomatic, Meriden, CT, USA) with a liquid flow cell. HPLC solvents were sparged with helium in a Perkin–Elmer SEC-4, Solvent Environment Control chamber, prior to use. The scintillation solvent used was Packard Radiomatic Flo-Scint 1 (Packard, Meriden, CT, USA) in a 1:1 ratio with the mobile phase. An ISCO Foxy II Fraction Collector (ISCO, Lincoln, NE, USA) was used to collect fractions for gas chromatographic analysis. The optimized solvent program used is presented in Table 1.

2.3. GC conditions

Because ^{14}C-radiolabeled compounds were unavailable for determination of mono-*ortho* PCB loading capacity, fractions were collected and analyzed by capillary gas chromatography. PCB fractions were analyzed using a HP 5890 II gas chromatograph (Hewlett–Packard, Avondale, PA, USA) equipped with a 30 m×0.25 mm, 0.25-μm DB-35 capillary column (J&W, Folsom, CA, USA) with a 1 m×0.53 mm deactivated retention gap connected to a ^{63}Ni electron-capture detection (ECD) system at a temperature of 330°C. Injections were 1 μl cool on-column, with H_2 carrier gas (11 p.s.i.g.), and temperature programmed as follows: initial temperature 90°C with no hold, ramp 10°C min^{-1} to 165°C, a second ramp of 3°C min^{-1} to 250°C, and a final ramp at 10°C min^{-1} to 300°C with a 5 min hold (1 p.s.i.=6894.76 Pa). Data were collected with a personal computer (80486) using PE Nelson model 900 interfaces and Turbochrom version 4.1 chromatography software (Perkin–Elmer).

2.4. Sample preparation and cleanup

Fish tissue and avian egg samples were extracted and processed through several cleanup steps before carbon column fractionation. The samples were extracted with methylene chloride and concentrated. These lipid extracts were processed through a two-stage reactive column cleanup. The first column was composed of sulfuric acid silica gel and potassium silicate; and a second column was composed of sulfuric acid silica gel, potassium silicate, and silica gel [6]. After the second column, the eluates were

concentrated and injected onto an automated gel permeation column (Phenogel; 250×22.5 mm; 10 μm particles; 100 A pore size; Phenomenex, Torrance, CA, USA) which further removes lipid and other biogenic material from the extracts [6]. This produces very clean PCB/PCDD/PCDF extracts (with other halogenated aromatic compounds present) which can then be fractionated on the PGC (or PX-21) column. For the sample extracts fractionated on the PGC column the first two fractions (bulk PCBs and mono-*ortho* PCBs, or one fraction when these were combined) were analyzed by GC–ECD; the third fraction and fourth fractions by GC with high-resolution mass spectrometry [5,6].

2.5. Methods for loading experiments

A variety of experiments were performed in order to test the loading capacity of the PGC column and are summarized in Table 2. In general, retention behavior of one target PCB congener was monitored as the amount of individual PCB congeners or mixtures of PCBs was increased. A change in retention time of 10% was considered significant [7].

3. Results and discussion

The results of the first set of loading experiments showed that the normalized retention of PCB 77 did not change when PCBs 153 or 157 were loaded up to 50 μg. However, loading a 5 μg amount of PCB 77 causes a 10% drop in retention. This drop is sufficient to cause a breakthrough of PCBs 81 and 77 into the mono-*ortho* PCB collect fraction. However, most total amounts of non-*ortho* PCBs in biotic environmental samples (Table 3) are below 1 μg and total amounts of PCDDs/PCDFs below 0.010 μg, so this

Table 2
Summary of loading experiments

Test compounds	Loading compounds	Amount (μg)	Experiment description (Figure)
[^{14}C] 3,4,3′,4′-TetraCB (PCB 77-1 μg)	PCB 153 2,4,5,2′,4′,5′-HCB	1–50	Hexane–toluene (70:30) at 2.5 ml min^{-1}
[^{14}C] 3,4,3′,4′-TetraCB (PCB 77-1 μg)	PCB 157 2,3,4,3′,4′,5′-HCB	1–50	Hexane–toluene (70:30) at 2.5 ml min^{-1}
[^{14}C] 3,4,3′,4′-TetraCB (PCB 77-1 μg)	PCB 77 3,4,3′,4′-TCB	1–50	Hexane–toluene (70:30) at 2.5 ml min^{-1}
[^{14}C] 2,2′,4,4′,5,5′-HexaCB (PCB 153) [^{14}C] 3,4,3′,4′-TetraCB (PCB 77) [^{14}C] 2,3,7,8-TCDF [^{14}C] OCDD	Aroclors 1242, 1248, 1254, 1260 in 1:1:1:1 (w/w) ratio	1–1000	Solvent program in Table 1. Monitor retention times of labeled compounds
PCB Window Mix (54, 170, 118, 189, 81, 169)	54, 170, 118, 189, 81, 169	0.1, 0.5	Solvent program, Table 1. Fractions analyzed by GC–ECD (Fig. 1)
PCB 105	PCB 105	0.5–8	Solvent program, Table 1. 50 fractions analyzed by GC–ECD (Fig. 2)
PCB 105 (0.5 μg 180, 0.5 μg 77)	PCB 180, PCB 105	10	Solvent program, Table 1. 50 fractions analyzed by GC–ECD (Fig. 3)
PCB 105, PCB 77	PCB 77, PCB 105	0.5, 1.0, 3.0	Solvent program, Table 1. 50 fractions analyzed by GC–ECD (Fig. 4)

Table 3
Concentrations of total-, mono-*ortho*-, non-*ortho*-PCBs, and 2378-PCDD/PCDFs *in biota*

Sample description	Total cPCBs[a] ($\mu g\,g^{-1}$)	Total mPCBs[b] ($\mu g\,g^{-1}$)	Total nPCBs[c] ($\mu g\,g^{-1}$)	Total PCBs[d] ($\mu g\,g^{-1}$)	Total 2378-DFs[e] ($\mu g\,g^{-1}$)
Aroclors					
Aroclor 1242	987 900	7000	5099	1000 000	0.598
Aroclor 1248	957 700	36 000	6282	1000 000	3.73
Aroclor 1254	872 700	126 600	655	1000 000	4.22
Aroclor 1260	975 700	24 000	263	1000 000	7.5
A1111(1242:1248:1254: 1260=1:1:1:1, w/w)	950 000	49 000	3100	1000 000	4
Fish					
Carp	6.6	0.5	0.005	7.1	0.0003
Channel Catfish	7.8	0.5	0.003	8.3	0.0002
Drum	0.6	0.02	0.001	0.6	0.00001
Gizzard Shad	2.2	0.05	0.008	2.3	0.0002
Log Perch	1.1	0.03	0.001	0.0	0.00004
Ouillback Carpsucker	1.0	0.04	0.008	1.1	0.00006
Smelt	0.4	0.02	0.0003	0.4	0.00002
Spottail Shiner	1.4	0.08	0.002	0.1	0.00008
Trout-Perch	0.6	0.03	0.0008	0.7	0.00004
Walleye	3.0	0.2	0.004	3.1	0.0001
White Bass	2.8	0.2	0.008	3.0	0.0002
White Sucker	0.6	0.03	0.0005	0.6	0.00002
Yellow Perch	0.7	0.05	0.0003	0.8	0.00004
Striped Bass	1.2	0.1	0.002	1.3	0.00005
White Perch	2.3	0.1	0.007	2.4	0.00016
Avian eggs					
Canada Goose	0.7	0.03	0.0002	0.7	0.00001
Mallard	5.5	0.05	0.0014	5.5	0.00005
Black-Crowned Night Heron	3.3	0.3	0.003	0.3	0.0001
Caspian Tern	7.6	0.8	0.006	0.8	0.0002
Double-Crested Cormorant	5.4	0.7	0.004	0.7	0.0002
Foresters Tern	12.0	0.7	0.006	0.7	0.0002
Great Egret	3.7	0.4	0.003	4.1	0.0001
Herring Gull	19.0	2.6	0.02	21.6	0.0004
Ring-necked Gull	2.4	0.3	0.003	0.3	0.00004
Double-Crested Cormorant	11.8	1.5	0.006	13.3	0.00043
Bald Eagle	4.3	0.5	0.005	4.8	0.00006

Note: Sample preparation as in Ref. [6].
[a] cPCBs=congener PCBs.
[b] mPCBs=mono-*ortho* PCBs.
[c] nPCBs=non-*ortho* PCBs.
[d] Total PCBs=Sum of cPCBs, mPCBs, nPCBs.

type of loading combination is unlikely to occur in environmental samples.

For the second set of experiments, when loading increasing amounts of A1111 up to 1 mg, retention of the four radiolabeled compounds did not significantly change. The retention behavior of the di-*ortho* PCB 153 showed variability of 5–10%, but did not change statistically over the course of the experiment. Likewise, the non-*ortho* PCB 77 and the 2378-TCDF/OCDD pair showed little or no shift in retention as amounts of A1111 are increased, 0.5–1.5% and 0.24%, respectively. Therefore, an en-

vironmental sample with PCB composition similar to the mixed A1111 standard would not cause overloading of the PGC column.

The injection of the PCB PGC-window mix, containing the first and last eluting congeners for each PGC collection window (Table 1), showed no retention shifts at 0.1 μg per component, but did show major retention shifts at 0.5 μg per component (Fig. 1). The di-*ortho* substituted congeners, PCBs 54 and 170, showed no shift in retention. Both mono-*ortho* substituted congeners, PCBs 118 and 189, were overloaded at 0.5 μg and eluted into the first fraction collected window (Fig. 1, Table 1). The non-*ortho* substituted congeners, PCBs 81 and 169, were not shifted, but did tail slightly. It was not clear if the shift in mono-*ortho* PCB retention was due to the amount of mono- or non-*ortho* substituted congeners. However, it did show that the mono-*ortho* PCBs were easily shifted at lower amounts than the non-*ortho* PCB congeners.

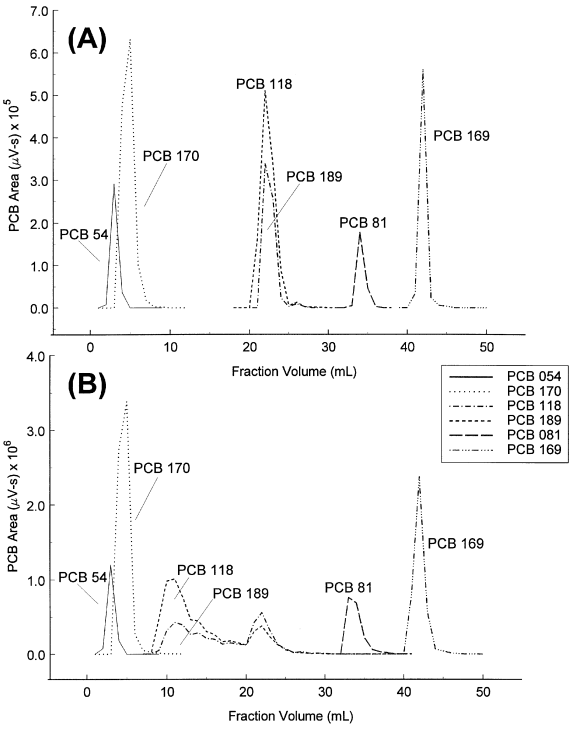

Fig. 1. Summary of GC–ECD analysis of 50 consecutive 1-ml fractions of HPLC eluent from PGC column using the solvent program in Table 1. PCB window mix elution showing mono-*ortho* PCBs, 118 and 189, overloading with each PCB component at 0.1 μg (A), compared to each PCB component at 0.5 μg (B).

The final set of experiments was designed to tease out the retention behavior of the mono-*ortho* PCBs when loading amounts of di-, mono-, and non-*ortho* PCBs. To determine the effect on loading by a mono-*ortho* PCB (PCB 105) alone, amounts of PCB 105 put on the column was increased from 0.5 μg to 8.0 μg. The column already exhibited overloading behavior by 2.0 μg of PCB 105 (Fig. 2B), the front edge of the peak has shifted about 20% earlier. Next, the impact of a large amount (10 μg) of di-*ortho* PCB 180 on a small amount (1.0 μg) of mono-*ortho* PCB 105 (Fig. 3) was evaluated. No overloading was observed. Finally, amounts of PCB 77 (3.0 μg, and 5.0 μg) were added to 1.0 μg of PCB 105 and retention monitored for both compounds. At the 3.0 μg amount of PCB 77, the retention of the peak has shifted to 18 ml from 20 ml already a 10% shift (Fig. 4A). At the 5.0 μg amount of PCB 77, peak splitting and broadening occurred to the extent that the PCB 105 peak begins to elute at 15 ml, a 25% shift in retention. This splitting phenomenon has been observed on another carbon column system, the PX-21/C_{18} column, with environmental samples that contain amounts of mono-*ortho* PCBs >3 μg total in a sample (Table 3).

The PGC column has different capacities for PCBs with differing degrees of *ortho* substitution. The greater the degree of *ortho* substitution of a congener, the greater the capacity. Applying Snyder's definition, the capacity for the planar non-*ortho* PCBs was between 3–5 μg. However, the total amount of planar components (non-*ortho* PCBs and dioxins/furans) in biotic, environmental samples are typically under 1 μg, and are typically around 0.05–0.5 μg (Table 3); therefore, these compounds are less of a concern than the level of mono-*ortho* PCBs when using the PGC column to fractionate samples.

The capacity of the PGC for mono-*ortho* PCBs was 2 μg, when amounts of non-*ortho* PCB are <0.1 μg. The PGC column retains its ability to fractionate mono- and non-*ortho*-substituted PCB congeners when tetra-/di-*ortho* PCBs (bulk PCBs) are loaded up to 1 mg. These bulk PCBs can be loaded at this level on the column because they have a weak interaction with the carbon surface and are essentially non-retained. They don't affect non-*ortho* PCB

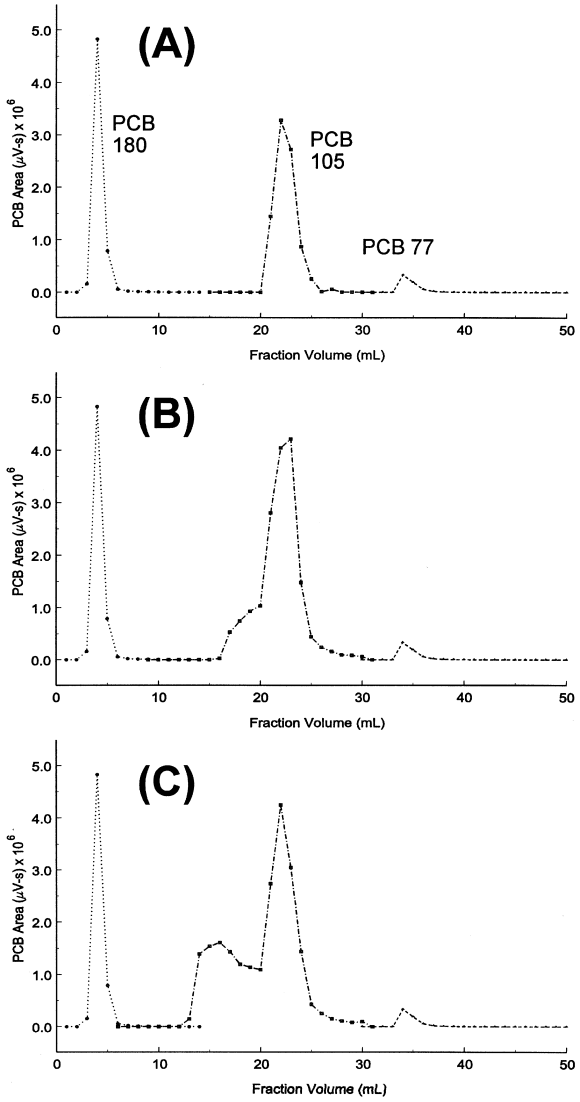

Fig. 2. Mono-*ortho* PCB 105 loading capacity problems demonstrated in summary GC–ECD analysis of 50 consecutive 1-ml fractions of HPLC eluent from PGC column using solvent program in Table 1. Showing, (A) 1 μg PCB 105; (B) 2 μg PCB 105; and (C) 3 μg PCB 105.

and PCDD/PCDF retention because they have left the column before these compounds start to migrate down the column. Thus, it is often difficult to fractionate the mono-*ortho* PCBs cleanly on the PGC column, under some conditions, 30–50% of the mono-*ortho* PCBs elute in the first (bulk PCB) fraction instead of the second (mono-*ortho*) fraction. Total mono-*ortho* PCB amounts can range from <1 μg to 100+ μg in environmental samples (Table 3), depending upon the sample type, and sample size.

From the results of the experiments, greater the amounts of non-*ortho* and/or mono-*ortho* substituted PCBs in environmental samples causes problems in getting definitive *ortho* substituted class fractions. Using the Aroclors as an example, between 87–99% (w/w) of the congeners in Aroclors have *ortho*-chlorine substitution of 2–4 [19]. Aroclors are contaminated with part per million quantities of chlorinated dibenzofurans [20,21]. This adds to the total amount of planar compounds and contributes to overloading the PGC column. Total planar component concentrations in Aroclors are estimated to be 5100 $\mu g\, g^{-1}$ for Aroclor 1242, 6300 $\mu g\, g^{-1}$ for Aroclor 1248, 700 $\mu g\, g^{-1}$ for Aroclor 1254, and 300 $\mu g\, g^{-1}$ for Aroclor 1260 from previously determined PCDF and planar PCB amounts in the literature (Table 3) [19–23]. The A1111 standard used in the loading experiments, has a summed planar concentration of 3100 $\mu g\, g^{-1}$ or about 0.3% (Table 3) [21]. At a total PCB (A1111) loading of 500 μg, 1.5 μg of planar PCBs/PCDDs/PCDFs would be loaded onto the PGC column. This approaches 50% of the amount (3–5 μg) estimated to degrade the chromatographic efficiency by 10%. At this same amount of A1111, the mono-*ortho* PCB loading would be 24 μg, over 20 times the maximum acceptable loading value of mono-*ortho* PCBs for the column. The net result is that loading of mono-*ortho* PCBs onto the PGC column tends to be the limiting factor for biotic samples from the environment. The greater the percentage of planar compounds in a mixture, the lower the total loading capacity will be. This can be used as a predictive tool for determining loading capacity of samples onto the PGC column, provided an estimate of the amount of planar compounds can be readily made before injection onto the column. Environmental sample extracts were taken through a rigorous cleanup procedure before PGC column injections to reduce or eliminate matrix effects on the fractionation. This type of reactive cleanup does destroy polycyclic aromatic hydrocarbons and some of the labile organochlorine pesticides that are co-extracted. There are small amounts of polychlorinated naphthalenes, and polychlorinated diphenyl ethers that are co-extracted and which are planar

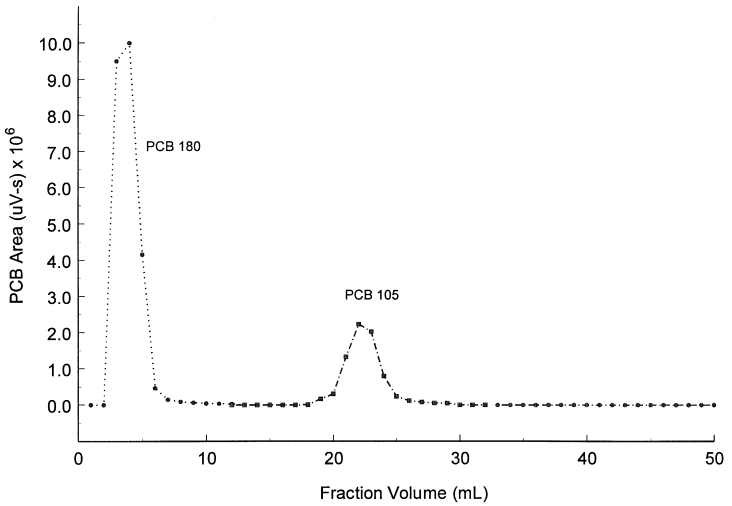

Fig. 3. Summary of GC–ECD analysis of 50 consecutive 1-ml fractions of HPLC eluent from PGC column using the solvent program in Table 1. Shows loading of PCB 180 at 10 μg while monitoring PCB 105 at 1 μg.

enough to potentially contribute to the loading on the PGC column. But these compounds are usually found at pg g^{-1} to ng g^{-1} levels [2,24].

An application of estimated total PCB loading as a predictive tool is shown using an in-house whole fish reference sample. Portions of a carp homogenate were fractionated on the PGC column after cleanup [5,6]. Pre-screening by GC–ECD indicated a total PCB concentration of about 7 μg g^{-1}. Using the 0.3% average concentration of co-planar PCBs in A1111, we predicted a co-planar concentration of 0.02 μg g^{-1}. Based on measured column capacity for non-*ortho* PCBs and PCDDs/PCDFs, a maximum of ~150 g of this sample would result in overloading the column. The total concentration of the four non-*ortho* PCBs-81, 77, 126, 169-was determined by GC–MS to be 20 ng g^{-1} and the total 2378-substituted PCDD/PCDF concentration was determined to be 0.3 ng g^{-1}. The measured total planar concentrations was approximately the same as that predicted from the A1111 standard mixture. The predicted total concentration of mono-*ortho* PCBs from the A1111 standard was 0.4 μg g^{-1}, and the measured concentration of mono-*ortho* PCB concentration was 0.5 μg g^{-1}. Based on the mono-*ortho* PCB concentrations, a sample of this fish weighing 4 g or less should be analyzed in order to prevent overloading of the PGC column by mono-*ortho* PCBs. For the carp sample we injected a 25 g-equivalent amount of extract onto the PGC column in order to specifically monitor what happened to the non- and mono-*ortho* PCBs. The non-*ortho* PCBs showed no breakthrough, but the mono-*ortho* PCBs did-for PCB 118 88% breakthrough; PCB 105 36% breakthrough; PCB 167 95% breakthrough. Injecting a smaller, 10 gram-equivalent of the extract eliminated the breakthrough for this fish sample.

For biotic samples with high concentrations of PCBs, such as fish and eggs of fish eating birds, a comparison of total PCBs, mono-*ortho* PCBs, non-*ortho* PCBs, and PCDDs/PCDFs fractionated on PGC and PX-21 carbon columns shows that relative concentrations of the groups varies somewhat with sample type. Total concentrations of mono-*ortho* PCB congeners range from 0.02 to 0.5 μg g^{-1} in most fish samples, average 0.04 μg g^{-1} for eggs of non-fish-eating birds, and range from 0.3 to 2.7 μg g^{-1} in eggs from fish-eating birds (Table 3). The concentrations of non-*ortho* PCBs range from 0.0003 to 0.008 μg g^{-1} for fish; 0.0002 to 0.001 μg g^{-1} for eggs of non-fish-eating birds; and 0.003 to 0.02 μg g^{-1} for eggs of fish-eating birds (Table 3). Adequate detection limits of planar congeners are generally achievable in fish samples while still fractionating mono-*ortho* substituted congeners. For egg samples, with high amounts of mono-*ortho*

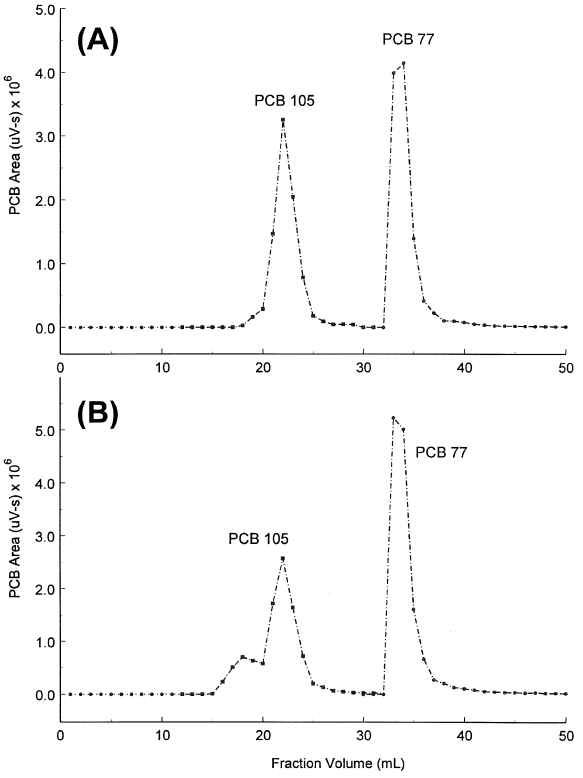

Fig. 4. Summary of GC–ECD analysis of 50 consecutive 1-ml fractions of HPLC eluent from PGC column using the solvent program in Table 1. Effect of non-*ortho* PCB 77 on mono-*ortho* PCB 105. Shows (A) 3 μg PCB 77, 1 μg PCB 105; (B) 5 μg PCB 77, 1 μg PCB 105.

PCBs it seems impractical to optimize the fractionation scheme for the mono-*ortho* PCBs, because the limits of detection for non-*ortho* PCBs and PCDDs/PCDFs may be unattainable. Most eggs of fish-eating birds that we have studied have greater concentrations of mono-*ortho* and non-*ortho* PCBs, and we typically analyze 10–25 g of these samples in order to achieve 1 pg g^{-1} detection limits for 2378-substituted PCDDs and PCDFs. However, mono-*ortho* PCB overloading problems thus occur. For example, a 10 g sample of a Caspian Tern egg can have 7 μg of total mono-*ortho* PCBs, which exceeds the PGC loading capacity of 2 μg.

Multiple injections on the PGC column of portions of a sample extract to eliminate overloading can be done, but this may only be practical if solvent use and time constraints are not an issue. An alternative would be to split samples or sample extracts that may have high mono-*ortho* and/or non-*ortho* PCBs. Inject the larger portion and collect non-*ortho* PCB and PCDD/PCDF fractions only then inject the smaller portion for bulk PCB and mono-*ortho* PCB fractionation. A third alternative would be to collect the bulk congener PCBs and the mono-*ortho* PCBs together in one fraction and rely on the GC to separate the mono-*ortho* PCB congeners from their interferences on a high-resolution capillary GC column.

Acknowledgements

This research was funded, in part, through the US Fish and Wildlife Service, Region 3 Cooperative Education Program. We would like to thank the following Environmental and Contaminants Research Center staff for their technical assistance: Jim Zaijcek, Paul Peterman. We would also like to thank Tim Kubiak and Diane Boellstorff of the East Lansing, MI, USA Field Office of the US Fish and Wildlife Service for collection of the native fish and bird samples. We would also like to thank Paul Peterman, Carl Orazio, and Lisa Williams for thoughtful comments on earlier drafts.

References

[1] P. de Voogt, D.E. Wells, L. Reutergårdh, U.A.Th. Brinkman, Int. J. Environ. Anal. Chem. 40 (1990) 1–46.
[2] P.H. Peterman, R.W. Gale, D.E. Tillitt, K.P. Feltz, in: G.K. Ostrander (Ed), Techniques in Aquatic Toxicology, Lewis Publishers, 1996, Ch. 27, p. 517.
[3] P. Hess, J. de Boer, W.P. Cofino, P.E.G. Leonards, D.E. Wells, J. Chromatogr. A 703 (1995) 417–465.
[4] P. De Voogt, P. Haglund, L.B. Reutergårdh, C. de Wit, F. Waern, Anal. Chem. 66 (1994) 305–311A.
[5] K. Echols, R. Gale, D. Tillitt, T. Schwartz, J. O'Laughlin, ETAC 16 (1997) 1590–1597.
[6] K.P. Feltz, D.E. Tillitt, R.W. Gale, P.H. Peterman, Environ. Sci. Technol. 29 (1995) 709–718.
[7] L.R. Snyder, Principles of Adsorption Chromatography, Marcel Dekker, 1968, pp. 75–96.
[8] L.M. Smith, Anal. Chem. 53 (1984) 2152–2154.
[9] J.N. Huckins, D.L. Stalling, J.D. Petty, J. Assoc. Off. Anal. Chem. 63 (1980) 750–755.
[10] N. Kannan, G. Petrick, D. Schulz, J. Duinker, Chemosphere 23 (1991) 1055–1076.

[11] N. Tanaka, T. Tanigawa, K. Kimata, K. Hosoya, T. Araki, J. Chromatogr. 549 (1991) 29–41.
[12] S.S. Atuma, Ö. Andersson, Chemosphere 27 (1993) 1–8.
[13] J.L. Sericano, A.M. El-Husseini, T.L. Wade, Chemosphere 23 (1991) 915–924.
[14] S.K. Poole, C.F. Poole, Anal. Commun. 34 (1997) 247–251.
[15] P. Haglund, L. Asplund, U. Järnberg, B. Jansson, J. Chromatogr. 507 (1990) 389–398.
[16] B.J. Bassler, R. Kaliszan, R.A. Hartwick, J. Chromatogr. 461 (1989) 139–147.
[17] J.H. Knox, B. Kaur, in: P.R. Brown, R.A. Hartwick (Eds.), High-Performance Liquid Chromatography, Wiley, 1989, Ch. 4, pp. 189–222.
[18] J.H. Knox, B. Kaur, G.R. Millward, J. Chromatogr. 352 (1986) 3–25.
[19] T.R. Schwartz, D.E. Tillitt, K. Feltz, P.H. Peterman, Chemosphere 26 (1993) 1443–1460.
[20] M.D. Erickson, Analytical Chemistry of PCBs, Butterworth, 1986.
[21] N. Kannan, S. Tanabe, T. Wakimoto, R. Tatsukawa, J. Assoc. Off. Anal. Chem. 70 (1987) 451–454.
[22] G.W. Bowles, M.J. Mulvihill, B.R.T. Simoneit, A.L. Burlingame, R.W. Risebrough, Nature 256 (1975) 305–307.
[23] T. Wakimoto, N. Kannan, M. Ono, R. Tatsukawa, Y. Masuda, Chemosphere 17 (1988) 743–750.
[24] P. H. Peterman, personal communication.