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Separation of extracts from biological tissues into polycyclic aromatic hydrocarbon, polychlorinated biphenyl and polychlorinated dibenzo-*p*-dioxin/polychlorinated dibenzofuran fractions prior to analysis

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Abstract

A low-pressure liquid chromatography method is presented for separating polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDDs/PCDFs) from biological tissue extracts. After removing lipid from extracts, the PAHs are separated from PCBs and PCDDs/PCDFs on a deactivated 13–24 μm silica gel column. The PCBs are subsequently separated from PCDDs/PCDFs by collecting the first fraction from an automated three column cleanup procedure for PCDDs/PCDFs. The complete method has been used to obtain high recoveries of the three compound classes for analysis by GC–electron capture detection (PCBs) or GC–MS (PAHs and PCDDs/PCDFs).

Keywords: Environmental analysis; Polynuclear aromatic hydrocarbons; Polychlorinated biphenyls; Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins (PCDDs)/polychlorinated dibenzofurans (PCDFs) are three related classes of toxic organic chemicals that are often found together in a variety of environmental samples. Unfortunately the three compound classes cannot be analyzed in one purified fraction since some PCB congeners [1,2] or their metabolites [3] can interfere with the analysis of PCDD compounds by low-resolution gas chromatography–mass spectrometry (GC–MS), e.g., the fragment ion at *m/z* 322 from a heptachlorobiphenyl can interfere with the analysis of the very toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) [1].

There are also close similarities in the chemical and physical properties of the PAHs, PCBs and PCDDs/PCDFs and consequently no single adsorbent has been found for separating them into compound classes. However two-dimensional HPLC (high-performance liquid chromatography) systems have been used to perform the separation [4,5]. In

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the first system [4], a column containing an amino-bonded phase was coupled to a porous graphitic carbon (PGC) column. It was possible to separate the PAHs containing three or more rings from the diaromatic compounds (PCBs and PCDDs/PCDFs) on the amino column and the diaromatic fraction was then separated by the PGC column, according to planarity, into di- to tetra-*ortho* PCBs, mono-*ortho* PCBs and a fraction containing non-*ortho* PCBs and PCDDs/PCDFs. More recently a nitro column coupled to a column with bonded pyrene (PYE) has been used to obtain the same separation in a more reproducible manner with the consumption of only 70 ml of solvent [5]. There are some disadvantages to the HPLC method. In the first place the pyrene column must be cycled between 0°C and 25°C during the fractionation process. Furthermore the procedure involves the use of analytical HPLC columns and these columns may be subjected to overloading with extracts from heavily contaminated samples. This overloading could lead to incomplete separation of the compound classes and cross contamination of other samples. In our laboratory, cleanup of PCDDs/PCDFs in extracts from environmental samples is carried out using a computer-controlled low pressure liquid chromatography system containing two alumina columns and a carbon column [6]. We find that cross-contamination is minimized in this system since the adsorbents are replaced between samples. In the present study we modified the original computer-controlled system to collect PCBs as a separate fraction. We also determined that the PAHs could be separated from the PCBs and PCDDs/PCDFs in a preliminary step. Two columns were investigated for this purpose, an intermediate particle size (13–24 μm) silica gel column and a charge-transfer HPLC column containing tetrachlorophthalimidopropyl (TCPP) bonded to silica.

2. Experimental

2.1. Materials

Silica gel, LPS-1, 13–24 μm particle size, was obtained from Whatman (Clifton, NJ, USA); silica gel, 100–200 mesh, Type 60 Å from Mallinckrodt (Paris, KY, USA); the 5-μm TCPP preparative (25 cm×1.0 cm I.D.) HPLC column from Shandon Scientific (Runcorn, UK via Keystone Scientific, Bellefonte, PA, USA); benzenesulfonylpropyl bonded silica (SCX) from Varian Sample Preparation Products (Harbor City, CA, USA); acidic aluminum oxide from J.T. Baker (Phillipsburg, NJ, USA); styrene–divinylbenzene polymer beads, Bio-Beads SX3, from Bio-Rad Labs. (Richmond, CA, USA) and Anderson AX21 graphitized carbon from colleagues at Research Triangle Labs. (Research Triangle Park, NC, USA). All solvents were obtained from Burdick and Jackson (Muskegon, MI, USA) as B&J Brand with the exception of benzene, which was obtained from J.T. Baker as "Baker Resi-Analyzed" grade. Unlabeled and labeled PAH and PCDD/PCDF compounds were obtained from Cambridge Isotope Labs. (Andover, MA, USA) (^{13}C labels for PCDD/PCDF compounds and deuterium labels for PAH compounds). Other chemicals were of reagent grade quality.

2.2. Extraction from biological tissues (Step 1)

The extraction step was carried out by homogenization with solvent followed by centrifugation as described previously [6]. However CH_2Cl_2–hexane (50:50, v/v) was used as the extraction solvent instead of benzene and the samples were spiked with a mixture of seventeen deuterated PAHs (10 ng/compound) in addition to eleven ^{13}C-labeled 2,3,7,8-substituted PCDD/PCDF compounds. Fig. 1 shows a flow-diagram for the steps involved in the isolation and analysis of PAHs, PCBs and PCDDs/PCDFs from biological tissues.

2.3. Removal of lipid from extracts by gel permeation chromatography (<100 mg) or polymer film dialysis (>100 mg) (Step 2)

The Bio-Beads SX3 for gel permeation chromatography (GPC) were allowed to swell overnight in CH_2Cl_2–cyclohexane (50:50, v/v) using a minimum of 3.75 ml solvent/g polymer. The swollen beads were pipetted into an 22 cm×8 mm I.D. glass column to give a bed length of 21.5 cm. The sample was added to the column in an 0.5 ml aliquot of the solvent and the column was eluted at a rate of 2 ml/min. The first 7 ml of eluent contained the lipids

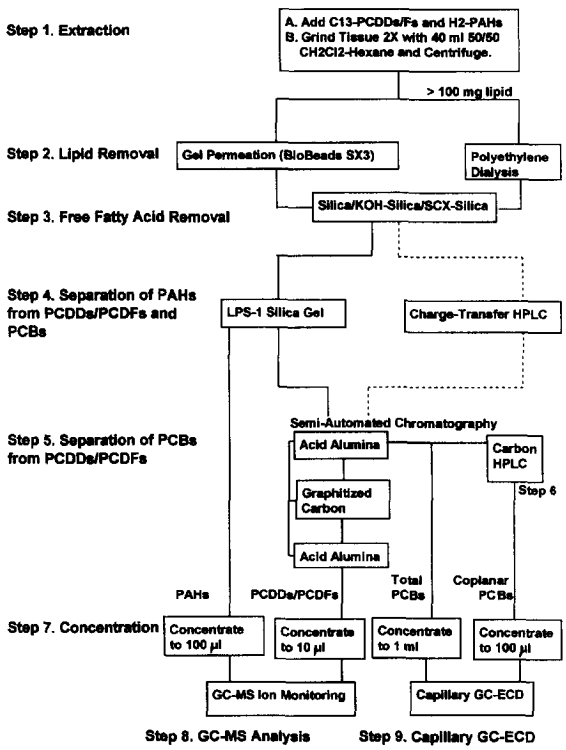

Fig. 1. Flow-diagram for steps involved in the isolation and analysis of PAHs, PCBs and PCDDs/PCDFs from biological tissues. Individual steps are referenced at appropriate places in Section 2.

and were discarded. The PAHs, PCBs and PCDDs/PCDFs were collected in the fraction eluting between 8 and 25 ml.

The procedure for polymer film dialysis has been described in the literature [7] and involves the addition of the extract in cyclopentane (11 ml) to a polyethylene bag, followed by placement of the bag in a beaker containing 200 ml cyclopentane. Over a 24-h time period the contaminants diffuse through the bag under the influence of osmotic pressure but the larger lipid molecules such as the triglycerides cannot diffuse as they have diameters exceeding the pore diameters of the film.

2.4. Removal of free fatty acids and other polar lipid contaminants (Step 3)

A series of adsorbents was added to a 2 cm I.D. column. In sequence from top to bottom these adsorbents were: (1) silica gel, 100–200 mesh, 5 cm; (2) sodium carbonate, 0.5 cm; (3) KOH-treated silica, 4 cm; (4) silica gel, 0.4 cm; (5) SCX silica, 3 cm; (6) silica gel, 0.4 cm and (7) KOH-treated silica gel, 0.4 cm. After prewashing with 100 ml hexane, a total extract from the gel permeation procedure or an extract from the dialysis procedure, concentrated to 10 ml, was added to the column. In the case of the dialysis extract the sample extract was followed by 18 ml CH_2Cl_2–hexane (50:50). The total eluent volume was brought to 100 ml by eluting with 72 ml of hexane for samples prepared by the dialysis method, and 82 ml of hexane for samples prepared by the GPC method.

2.5. Separation of PAHs from PCBs and PCDDs/PCDFs (Step 4)

An 8 mm I.D. column was filled with 21.5 cm of activated (120°C for 16 h) LPS-1 silica gel and the silica gel was partially deactivated with 100 ml of water-saturated toluene–alumina-dried hexane–water-saturated hexane (1:49.5:49.5, v/v). Water-saturated solvents were prepared by equilibrating each solvent with water for 5 min in a separatory funnel. Hexane was dried by pumping 4 l at 10 ml/min through two coupled 8 mm I.D. columns containing 21 cm and 24 cm acid alumina, respectively. Nitrogen at 275 kPa was used to achieve a flow-rate of 1.5 ml/min in the silica gel column. An extract from the multiadsorbent column in 1 ml cyclohexane was added to the column and the PCBs and PCDDs/PCDFs were eluted with 32 ml of water-saturated toluene–alumina-dried hexane–water-saturated hexane (1:49.5:49.5, v/v). The PAHs were then eluted with 25 ml CH_2Cl_2–hexane (20:80, v/v).

Charge-transfer chromatography was also used to separate PAHs from PCBs and PCDDs/PCDFs. The preparative TCPP column was connected to a Waters ALC/GPC-204 liquid chromatograph (Millipore, Waters Chromatography Division, Milford, MA, USA) and equilibrated with toluene–hexane (50:50, v/v) at a flow-rate of 1.5 ml/min. Samples were injected in 1 ml volumes and elution of the compounds was monitored by measuring UV absorbance at 313 nm. The PCBs and PCDDs/PCDFs were

eluted in 19.5 ml from the point of injection and the PAHs were then eluted with 45 ml CH_2Cl_2.

2.6. Separation of PCBs from PCDDs/PCDFs (Step 5)

The first fraction from the LPS-1 column was dried over Na_2SO_4 and made up to a volume of 100 ml as a toluene–hexane (10:90, v/v) solution. This solution was cleaned up using a computer-controlled chromatography system developed for PCDD/PCDF cleanup, consisting of acid alumina, carbon and acid alumina columns connected in series [6]. The PCBs were collected in the first fraction from the first alumina column. As described in Section 3.3, the system was optimized to achieve a complete separation of PCBs from PCDDs/PCDFs and to recover certain TCDFs that are not strongly adsorbed to alumina. The first column consisted of two directly-coupled acid alumina columns and the sample was added to the system in 100 ml of toluene–hexane (30:70, v/v). The first eluent for this column was 85 ml of CH_2Cl_2–hexane (8:92, v/v) and the PCDDs/PCDFs were then eluted from the two columns using 130 ml of CH_2Cl_2–hexane (50:50, v/v). The final acid alumina column had a diameter of 11 mm. Interfering compounds were eluted from the column with 74 ml of CH_2Cl_2–hexane (3:97, v/v). followed by the elution of the PCDDs/PCDFs with 150 ml of CH_2Cl_2–hexane (50:50, v/v). The remaining steps were carried out as described in the original procedure [6].

2.7. Separation of non-ortho- and mono-ortho PCBs from di-ortho PCBs (Step 6)

This separation was accomplished by HPLC using the Waters ALC/GPC-204 liquid chromatograph together with a PGC column (100×4.7 mm, 7-μm particle size, Hypercarb, Shandon Scientific). A Rheodyne 7040 valve (Rheodyne, Cotati, CA, USA) was used for reversing solvent flow through the column. The toluene in the PCB fraction from the alumina column was solvent-exchanged to nonane and the extract was then concentrated to a volume of 100 μl and injected onto the Hypercarb column. The nonplanar PCBs were eluted from the column in 6 ml hexane at a flow-rate of 2 ml/min, and the mono-*ortho* and non-*ortho* PCBs were eluted in the reverse direction with 18 ml toluene. The column was washed with an additional 30 ml toluene and, after reversing the solvent flow and washing with 30 ml hexane, the system was ready for the next sample. This procedure is a modification of methodology described elsewhere [8].

2.8. Concentration of fractions for analysis (Step 7)

Solvent concentration was accomplished by boiling with partial reflux on hot water baths followed by nitrogen evaporation. The PAH and PCDD/PCDF fractions were concentrated to final volumes of 100 μl and 10 μl, respectively. After concentration to 100 μl the PCB fraction was diluted to a final volume of 1 ml with hexane since the chromatographic separation on a capillary GC column was adversely affected by more than 10% toluene in the injection solution.

2.9. Analysis of PAHs and PCDDs/PCDFs by GC–MS (Step 8)

Final analysis of each fraction was carried out by capillary GC–low-resolution MS using a DB5 column coupled to a Hewlett-Packard 5970 mass selective detector (Hewlett-Packard, Wilmington, DE, USA). In the case of the PAHs the most prominent molecular ions for fourteen US Environmental Protection Agency (EPA) priority pollutant PAHs and their deuterated analogs were monitored. The PCDD/PCDF analysis was restricted to compounds containing from four to eight chlorine atoms, and for each congener group the two most prominent molecular ions for the native compounds and the ^{13}C-labeled standards were monitored. The tolerance range for the molecular ion isotope ratios was ±20% for this study but it has now been reduced to ±15%, in agreement with the requirements of EPA Method 8280.

2.10. Analysis of PCBs by capillary GC–electron capture detection (Step 9)

GC analysis was carried out with a Hewlett-Packard 5840A instrument with a splitless glass capil-

lary inlet, a Hewlett-Packard HP-5 (Ultra 2) capillary column, a ^{63}Ni electron capture detector and an ASCII interface board, through which the calibrated data tables were transmitted to a personal computer. The computer generated reports of congener structure identification and concentrations using a Fortran program. The Biomedical Data Package (BMDP) was used for data analysis and quality control [9].

3. Results and discussion

3.1. Selection of adsorbents to separate PAHs from PCDDs/PCDFs

Earlier work in our laboratory showed that a computer-controlled chromatographic system of alumina and carbon columns efficiently removed contaminants that interfered with PCDD/PCDF analysis. When attempts were made to separate PAHs using this chromatography system it was found that nine of the PAHs (anthracene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, benzo[*ghi*]perylene and dibenz[*a,h*]anthracene) were irreversibly adsorbed to one of the adsorbents, probably carbon, since this adsorbent has a strong affinity for PAHs. The other PAHs were eluted in waste solvents (naphthalene and fluorene) or in the PCDD/PCDF fraction (phenanthrene and fluoranthene).

The results from these experiments indicated that the PAHs would have to be separated from both the PCBs and the PCDDs/PCDFs prior to using the computer-controlled chromatography system to separate the PCBs from the PCDDs/PCDFs. Large particle-size (>100 μm) silica gels and silica derivatized with polar groups (amino and hydroxyl) were initially used to separate the PAHs from the PCDDs/PCDFs. However, even with the adsorbent that gave the best separation, a diaminopropyl silica, several of the lower-molecular-mass PAHs were eluted with hexane prior to the last eluting TCDD isomer pair (1,2,6,7- and 1,2,8,9-) [10]. When a smaller-particle-size (13–24 μm) silica gel was used, with dry hexane as an eluent, only naphthalene was eluted prior to the 1,2,6,7- and 1,2,8,9-TCDD isomers. It was found that solvent flow-rates of 1.5 ml/min could be achieved with this particle-size silica using pressures of 20 p.s.i. (1 p.s.i. = 6894.76 Pa). This pressure is below the upper pressure limit of the glass chromatography columns and does not result in leaks from the PTFE chromatography fittings.

Electron-acceptor (EA) adsorbents were also investigated as alternative adsorbents for the same separation since the PAHs are strong electron donors (EDs) [11] while the PCDDs are weak EDs and interact with EA phases primarily by dipole forces [12]. An HPLC column, containing the EA compound TCPP bonded to silica, was successfully used to separate TCDD isomers from the sixteen priority pollutant PAHs. However the recoveries of the PAHs and the 1,2,6,7/1,2,8,9-TCDD isomer pair were lower (75±7% and 78%, respectively) than those found with the silica adsorbent (98±26% and 92%, respectively).

3.2. Separation of PAHs from PCBs and from PCDDs/PCDFs (Step 4)

The PCB formulations, Aroclors 1016, 1221, 1254 and 1260, contain the range of PCB congeners found in environmental samples in the USA. When a 1:1:1:1 mixture was separated by the silica column it was anticipated that the PCBs would elute in the same fraction as the PCDDs/PCDDs. However, it was found that congeners containing three or more chlorine atoms co-eluted with the PCDDs/PCDFs, whereas monochloro and dichloro congeners were more strongly retained and co-eluted with the PAHs. The silica was deactivated with various amounts of water to recover the complete range of PCB congeners in the PCDD/PCDF fraction. The optimum separation was achieved when the column was equilibrated with a 50:50 mixture of dry hexane and water-saturated hexane containing 1% water-saturated toluene. With this solvent system it was found that the two-ring PAH compounds were eluted almost entirely in the PCB/PCDD/PCDF fraction. These compounds were therefore omitted from the analysis procedure. The three-ring compounds, anthracene and phenanthrene, and the four-ring compound, pyrene, were recovered primarily in the PAH fraction, but 30 to 40% of the compounds were also present in the PCB/PCDD/PCDF fraction. The highly delocalized π electron systems of these

compounds [13] could have the effect of reducing the strength of their interactions with silica OH groups. The other ten PAHs were present with high recovery values (99±6%) in the PAH fraction and the two PCB test compounds, 2-monochlorobiphenyl and 2,4-dichlorobiphenyl, were recovered primarily in the PCDD/PCDF fraction (54% and 59%, respectively).

The recoveries of the PAHs in the experiments described above were determined by adding deuterated analogs as internal recovery standards after completion of the chromatographic fractionation step. Additional experiments were carried out using native PAHs as internal recovery standards for deuterated compounds. When the data from the two types of analysis were compared, it was apparent that the recoveries of the deuterated analogs of anthracene, phenanthrene and pyrene were higher than the recoveries of the native compounds (Fig. 2). As a practical matter the use of deuterium-labeled PAHs as internal standards may lead to a 10 to 20% underestimation of the recoveries for anthracene, phenanthrene and pyrene. The isotope effect does not occur with the other PAH compounds since they are strongly adsorbed to silica gel.

3.3. Separation of PCBs from PCDDs/PCDFs (Step 5)

It was previously found in our laboratory that μg/g quantities of PCBs in environmental samples do not interfere with the analysis of pg/g quantities of PCDD/PCDF compounds when sample extracts are cleaned up with a computer-controlled three column chromatography system. However, the PCB removal efficiencies of each step in the cleanup method were unknown. In this study data on the removal efficiencies were obtained using a 1:1:1:1 mixture of the PCB formulations, Aroclors 1016, 1221, 1254 and 1260. While it was found that the PCBs were effectively removed (>98%) by the chromatographic process, no single waste fraction contained all of the PCBs. The highest total PCB recovery (76%) was found in the first fraction from the first alumina column. However four mono-*ortho* *para*-substituted PCB congeners (2,3,4′-, 2,4,4′-, 2,3,3′,4,4′- and 2,3′,4,4′,5-) were not detected in this fraction but were recovered completely in the next

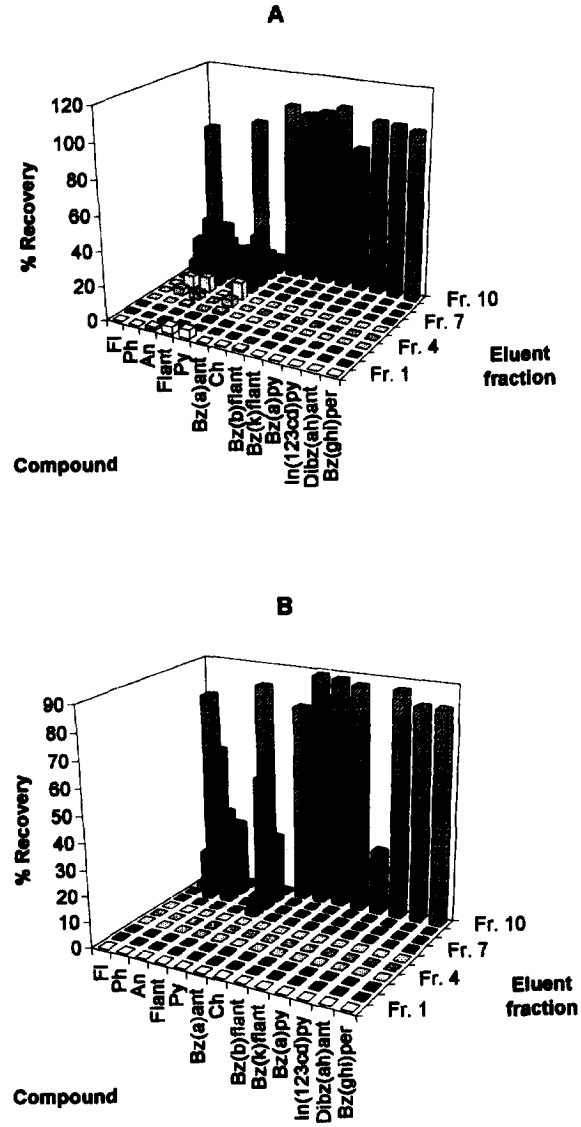

Fig. 2. Elution profiles of PAHs from LPS-1 silica gel deactivated with water-saturated hexane. The eluents were as follows: fraction 1, 20 ml of water-saturated toluene–alumina-dried hexane–water-saturated hexane (1:49.5:49.5, v/v), fractions 2–9, 2 ml of water-saturated toluene–alumina-dried hexane–water-saturated hexane (1:49.5:49.5, v/v) and fraction 10, 20 ml CH_2Cl_2–hexane (20:80, v/v). The chromatographic conditions were optimized to collect PCBs in fractions 1–7 and PAHs in fractions 8–10. (A) Shows the separation of unlabeled PAHs with recoveries determined using labeled PAHs and (B) shows the separation of labeled PAHs with recoveries determined using unlabeled PAHs. Analysis was by capillary GC–MS.

fraction, the first fraction from the carbon column. The trace quantities of toxic coplanar (non-*ortho*) PCBs (3,3′,4,4′-, 3,3′,4,4′5- and 3,3′4,4′5,5′-) were not determined in these analyses but on the basis of their planarity and the delocalization of charge to the para positions, it was expected that they would be even more strongly retained than the mono-*ortho para*-substituted congeners.

The resolution of the first chromatographic step in the automated system was increased in order to obtain higher PCB recoveries at this step. The increase in resolution was achieved by replacing the single acid alumina column by two acid alumina columns in-series. A mixture of the three toxic coplanar PCBs, eight mono-*ortho* PCBs (2′,3,4,4′,5-; 2,3′,4,4′5-; 2,3,4,4′,5-; 2,3,3′,4,4′-; 2,3′,4,4′,5,5′-; 2,3,3′,4,4′,5-; 2,3,3′,4,4′,5′- and 2,3,3′,4,4′5,5′-) and two di-*ortho* PCBs (2,2′,3,4,4′,5,5′- and 2,2′,3,3′4,4′,5-) were loaded on the coupled columns in 130 ml toluene–hexane (30:70, v/v) followed by a wash of 85 ml CH_2Cl_2–hexane (8:92, v/v). It was found that all the PCB congeners were recovered in the eluent in the range of 76 to 122%, with 3,3′,4,4′-TCB having the lowest recovery.

The potential for PCDDs/PCDFs to elute with the PCBs was investigated with 1,2,3,4-TCDF. This compound was selected as TCDF isomers with chlorines in the 4 or 6 positions are weakly adsorbed to alumina [14]. No traces of 1,2,3,4-TCDF were found in the PCB fraction and high recoveries were obtained in the next fraction, the PCDD/PCDF fraction. The final acid alumina column in the automated system also had to be modified to obtain high recoveries of this PCDF congener (see Section 2.6).

3.4. Method validation for PAH, PCB and PCDD/PCDF recoveries from biological tissues

The liquid chromatography separation steps were incorporated into a cleanup method for isolating the PAH, PCB and PCDD/PCDF compounds from biological tissues. Extracts were obtained by high speed homogenization of tissues with CH_2Cl_2 and hexane (Step 1). For those extracts containing less than 100 mg lipid, the lipid was removed from the extracts using an 8 mm I.D. GPC column (Step 2). Larger quantities of lipid were removed by dialysis (Step 2). After removal of the neutral lipids, free fatty acids, phenolics and any basic compounds were removed from the extract using a column containing silica gel, KOH-treated silica gel and benzenesulfonic acid-bonded silica gel (Step 3). These procedures do not involve the use of strong acids such as sulfuric acid that can react with PAHs, or alkaline digestion, which can dechlorinate higher-chlorinated PCDD/PCDF compounds.

For the purposes of method validation two aquatic sample types were selected for spiking experiments: (1) larvae of the aquatic midge, *Chironomus tentans*, as representative of samples containing <100 mg lipid and (2) oil from chinook salmon (*Oncorhynchus tshawytscha*) as representative of samples containing high levels of lipid (≥ 1 g). These samples were obtained respectively from laboratory cultures and the Pacific Northwest coast of the USA and they generally contained low or non-detectable background concentrations of the test compounds. In addition to the biological matrices, spiked method blanks were prepared for the low-level lipid (larvae) procedure.

3.4.1. PAHs

Data for the recovery of fourteen PAHs from spiked samples are shown in Table 1. Recoveries from the larvae method blanks varied from 41% to 76% with the exception of dibenzo[*a*,*h*]anthracene, where the recoveries from duplicate samples were only 24% and 33%. In the absence of sample matrix this compound appears to be more strongly adsorbed to silica gel than the other PAHs. Background contributions to the spike level of 40 ng varied from 6.8 to 8.8 ng for the three most ubiquitous PAHs (phenanthrene, fluoranthene and pyrene). Background contributions for the other PAHs were less than 1.6 ng. The larvae were spiked with a mixture of the native and deuterated PAHs to simulate environmental analyses where labeled internal standards are added at the start of the sample preparation procedure. The deuterated standards are then used to correct for recovery losses of the native compounds. Since the primary objective of the validation experiments was to determine absolute recoveries, data in Table 1 are presented for recoveries of the deuterated compounds, using 2,2′,5,5′-TCB as an injection

Table 1
Recovery of PAHs from spiked samples using a procedure for separating PAHs, PCBs and PCDDs/PCDFs from biological tissue extracts

Compound	Recovery (%)[a]					
	Larvae method blanks		Larvae (1.2–1.5 g)	Salmon oil (1.0 g)		
	Blank background corrected as % of spike	Background corrected	Deuterated standards (av.±S.D.)	Blank background as % of spike	Background corrected low level	High level
Fluorene	3.6	48, 63	76±26	210	9, 51	75, 65
Phenanthrene	22	53, 78	71±20	340	32, 53	63, 35
Anthracene	0.0	47, 64	61±23	16	11, 31	38, 21
Fluoranthene	21	47, 76	78±11	138	46, 68	81, 61
Pyrene	17	45, 74	66±19	59	21, 30	40, 24
Benzo[*a*]anthracene	0.0	50, 66	67±6.7	2.9	41, 64	67, 54
Chrysene	0.6	52, 66	67±7.0	7.1	56, 87	89, 70
Benzo[*b*]fluoranthene	2.6	44, 64	66±5.3	8.0	44, 62	67, 53
Benzo[*k*]fluoranthene	0.0	46, 65	60±6.7	2.4	44, 66	71, 56
Benzo[*e*]pyrene	0.7	45, 69	NA	NA	NA	NA
Benzo[*a*]pyrene	0.0	41, 59	49±7.8	25	36, 68	75, 58
Indeno[1,2,3-*cd*]pyrene	0.7	41, 57	46±7.8	4.7	41, 63	86, 67
Dibenzo[*a,h*]anthracene	0.0	24, 33	43±9.5	0.0	41, 63	69, 53
Benzo[*ghi*]perylene	0.5	41, 60	51±7.0	4.7	39, 65	71, 56

NA = Not analyzed.

[a] The larvae method blanks and salmon oil were spiked with a mixture of native PAHs, and recoveries were determined by capillary GC–low-resolution MS using deuterated PAHs as injection standards. Spike levels were 40 ng/compound for the method blanks and 10 ng and 40 μg for the low-level and high-level spiked salmon oil samples, respectively. The larvae were spiked with a mixture of deuterated PAHs at a level of 10 ng/compound and the recoveries were determined by capillary GC–low-resolution MS using 2,2′5,5′-TCB as an injection standard. Two replicates were used for the larvae method blank and salmon oil experiments and three replicates for the larvae experiments.

standard. The deuterated-compound recoveries varied from 43% to 76%.

The salmon oil samples were spiked at two different levels, 10 ng and 40 μg. It is apparent that the background concentrations of fluorene, phenanthrene and fluoranthene in the oil exceeded the low-level spike concentration, and therefore the background corrected low-level recoveries for these compounds are subject to error. However background contributions to the high-level spiked samples were negligible and it was found that all the PAHs in these samples had recoveries varying from 53% to 89% with the exception of anthracene, phenanthrene and pyrene where recoveries from duplicate samples varied from 21% for anthracene to 63% for phenanthrene. As discussed above, these compounds are not as strongly retained by silica gel as the other PAHs and consequently can be partially lost into the PCB/PCDD/PCDF fraction.

3.4.2. PCBs

The larvae method blanks were spiked with a mixture of eighteen di- to tetra-*ortho* PCBs, eight mono-*ortho* PCBs and four non-*ortho* PCBs. The first PCB eluting from the GC column, 4-MCB, was not determined since it was obscured by an impurity associated with the injection solvent front. Background contributions from the method blanks to the other congeners were less than 15% of the spike levels except for the three congeners, 2,3′,4,4′,5-, 2,2′,3,4,4′,5′- and 2,2′,3,3′,4,5′,6,6′-, which had contributions ranging from 36% to 44%. After correcting for these contributions recoveries ranged from 40% to 126% (Table 2). The two recovery values below 50% were associated with a monochloro and a dichloro congener. The losses could have occurred as a result of volatilization during the concentration of extracts or as a result of elution of the congeners in the PAH fraction from silica gel.

The larvae were spiked with a 1:1:1:1 mixture of Aroclors 1016, 1221, 1254 and 1260. While 68 peaks were monitored during the analyses, for ease of presentation data are only reported for those congeners that were spiked into the method blanks.

Table 2
Recovery of PCBs from spiked samples using a procedure for separating PAHs, PCBs and PCDDs/PCDFs from biological tissue extracts

Congener	Recovery (%)			
	Larvae[a]	Larvae[b]	Salmon oil ($n=3$, av.±S.D.)[c]	
	blanks ($n=2$)	(Aroclor spike) ($n=3$, av.±S.D.)	Background as % of spike	Background corrected
4	37, 52	51±22		
2,2′ (+2,6)	40, 41	50±14		
2,4′ (+2,3)	111, 126	60±14		
2,2′,5	97, 98	69±29		
2,4,4′	102, 96	59±14		
2,2′,5,5′	91, 92	67±25		
2,2′,3,5′	91, 92	66±23		
2,2′,4,5,5′	91, 87	78±31		
2,2′,3,4,5′	83, 85	79±30		
3,4,4′,5	71, 80		17	92±16
3,3′,4,4′ (+2,3,3′,4′,6)	97, 97	83±28	130	57±5.9
2,3′,4,4′,5′	106, 104		111	69±23
2,3′,4,4′,5	46, 67	83±29	211	101±12
2,3,4,4′,5	96, 92		8.1	67±3.1
2,2′,4,4′5,5′ (+2,2′,3,3′,4,6′)	81, 80	69±25		
2,3,3′,4,4′	78, 75		97	64±11
2,2′,3,4,4′,5′	78, 84	112±86		
3,3′,4,4′,5	94, 87		19	88±1.4
2,2′,3,4,4′,5′,6	93, 88	79±40		
2,3′,4,4′,5,5′	102, 97		26	87±13
2,3,3′4,4′,5 (+2,2′,3,3′,4,4′,6)	102, 95	83±39		
2,2′,3,3′,4,5′,6,6′	81, 77			
2,3′,3,4,4′,5′	95, 94		23	85±7.2
2,2′,3,4,4′,5,5′	84, 77	87±51	12	103±15
3,3′,4,4′,5,5′	120, 113		<1.0	112±12
2,3,3′,4,4′,5,5′	103, 96		<1.0	104±2.1
2,2′3,3′,4,4′5,5′	93, 89	92±51		
2,2′3,3′4,4′,5,6,6′	95, 90			
2,2′,3,3′,4,4′5,5′,6,6′	94, 88			
Total PCBs	95, 118	69±28		

All samples were analyzed by capillary GC–ECD.
[a] Samples were spiked with 4 non-*ortho*-, 8 mono-*ortho*- and a group of 18 di- and tri-*ortho*-substituted PCBs at a level of 5 ng/congener.
[b] Samples (1.2–1.6 g) were spiked with a mixture of four Aroclors (1016, 1221, 1254 and 1260) at a level of 200 ng/Aroclor. A total of 68 peaks were monitored but only data from congeners in common with the method blank spikes are presented. Where two congeners coelute, the second congener is shown in parentheses in column 1.
[c] Samples (1.0 g) were spiked with 4 non-*ortho*- and 8 mono-*ortho*-substituted PCBs at a level of 5 ng/congener.

Background contributions from the larvae were similar to those reported above for the method blanks and, after correction for this background, the average recoveries varied from 50% to 112%. However, the standard deviations were large and, in the case of four higher-chlorinated congeners, the relative standard deviation (R.S.D.) values were greater than 50%. These data reflect the fact that one of the three replicates had recovery values exceeding 100%, whereas the other two replicates had average recoveries near 50%, and the R.S.D. values for these two samples did not exceed 20%. The PCB analyses (Step 8) were carried out without benefit of internal standards and therefore they were not as reproducible as the PAH and PCB analyses (Step 9) where isotopically labeled internal standards were used.

Most toxic responses to PCBs can be attributed to the presence of one or more of a group of eight mono-*ortho* and four non-*ortho* PCB congeners. When a mixture of these congeners was spiked into

salmon oil, recoveries of the individual congeners exceeded 57%, although large corrections for background contributions were required for four congeners (Table 2).

3.4.3. PCDDs/PCDFs

Since these compounds are present in the environment at lower concentrations than PAHs or PCBs, they were spiked into the validation samples in amounts varying between 0.5 ng and 1 ng. The larvae method blanks were spiked with all the 2,3,7,8-substituted PCDDs/PCDFs together with three PCDDs/PCDFs which are strongly adsorbed by silica gel (1,2,6,7-TCDD, 1,2,8,9-TCDD and 1,2,8,9-TCDF) and 1,2,3,4-TCDF, the PCDD/PCDF compound in the tetra to octa group with the weakest adsorption on alumina. Salmon oil was spiked with a mixture which contained all the compounds spiked into the larvae method blanks with the exception of the 1,2,6,7- and 1,2,8,9-TCDDs. Finally the larvae were spiked to simulate an environmental sample, using a mixture of native PCDD/PCDF compounds and a group of ^{13}C-labeled PCDD/PCDF compounds for quantitation. Recoveries from the larvae were determined only for the ^{13}C-labeled compounds for reasons discussed above for the PAHs. Acceptable recoveries were obtained for all the spiked compounds (Table 3), with 1,2,3,4-TCDF having the lowest recovery (51% for the larvae method blanks and 42% for the salmon oil). The most likely explanation for this finding is that a certain amount of the compound is being eluted with the PCB fraction. Background signals for the spiked congeners were not detected in the larvae method blanks or larvae at detection limits close to 1 pg although trace background signals (2 to 30 pg) for hepta- and

Table 3
Recovery of PCDDs/PCDFs from spiked samples using a proceedure for separating PAHs, PCBs and PCDDs/PCDFs from biological tissue extracts

Congener	Recovery (%)		
	Salmon oil (1.0 g)[a] (n=4, av.±S.D.)	Larvae method blanks[a] (n=2)	Larvae (1.2–1.6 g)[b] (n=3, av.±S.D.)
2,3,7,8-TCDD	72±6.5	70, 94	60±24
1,2,6,7-TCDD		60, 83	
1,2,8,9-TCDD		55, 99	
1,2,3,7,8-PCDD	79±8.1	74, 92	74±16
1,2,3,4,7,8-HXCDD	77±10	78, 101	
1,2,3,6,7,8-HXCDD	82±9.4	64, 98	76±12
1,2,3,7,8,9-HXCDD	87±5.3	76, 103	
1,2,3,4,6,7,8-HPCDD	81±12	62, 98	58±12
1,2,3,4,6,7,8,9-OCDD	74±12	68, 96	61±13
1,2,3,4-TCDF	42±19	51, 62	
2,3,7,8-TCDF	76±6.2	58, 93	80±15
1,2,8,9-TCDF	60±1.9	52, 79	
1,2,3,7,8-PCDF	96±6.7	90, 150	87±17
2,3,4,7,8-PCDF	78±16	65, 93	
1,2,3,4,7,8-HXCDF	73±9.8	67, 87	74±16
1,2,3,6,7,8-HXCDF	81±9.5	63, 97	
2,3,4,6,7,8-HXCDF	72±8.2	54, 81	
1,2,3,7,8,9-HXCDF	76±9.3	55, 85	
1,2,3,4,6,7,8-HPCDF	77±15	64, 95	65±13
1,2,3,4,7,8,9-HPCDF	91±12	65, 99	
1,2,3,4,6,7,8,9-OCDF	83±9.2	61, 90	53±12

All samples were analyzed by capillary GC–low-resolution MS.

[a] Samples were spiked with a mixture of the native congeners at a concentration of 1 ng/congener and recoveries were determined by adding a mixture of 11 ^{13}C-labeled PCDDs/PCDFs as injection standards.

[b] Samples were spiked with a mixture of 11 ^{13}C-labeled PCDDs/PCDFs at a concentration of 1 ng/congener and recoveries were determined by adding ^{13}C-labeled 1,2,3,4-TCDD as an injection standard.

octa-CDDs and hexa-, hepta- and octa-CDFs were found in the salmon oil.

4. Conclusions

Results from this study show that PAHs, PCBs and PCDDs/PCDFs can be separated into compound classes by low-pressure liquid adsorption chromatography. By carefully adjusting the activity of 13–24 μm particle-size silica gel with 50% water-saturated hexane the adsorbent can be used to separate mono- to decachlorinated BPs and tetra- to octa-CDDs/CDFs from three- to six-ring PAHs. The separation of the PCBs from the PCDDs/PCDFs can subsequently be achieved using acid alumina in the first stage of a three-column automated system for cleanup of PCDD/PCDF compounds from environmental sample extracts. However, in order to separate the most retentive PCBs (the non-*ortho* congeners) from the least retentive PCDDs/PCDFs (TCDFs substituted at the 4 and 6 positions), the chromatographic resolution must be increased by using two columns in-series instead of one column.

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