ELSEVIER

Journal of Chromatography A, 816 (1998) 213–220

JOURNAL OF CHROMATOGRAPHY A

Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of polychlorinated biphenyls in environmental matrix standard reference materials

Michele M. Schantz[a,*], Søren Bøwadt[b,1], Bruce A. Benner Jr.[a], Stephen A. Wise[a], Steven B. Hawthorne[b]

[a]*Analytical Chemistry Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA*
[b]*Energy and Environmental Research Center, University of North Dakota, Grand Forks, ND 58202, USA*

Received 27 January 1998; received in revised form 23 March 1998; accepted 16 June 1998

Abstract

Supercritical fluid extraction (SFE) was compared to traditional Soxhlet extraction for the determination of polychlorinated biphenyl congeners in three standard reference materials: SRM 1941a (Organics in Marine Sediment), SRM 1944 (New York/New Jersey Waterway Sediment) and SRM 2974 [Organics in Mussel Tissue (*Mytilus edulis*) (Freeze-Dried)]. The concentrations determined using SFE compared well with the certified concentrations for the majority of the polychlorinated biphenyl congeners. Published by Elsevier Science B.V.

Keywords: Extraction methods; Polychlorinated biphenyls

1. Introduction

Soxhlet extraction has been the traditional method used for the extraction of polychlorinated biphenyl (PCB) congeners from environmental samples. When carried out for an adequate amount of time (>12 h) using a mixture of polar and nonpolar solvents, Soxhlet extraction is expected to give quantitative recoveries for the PCB congeners from environmental matrices [1]. During the certification of reference materials at the National Institute of Standards and Technology (NIST), the results from at least two independent analytical techniques are typically used to determine the certified concentrations of the analytes. For the extraction of PCB congeners from environmental matrices, however, Soxhlet extraction with differing solvents has been the only extraction method used in the certification procedures at NIST [2].

Alternative extraction techniques include supercritical fluid extraction (SFE) [3,4] and pressurized fluid extraction (PFE) [5,6]. SFE with on-line clean-up of natural environmental samples using solid-phase trapping has been described by several workers [7–9]. Bøwadt et al. [10] discussed an independent comparison of SFE and Soxhlet extraction for the determination of PCBs in soil (CRM 481, available from the Community Bureau of Reference). This study pointed out no significant differences

*Corresponding author.
[1]Present address: VKI, Agern Allé 11, DK 2970, Hørsholm, Denmark.

0021-9673/98/$19.00 Published by Elsevier Science B.V.
PII: S0021-9673(98)00528-7

between the results obtained using SFE or Soxhlet extraction.

In the present study, three natural matrix standard reference materials (SRMs) were chosen for evaluation of SFE for determination of PCB congeners: SRM 1941a (Organics in Marine Sediment), SRM 1944 (New York/New Jersey Waterway Sediment) and SRM 2974 [Organics in Mussel Tissue (*Mytilus edulis*) (Freeze-Dried)]. SRM 1941a and SRM 1944 are both marine sediments that differ in PCB content by about a factor of five. It is advantageous to use an SRM for this type of evaluation because an SRM is a large batch of homogeneous material that has been well characterized at NIST and is widely available to other laboratories. This study was a collaborative effort between the Energy and Environmental Research Center (EERC) at the University of North Dakota and NIST. SFE experiments were performed at EERC along with a limited number of Soxhlet extractions while the majority of Soxhlet extraction experiments were done at NIST along with a limited number of SFE experiments designed to duplicate the experimental conditions employed at EERC. A summary of the methods used is given in Table 1 and described below in detail. Both extraction techniques and the analyses were performed at EERC and NIST to eliminate differences in the analytical measurement step.

2. Experimental

2.1. Materials

SRM 1941a, SRM 1944 and SRM 2974 were obtained from the Standard Reference Materials Program (SRMP), NIST. SRM 2262, Chlorinated Biphenyl Congeners in 2,2,4-Trimethylpentane (nominal concentration 2 μg/ml), which was used by both laboratories as a calibration solution, was also obtained from the SRMP, NIST. Additional PCB congeners were obtained from AccuStandard (New Haven, CT, USA) and Ultra Scientific (New Kingston, RI, USA). All solvents were HPLC-grade.

2.2. Soxhlet extraction, SFE and analysis at NIST

2.2.1. Soxhlet extraction at NIST

For Soxhlet extraction, three to six weighed aliquots of sediment SRM 1941a (~10 g each), sediment SRM 1944 (~2 g each), and mussel tissue SRM 2974 (~2 g each) were mixed with ~50 g of pre-extracted sodium sulfate. The mixtures were placed in glass extraction thimbles and Soxhlet extracted for 18 h using 250 ml of dichloromethane. The extracts were concentrated to ~0.5 ml. Each concentrated sediment extract was placed on a silica solid-phase extraction (SPE) column (Sep-Pak, Wa-

Table 1
Summary of the methods used

Laboratory	Extraction	Clean-up	GC–ECD column used
NIST	Soxhlet Dichloromethane	Copper for sediments SEC for mussel tissue Normal-phase LC fractionation	DB-5 column
EERC	Soxhlet Hexane–acetone (1:1, v/v)	Column chromatography with activated silica impregnated with 40% (w/w) sulfuric acid plus copper powder	DB-17 column and parallel coupled HP-5 with HT-5 column
NIST	SFE–CO_2 150°C for SRM 1944 80°C for SRM 1941a and 2974	SPE for mussel tissue	DB-1701
EERC	SFE–CO_2 80°C, 97°C and 150°C for SRM 1944 80°C for SRM 1941a and 2974	Florisil trap	DB-17 column and parallel coupled HP-5 with HT-5 column

ters, Milford, MA, USA), which had been precleaned with 15 ml of dichloromethane–hexane (1:10, v/v) to separate the more polar material from the fraction of interest. Copper powder was then added to the fraction to remove sulfur contamination. The fraction of interest was decanted from the copper and concentrated to ~400 μl for normal-phase liquid chromatographic fractionation on a semipreparative aminopropylsilane column (μBondapak NH_2, 30 cm×9 mm I.D., Waters) (LC-NH_2).

The LC-NH_2 procedure was used to isolate two fractions containing: (1) the PCBs and lower polarity chlorinated pesticides and (2) the more polar chlorinated pesticides. Hexane was used as the mobile phase for the isolation of the PCBs and lower polarity pesticides, and dichloromethane–hexane (1:20, v/v) was used for the isolation of the more polar pesticide fraction. Each fraction was then concentrated to ~300 μl for GC analysis.

For the mussel tissue extract, the majority of the lipid and biogenic material was removed using size exclusion chromatography (SEC) on a preparative divinylbenzene–polystyrene column (10 μm particle size, 100 Å pore size, 60 cm×2.5 cm I.D., PL-Gel, Polymer Labs., Amherst, MA, USA). Dichloromethane was used as the mobile phase at a flow-rate of 10 ml/min. The eluent (70 ml) was concentrated to ~400 μl with a solvent change to hexane for the LC-NH_2 fractionation described above for the sediment extracts.

A known amount of internal standard (I.S.) solution containing PCB 103 (2,2′,4,5′,6-pentachlorobiphenyl) and PCB 198 (2,2′,3,3′,4,5,5′,6-octachlorobiphenyl) was added to each sediment and mussel tissue sample prior to Soxhlet extraction. Response/recovery solutions were prepared by gravimetrically diluting SRM 2262 and a supplemental PCB solution with hexane and then adding a known amount of I.S. solution to this mixture. Procedural blanks were prepared by adding a known amount of I.S. solution to hexane. The response/recovery solutions and procedural blanks were processed in the same manner as the respective samples.

The samples were analyzed by gas chromatography with electron capture detection (GC–ECD) using a 60 m×0.25 mm column with a 5% phenyl-substituted methylpolysiloxane phase (DB-5, J&W Scientific, Folsom, CA, USA) (0.25 μm film thickness). Helium was used as the carrier gas at a linear velocity of 37 cm/s. Injections were performed in the split mode at a ratio of 25:1. The column temperature was held isothermally at 200°C for 30 min and then temperature programmed at 2°C/min to 270°C where it was held for 10 min. The injection port was maintained at 280°C while the ECD system was maintained at 310°C.

2.2.2. SFE at NIST

For SFE, four weighed portions of sediment SRM 1944 (~1 g each), five weighed portions of sediment SRM 1941a (~1 g each), and six weighed portions of mussel tissue SRM 2974 (~1 g each) were placed in an extractor cell along with copper (for the sediment samples) and precleaned SFE Wet Support (Isco, Lincoln, NE, USA) to decrease the dead volume of the cell. The use of copper directly in the extraction process has previously been shown to prevent the interference from elemental sulfur contained in sediment samples [11]. The samples of SRM 1944 were extracted using CO_2 for 10 min static and 40 min dynamic at 415 bar, 150°C (0.62 g/ml) with a flow-rate of 1 ml/min, a restrictor temperature of 60°C, and collection in 15 ml of hexane. The samples of SRM 1941a and SRM 2974 were extracted using CO_2 for 10 min static and 40 min dynamic at 305 bar, 80°C (0.75 g/ml) with a flow-rate of 2.5 ml/min, a restrictor temperature of 60°C, and collection in 15 ml of hexane.

A known amount of I.S. solution containing PCB 103, PCB 198, and perdeuterated 4,4′-DDT was added to the extracts. Response/recovery solutions were prepared from SRM 2262 and a supplemental PCB solution as described above. Procedural blanks were also extracted. The extracts were then concentrated to ~400 μl for GC analysis in the case of the sediment extracts and for fractionation on an aminopropylsilane SPE column in the case of the mussel extracts. The mussel extracts were eluted from a precleaned aminopropylsilane SPE column using 20 ml dichloromethane–hexane (1:50, v/v). The fraction was concentrated to ~400 μl for GC analysis.

The samples were analyzed by GC–ECD using a 60 m×0.25 mm column with a 14%-cyanopropylphenyl-substituted methylpolysiloxane phase (DB-1701, J&W Scientific) (0.25 μm film thickness). Helium was used as the carrier gas at a linear

velocity of 37 cm/s. Injections were performed in the split mode at a ratio of 25:1. The column was held isothermally at 50°C for 1 min, then temperature programmed at 45°C per min to 200°C for 30 min, and then temperature programmed at 2°C per min to 280°C for 15 min. The injection port was maintained at 250°C while the ECD system was maintained at 310°C.

2.3. SFE, Soxhlet extraction, and analysis at EERC

2.3.1. SFE at EERC

For SFE, seven weighed portions, each ~1 g, of SRM 1941a, SRM 1944 or SRM 2974 were mixed with ~7 g of sodium sulfate (SRM 2974) or ~6 g of sodium sulfate and 1.5 g of prerinsed copper powder (SRM 1941a and SRM 1944) and packed into 7-ml extraction cells. The samples were extracted with pure CO_2 for 10 min static and 40 min dynamic using the following conditions: SRM 1941a and SRM 2974 at a density of 0.75 g/ml (305 bar) at 80°C with a flow-rate of 2.5 ml/min (the conditions used in the newly approved US Environmental Protection Agency (EPA) method 3562) and SRM 1944 at 0.75 g/ml (305 bar) at 80°C, 0.75 g/ml (378 bar) at 97°C and 0.62 g/ml (415 bar) at 150°C, all with a flow-rate of 1 ml/min. The completeness of the extractions was examined using sequential extractions with CO_2 modified with 5% (v/v) MeOH for a 30-min dynamic extraction using the above-mentioned conditions.

The nozzle and trap temperatures were kept constant at 45°C and 20°C, respectively, (except for the extractions with 5% MeOH where the trap temperature was kept at 65°C). The trap was filled with approximately 1 ml Florisil (0.16–0.25 mm particle size) as trapping material and was eluted twice with 1.5 ml *n*-heptane, then with 3 ml of acetone–dichloromethane (1:1, v/v) followed by 1.5 ml *n*-heptane after the end of each individual extraction. Only the first 1.5 ml of *n*-heptane was used for the GC analysis (with the addition of PCB 103 and 198 as internal standards and adjustment of volume to 1.8 ml). The residual elution fractions were used to check the completeness of the elution of the trap and for cleaning of the trap between samples.

2.3.2. Soxhlet extractions at EERC

For the Soxhlet extractions, three aliquots, each ~1 g, of SRMs 1941a, 1944 and 2974 were mixed with ~10 g of sodium sulfate and extracted using 250 ml of *n*-hexane–acetone (1:1, v/v) for 24 h. The solvents were evaporated on a rotary evaporator at 30°C and redissolved in 10 ml of *n*-hexane. Extracts were loaded on a 45 cm×20 mm column with 6 cm of activated silica impregnated with 40% (w/w) sulfuric acid and 1 cm activated copper powder and eluted with 100 ml *n*-hexane. The eluent was evaporated and the residues were redissolved in 1.5 ml *n*-heptane. Internal standards were added (PCB 103 and 198, as for the supercritical fluid extractions), and the final volume was adjusted to 1.8 ml with *n*-heptane.

The SFE and Soxhlet extracts were analyzed using a GC equipped with an autosampler and two ECD systems held at a temperature of 300°C (each purged with 60 ml/min of nitrogen). Aliquots (1 μl) of the extracts were injected on-column on two parallel coupled columns, a 60 m×0.25 mm, 0.25 μm phase, 50% diphenyldimethylsiloxane DB-17 (J&W Scientific) column and a series combination of a 25 m×0.25 mm, 0.25 μm 5% diphenyldimethylsiloxane HP-5 (Hewlett-Packard, Wilmington, DE, USA) column and a 25 m×0.22 mm, 0.10 μm 1,7-dicarbaclosododecarborane-dimethylpolysiloxane HT-5 (SGE, Austin, TX, USA) column. The columns were installed in the GC oven together with a deactivated 2 m×0.53 mm fused-silica retention gap.

The GC oven program was: initial temperature 90°C for 2 min, then increased at a rate of 20°C/min to 170°C for 7.5 min, then increased at a rate of 3°C/min to 275°C for 10 min. The hydrogen carrier gas linear velocity was approximately 43 cm/s, held constant by the pressure controlled inlet throughout the whole temperature program (starting pressure 1.7 bar at 90°C). This choice of columns and GC conditions has previously been shown to give optimum separation of PCB congeners and organochlorine pesticides [12,13]

3. Results and discussion

Natural matrix environmental SRMs are homogenous, stable materials that have been well-character-

ized for the concentration of selected chemical compounds. These materials are generally produced in sufficient quantities to provide a 5–10 year supply depending on the stability of the matrix. Because SRMs are homogenous and available in large quantities, they are valuable materials for the evaluation of new analytical methods, including extraction techniques. Samples of three natural matrix SRMs, SRM 1941a, SRM 1944, and SRM 2974, all of which contain natural levels of PCB contamination, were provided to EERC for evaluation of SFE and comparison to Soxhlet extraction. Samples of the same three SRMs were extracted using Soxhlet and supercritical fluids and analyzed at NIST thus leading to an inter-laboratory study between EERC and NIST comparing Soxhlet extraction and SFE. Because the analytical measurements were done in different laboratories, the calibration of the ECD system and the chromatographic separations on different columns often resulted in some differences between the results from the two laboratories. These inter-laboratory differences for PCB measurements have been discussed in detail in summaries of studies conducted within the International Council for the Exploration of the Sea (ICES) [14–16], the QUASIMEME program [17], and a recent marine mammal tissue inter-laboratory study [18].

Different extraction temperatures (80, 97 and 150°C) using supercritical CO_2 were evaluated for extraction of PCB congeners from SRM 1944 (Table 2) [19]. As shown in Table 2, there is a general trend that more of a specific congener is extracted at the higher temperatures; however, the differences are small, typically less than 15%. The largest difference (15%) is for the decachlorobiphenyl PCB 209. Also included in Table 2 are the concentrations of PCB congeners in SRM 1944 determined at EERC using Soxhlet extraction. The concentrations determined using Soxhlet extraction agree well with those determined using supercritical CO_2 at 150°C.

One of the advantages of SFE over Soxhlet extraction is the reduced sample clean-up required. This advantage is demonstrated in Table 3, where it can be seen that increased clean-up of the resulting Soxhlet extracts eventually leads to results similar to those obtained by SFE after elution from the Florisil

Table 2
Concentrations of PCB congeners in SRM 1944 determined using different extraction temperatures

Compound	Concentration (μg/kg dry mass)			
	SFE (80°C)[a]	SFE (97°C)[a]	SFE (150°C)[a]	Soxhlet[b]
PCB 18 (2,2′,5-trichlorobiphenyl)[c]	51 (2)	51 (2)	54 (3)	52 (1)
PCB 28 (2,4,4′-trichlorobiphenyl)[c]	75 (2)	78 (2)	83 (2)	82 (2)
PCB 31 (2,4′,5-trichlorobiphenyl)[c]	70 (1)	73 (1)	77 (2)	75 (1)
PCB 52 (2,2′,5,5′-tetrachlorobiphenyl)[c]	68 (3)	68 (2)	71 (4)	69 (2)
PCB 101 (2,2′,4,5,5′-pentachlorobiphenyl)[c]	56 (3)	55 (2)	57 (3)	56 (2)
PCB 105 (2,3,3′,4,4′-pentachlorobiphenyl)[c]	19 (1)	20 (1)	20 (1)	22 (1)
PCB 118 (2,3′,4,4′,5-pentachlorobiphenyl)[c]	47 (3)	48 (1)	49 (2)	49 (3)
PCB 138 (2,2′,3,4,4′,5′-hexachlorobiphenyl)[d]	32 (2)	32 (2)	31 (2)	33 (1)
PCB 149 (2,2′,3,4′,5′,6-hexachlorobiphenyl)[c]	47 (2)	47 (1)	49 (2)	48 (2)
PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl)[c]	54 (2)	54 (2)	56 (2)	53 (2)
PCB 170 (2,2′,3,3′,4,4′,5-heptachlorobiphenyl)[c]	14 (1)	14 (1)	15 (1)	15 (1)
PCB 180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl)[c]	37 (1)	38 (1)	39 (1)	41 (2)
PCB 194 (2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl)[c]	9.7 (0.3)	10 (1)	11 (1)	12 (1)
PCB 206 (2,2′,3,3′,4,4′,5,5′,6-nonachlorobiphenyl)[c]	8.0 (0.1)	8.3 (0.3)	8.6 (0.2)	9.3 (0.5)
PCB 209 (decachlorobiphenyl)[c]	6.6 (0.1)	7.2 (0.3)	7.8 (1.2)	8.8 (0.6)

[a] Seven samples were extracted at EERC using carbon dioxide at the temperature indicated. Analyses were performed at EERC. Concentrations are the means, and the numbers in parentheses are one standard deviation of a single measurement.
[b] Three samples were extracted and analyzed at EERC using hexane–acetone (1:1, v/v). Concentrations are the averages, and the numbers in parentheses are one standard deviation of a single measurement.
[c] Data from the analyses done using the HP5-HT5 column.
[d] Data from the analyses done using the DB-17 column.

Table 3
Impact of sequential clean-up and baseline manipulation on the Soxhlet extract for SRM 1941a[a]

Compound	Concentrations (μg/kg dry mass)				
	Soxhlet			SFE Raw extract[e]	Certified concentration[f]
	Clean-up (1×)[b]	Clean-up (2×)[c]	Baseline[d]		
PCB 28	22 (8)	9.5 (0.6)	6.2 (1.6)	6.4 (0.1)	9.8±1.3
PCB 52	70 (60)	25 (16)	21 (16)	7.5 (0.3)	6.89±0.56
PCB 101	19 (6)	11 (1)	10 (1)	10 (1)	11.0±1.6
PCB 118	14 (2)	6.8 (1.2)	6.9 (0.3)	6.8 (0.2)	10.0±1.1
PCB 149	11 (2)	9.2 (1.7)	9.5 (0.6)	9.9 (0.1)	9.2±1.1
PCB 156	1.7 (0.7)	1.1 (0.2)	1.0 (0.1)	0.9 (0.1)	0.93±0.14
PCB 170	3.9 (0.3)	4.0 (0.5)	3.2 (0.3)	3.1. (0.1)	3.00±0.46

[a] All extractions and analyses were performed at EERC.
[b] Three extractions were done and each extract was cleaned over acid silica and copper once. Concentrations are the means and the numbers in parentheses are one standard deviation of a single measurement.
[c] Three extractions were done and each extract was cleaned over acid silica and copper twice. Concentrations are the means and the numbers in parentheses are one standard deviation of a single measurement.
[d] Three extractions were done and each extract was cleaned over acid silica and copper twice with baseline adjustment after chromatography. Concentrations are the means and the numbers in parentheses are one standard deviation of a single measurement.
[e] Seven extractions were done using 1.5 g of copper in the extraction cell. Concentrations are the means and the numbers in parentheses are one standard deviation of a single measurement.
[f] Certified concentrations with associated uncertainties as described in the Certificate of Analysis for SRM 1941a [20], except for PCB 28 which is a noncertified concentration.

trap. The Soxhlet extracts done at NIST for SRM 1941a were processed through a SPE column, copper, and an LC-NH_2 clean-up prior to analysis.

The comparison between Soxhlet extractions and SFE done at NIST is shown in Table 4 for SRM 1941a and SRM 1944. For SRM 1941a, the concentrations determined from the Soxhlet and SFE are compared to the certified concentrations. The agreement is good with all of the concentrations falling within the 95% confidence interval of the certified concentration. For SRM 1944, the concentrations determined from Soxhlet extraction are compared to those determined from SFE. (The certification of SRM 1944 is currently in progress and the final certified concentrations of the PCB congeners are not yet available.) Again, the agreement between the two extraction techniques is good. For the majority of the compounds, the uncertainties determined from the SFE are larger than those determined from the Soxhlet extractions. The larger uncertainties are a result of more matrix interferences since the samples from the SFE were not cleaned-up as much as the samples from the Soxhlet extraction. When the SFE samples are cleaned-up in a similar fashion to the Soxhlet extracts the uncertainties are comparable between the two sets of samples (shown in Table 4 for SRM 1944). The clean-up procedures are not necessary with the use of the EERC procedures, where solid-phase trapping and subsequent elution with a small volume of solvent has the same effect as a clean-up. Four chlorinated pesticides are included in the comparison shown in Table 4. The results were in very good agreement, within the uncertainties of the measurements, between the concentrations determined using the two extraction techniques. These pesticides have polarities similar to the PCBs. More polar pesticides may not give comparable results from the two extraction techniques.

The freeze-dried mussel tissue investigated, SRM 2974, has concentrations of individual PCB congeners ranging from ≈2–150 ng/g dry mass. The concentrations determined at NIST using Soxhlet extraction and SFE are compared to the certified concentrations in Table 5. As was found for the sediments, the results for the mussel tissue are comparable using the two extraction techniques and are comparable to the certified concentrations, i.e., within the 95% confidence level of the certified

Table 4
Concentrations (μg/kg dry mass) of selected PCB congeners and chlorinated pesticides in SRM 1941a and SRM 1944 determined at NIST using Soxhlet and SFE

Compound	SRM 1941a			SRM 1944		
	Certified concentrations[a]	Soxhlet[b]	SFE[c]	Soxhlet[a]	SFE[c]	
					Before clean-up	After clean-up
PCB 28				76 (2)	73 (2)	75 (2)
PCB 31				78 (2)	75 (6)	77 (2)
PCB 49	9.5±2.1	8.0 (0.1)	8.9 (0.2)	53 (2)	55 (2)	54(2)
PCB 52	6.89±0.56	7.0 (0.1)	7.2 (0.2)	79 (2)	83 (2)	80 (2)
PCB 101	11.0±1.6	11 (1)	11 (1)	73 (2)	68 (2)	70 (2)
PCB 105	3.65±0.27	3.4 (0.1)	3.5 (0.2)	22 (1)	20 (2)	21 (1)
PCB 118	10.0±1.1	9.4 (0.1)	10 (1)	58 (1)	55 (3)	57 (2)
PCB 138/163/164	13.38±0.97	12 (1)	14 (1)	60 (2)	62 (5)	61 (2)
PCB 149	9.2±1.1	9.6 (0.3)	10 (1)	49 (2)	46 (4)	49 (2)
PCB 153	17.6±1.9	16 (1)	17 (1)	74 (2)	78 (7)	74 (3)
PCB 170/190	3.00±0.46	3.1 (0.1)	3.5 (0.2)	23 (1)	21 (2)	21 (1)
PCB 180	5.83±0.58	6.1 (0.1)	5.7 (0.3)	42 (1)	43 (3)	40 (1)
PCB 194	1.78±0.23	1.9 (0.1)	1.9 (0.1)	11 (1)	11 (1)	11 (1)
PCB 206	3.67±0.87	3.2 (0.1)	3.6 (0.1)	9.3 (0.2)	9.5 (0.4)	9.3 (0.2)
PCB 209	8.34±0.49	8.5 (0.2)	8.1 (0.1)	6.9 (0.3)	6.3 (0.5)	6.7 (0.3)
Hexachlorobenzene	70±25	68 (4)	77 (6)	5.5 (0.5)	5.7 (0.5)	5.6 (0.5)
4,4′-DDE	6.59±0.56	6.7 (0.2)	7.0 (0.5)	96 (2)	97 (11)	95 (3)
4,4′-DDD	5.06±0.58	5.0 (0.2)	5.2 (0.3)	120 (8)	126 (20)	124 (11)
4,4′-DDT				125 (6)	117 (6)	119 (6)

[a] The certified values as reported on the Certificate of Analysis [20] are weighted means of results from two or more analytical techniques as described by Schiller and Eberhardt [21]. The uncertainty is based on a 95% confidence interval for the true concentration, and includes an allowance for differences between the analytical methods used.
[b] Three samples were Soxhlet extracted at NIST using dichloromethane. Concentrations are the means, and the numbers in parentheses are one standard deviation of a single measurement.
[c] Five samples of SRM 1941a and four samples of SRM 1944 were extracted at NIST using supercritical CO_2 at 80°C for SRM 1941a and at 150°C for SRM 1944. Concentrations are the means, and the numbers in parentheses are one standard deviation of a single measurement. For SRM 1944, the concentrations are given before and after clean-up of the extracts.

concentrations. The uncertainties associated with the results are again generally larger for the concentrations determined from the SFE. The mussel tissue extracts contain some lipid and pigments so the SFE samples were cleaned-up on an SPE column prior to GC analysis, but when these samples are also run through the SEC column, the uncertainties are similar to the uncertainties associated with the Soxhlet extracts.

4. Conclusions

SFE and Soxhlet extractions of PCB congeners from sediment and mussel tissue SRMs (SRM 1941a, SRM 1944, and SRM 2974) give comparable results. The SRMs are ideal materials for this type of comparison because they are well characterized, homogeneous, and widely available. SFE has several advantages over Soxhlet for PCB determinations including reduced sample clean-up, reduced extraction time (50 min compared to 18 h to 24 h), and reduced organic solvent usage (7.5 ml compared to 250 ml).

Disclaimer: Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are the best available for the purpose.

Table 5
Concentrations of PCB congeners in SRM 2974 determined using different extraction methods

Compound	Concentration (μg/kg dry mass)		
	Certificate[a]	Soxhlet[b]	SFE[c]
PCB 28	79±15	78 (2)	86 (4)
PCB 31	76±21	76 (2)	69 (3)
PCB 49	88.8±5.7	87 (2)	90 (3)
PCB 52	115±12	113 (3)	114 (7)
PCB 101	128±10	125 (3)	125 (7)
PCB 105	53.0±3.8	54 (3)	59 (4)
PCB 118	130.8±5.3	122 (2)	127 (4)
PCB 138/163/164	133.5±10	127 (4)	135 (9)
PCB 149	87.6±3.5	86 (4)	84 (4)
PCB 153	145.2±8.8	143 (5)	152 (10)
PCB 170/190	5.5±1.1	5.3 (0.4)	5.0 (0.6)
PCB 180	17.1±3.8	17 (1)	16 (1)

[a] From the Certificate of Analysis for SRM 2974 [22].
[b] Three samples were Soxhlet extracted using dichloromethane and analyzed at NIST. Concentrations are the means, and the numbers in parentheses are one standard deviation of a single measurement.
[c] Six samples were extracted at 80°C and 301 bar for 10 min static and 40 min dynamic with a CO_2 flow of 2.5 ml/min. Concentrations are the means, and the numbers in parentheses are one standard deviation of a single measurement.

Acknowledgements

S.B. gratefully acknowledges the support from Hewlett-Packard with SFE equipment, GC software, and GC columns as well as the financial support from the Danish Research Council during the course of this work.

References

[1] J. de Boer, Chemosphere 17 (1988) 1803.
[2] M.M. Schantz, B.J. Koster, L.M. Oakley, S.B. Schiller, S.A. Wise, Anal. Chem. 67 (1995) 901.
[3] J.W. King, J.E. France, in: B. Wenclaviak (Editor), Analysis with Supercritical Fluids: Extraction and Chromatography, Springer, Berlin, 1992, p. 32.
[4] S. Bøwadt, S.B. Hawthorne, J. Chromatogr. A 703 (1995) 549.
[5] B.E. Richter, B.A. Jones, J.L. Ezzell, N.L. Porter, N. Avdalovic, C. Pohl, Anal. Chem. 68 (1996) 1033.
[6] M.M. Schantz, J.J. Nichols, S.A. Wise, Anal. Chem. 69 (1997) 4210.
[7] F. David, M. Vershuere, P. Sandra, Fresenius J. Anal. Chem. 344 (1992) 479.
[8] S. Bøwadt, B. Johansson, F. Pelusio, B. Larsen, C. Rovida, J. Chromatogr. A 662 (1994) 424.
[9] N.L. Porter, A.F. Rynaski, R.E. Campbell, M. Saunders, B.E. Richter, J.T. Swanson, R.B. Nielsen, B.J. Murphy, J. Chromatogr. Sci. 30 (1992) 367.
[10] S. Bøwadt, B. Johansson, S. Wunderli, M. Zennegg, L.F. De Alencastro, D. Grandjean, Anal. Chem. 67 (1995) 2424.
[11] S. Bøwadt, B. Johansson, Anal Chem. 66 (1994) 667.
[12] M.S. Rahman, S. Bøwadt, B. Larsen, J. High Resolut. Chromatogr. 16 (1993) 731.
[13] S. Bøwadt, H. Skejø-Andresen, L. Montanarella, B. Larsen, J. Environ. Anal. Chem. 56 (1994) 87.
[14] J. de Boer, J. van der Meer, Report on the Results of the ICES/IOC/OSPARCOM, Intercomparison Exercise on the Determination of Chlorobiphenyl Congeners in Marine Media Steps 3 and 4, International Council for the Exploration of the Sea, Copenhagen, in press.
[15] J. de Boer, J. Duinker, J.A. Calder, M. van der Meer, J. Assoc. Off. Anal. Chem. 75 (1992) 1054.
[16] J. de Boer, J. van der Meer, L. Reutergårdh, J.A. Calder, J. Assoc. Off. Anal. Chem. 77 (1994) 1411.
[17] D.E. Wells, J. de Boer, Mar. Pollut. Bull. 29 (1994) 174.
[18] M.M. Schantz, B.J. Porter, S.A. Wise, M. Segstro, D.C.G. Muir, S. Mössner, K. Ballschmiter, P.R. Becker, Chemosphere 33 (1996) 1369.
[19] S.B. Hawthorne, D.J. Miller, Anal. Chem. 66 (1994) 4005.
[20] Certificate of Analysis for SRM 1941a: Organics in Marine Sediment. Standard Reference Materials Program, National Institute of Standards and Technology, Gaithersburg, MD, 1994.
[21] S.B. Schiller, K.R. Eberhardt, Spectrochim. Acta 46 (1991) 1607.
[22] Certificate of Analysis for SRM 2974: Organics in Freeze-Dried Mussel Tissue (*Mytilus edulis*). Standard Reference Materials Program, National Institute of Standards and Technology, Gaithersburg, MD, 1997.