QUANTITATION OF NON-*ORTHO* POLYCHLORINATED BIPHENYLS IN STANDARD REFERENCE MATERIALS

W. Wayne Brubaker, Jr., Michele M. Schantz, and Stephen A. Wise

Analytical Chemistry Division, National Institute of Standards and Technology, Gaithersburg, MD 20899-8392, USA

Introduction

Polychlorinated biphenyls (PCBs) are industrial pollutants that have become ubiquitous in nearly all environmental compartments. Those without *ortho*-substituted chlorines (known as non-*ortho* or "coplanar" PCBs) are of particular interest because they are potentially the most toxic PCBs (*1*). Over the past decade, laboratories have refined liquid chromatographic methods to fractionate the non-*ortho* PCBs (NOPCBs) from other interfering PCBs (*2*, *3*) which then allow for subsequent analysis by gas chromatography-electron capture detection (GC-ECD) or gas chromatography/mass spectrometry (GC/MS). Measurements of NOPCBs have now been reported in a variety of environmental matrices, including sediments, fish, marine mammals, and even polecats (*4-6*).

To assist U.S. and international laboratories in validating their measurements of PCBs, the National Institute of Standards and Technology (NIST) has issued several natural-matrix Standard Reference Materials (SRMs) with both certified and noncertified concentrations of individual PCB congeners (*7-9*). These SRMs include SRM 1588a (Organics in Cod Liver Oil), SRM 1944 (New York/NewJersey Waterway Sediment), SRM 1945 (Organics in Whale Blubber), and SRM 1974a (Organics in Mussel Tissue [*Mytilus edulis*]). Unfortunately, concentrations have not been reported for the NOPCBs in these SRMs or in other natural-matrix reference materials which are available from sources similar to NIST. Here we present the methods and preliminary results of the quantitative analysis of SRMs 1588a, 1944, and 1945 for NOPCBs.

Materials and Methods

Four to six samples of SRM 1588a, SRM 1944, and SRM 1945 were processed and analyzed for three NOPCBs: 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 3,3',4,4',5-pentachlorobiphenyl (PCB 126), and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169). Detailed information on the collection and preparation of each SRM can be found in the SRM's Certificate of Analysis (*10*). Individual sample sizes in this study were approximately 0.5 g, 10 g, and 1 g for SRMs 1588a, 1944, and 1945, respectively. Prior to sample cleanup, samples were spiked with appropriate amounts of ^{13}C-labelled PCB 77, PCB 126, and PCB 169 (Cambridge, Andover, MA) for use as internal standards. A separate ^{13}C-spiking solution was prepared for each SRM based on the levels of individual NOPCBs detected in a preliminary analysis.

Samples of SRM 1944 and SRM 1945 were each Soxhlet extracted overnight with approximately 200 mL of methylene chloride. Each whale blubber extract was then reduced and subjected to size

exclusion chromatography (SEC) on a preparative scale divinylbenzene/polystyrene column (10 μm particle size, 100 Å pore size, 2.5 cm i.d. × 60 cm, PL-Gel, Polymer Labs, Amhearst, MA) to remove lipid and biogenic materials. The sediment extracts of SRM 1944 did not undergo SEC, but they were reduced and eluted through separate silica solid phase extraction (SPE) columns (Sep-Pak, Waters, Milford, MA) with 15 mL each of 10% methylene chloride in hexane. The SRM 1588a samples were initially processed on the SEC column to remove lipids and did not require Soxhlet extraction.

All samples were reduced and fractionated on a semi-preparative aminopropylsilane column (μBondapak NH_2, 9 mm i.d. × 30 cm, Waters). Each fraction in which PCBs were isolated was reduced to approximately 25 μL and injected onto a 2-(1-pyrenyl)ethyldimethylsilylated silica (PYE) column (5 μm particle size, 4.6 mm i.d. × 25 cm, Comosil 5-PYE, Nacalai Tesque, Kyoto, Japan) (*2*). The PYE column separated the NOPCBs of each sample from the majority of other PCBs present using a 1-mL/min flow of hexane. The NOPCB fractions were each reduced to about 25 μL in hexadecane for GC/MS analysis.

Each sample set also included a method blank and a subset of 3 to 4 calibration samples specific to the SRM being processed. The calibration samples consisted of the same amount of ^{13}C-spiking solution applied to each SRM sample and known amounts of the unlabelled NOPCBs. The results of these calibration samples provided calibration curves for quantitation by GC/MS.

GC/MS analysis was carried out on a Hewlett-Packard (HP) 6890 gas chromatograph coupled to a HP 5973 mass spectrometer, operating in either its electron impact ionization (EI) or negative chemical ionization (NCI) mode. Injections of 2 μL were made on-column with helium as the carrier gas. Chromatographic separations were performed with one of the three GC columns given in Tables 1 and 2. Methane served as the reagent gas for NCI. A quantitation ion close to the molecular weight of each analyte and a confirmation ion of the same chlorine isotope pattern were monitored by the HP 5973 in its selected ion monitoring (SIM) mode. Chromatographic peaks identified as NOPCBs were only quantitated if the confirmation ion was present and the observed chlorine isotope ratio was within 15% of the appropriate theoretical value.

Results and Discussion

All three NOPCBs were detected at a signal-to-noise level of 3 or greater in each of the SRMs studied. Quantitative results for SRM 1588a are given in Table 1, and Table 2 summarizes the preliminary data for SRMs 1944 and 1945. Values are not reported for PCB 169 in SRM 1944 due to inconsistent values and apparent contamination within the sample set for that particular congener. Likewise, the same was observed for PCB 77 in the SRM 1945 samples. Significant amounts of NOPCBs (especially PCB 77) in blanks have been reported in the literature (*11*).

Our measurements for the cod liver oil (see Table 1) were in excellent agreement with those of Muir (*12*) and Krahn et al. (*13*) who used GC-ECD and gas chromatography/high resolution mass spectrometry (GC/HRMS), respectively. Since SRM 1944 was only recently issued, there are no values reported by other researchers, but our values for SRM 1944 do compare favorably with the concentrations reported for surface sediments of the Newark Bay Estuary (*14*) where the sediment

Table 1. Non-*ortho* PCBs in SRM 1588a (Organics in Cod Liver Oil).

	Concentration (ng/g) [a]		
	PCB 77	**PCB 126**	**PCB 169**
This study [b]			
EI [c]; DB-5MS [d]	1.33 ± 0.02	1.30 ± 0.02	0.21 ± 0.02
NCI [e]; HP-5MS [f]	1.31 ± 0.04	1.31 ± 0.02	0.22 ± 0.02
Other Studies			
Muir [g]	1.4 ± 0.3	1.3 ± 0.3	0.21 ± 0.09
Krahn et al. [h]	1.3	1.5	0.33

[a] Errors represent 95% confidence limits. [b] Both sets of measurements were obtained from the same sample set by GC/MS with the ionization and GC column given. [c] EI = electron impact ionization. [d] (5%-Phenyl)-methylpolysiloxane; 0.25 mm i.d. × 60 m, 0.25 μm flim thickness; J&W Scientific, Folsom, CA. [e] NCI = negative chemical ionization. [f] (5%-Diphenyl)-dimethylsiloxane; 0.25 mm i.d. × 30 m, 0.25 μm film thickness; Hewlett-Packard, Palo Alto, CA. [g] Ref. 12; samples were analyzed by GC-ECD. [h] Ref. 13; sample analyzed by GC/HRMS.

Table 2. Preliminary Results for Non-*ortho* PCBs in SRM 1944 (New York/New Jersey Waterway Sediment) and SRM 1945 (Organics in Whale Blubber).

	Concentration (ng/g) [a]		
	PCB 77	**PCB 126**	**PCB 169**
SRM 1944 [b]			
EI; DB-5MS	9.2 ± 0.6	0.26 ± 0.08	—
EI; DB-XLB [c]	9.0 ± 0.6	0.27 ± 0.07	—
NCI; HP-5	8.8 ± 0.5	0.29 ± 0.09	—
SRM 1945 [d]			
EI; DB-XLB	—	0.18 ± 0.07	0.20 ± 0.06
NCI; HP-5	—	0.16 ± 0.07	0.17 ± 0.05
Canadian Wildlife Service [e]	0.35 ± 0.03	0.14 ± 0.01	0.10 ± 0.01

[a] Errors represent 95% confidence limits. [b, d] Measurements were obtained from the same sample set by GC/MS with the ionization and GC column given. [c] 0.25 mm i.d. × 60 m, 0.25 μm film thickness; J&W Scientific. [e] Ref. 15; samples were analyzed by GC/HRMS.

for this SRM originated. Our values for PCB 126 in SRM 1945 agree with those of the Canadian Wildlife Service (*15*); however, those for PCB 169 differ by an approximate factor of 2.

The contamination problems mentioned above are currently being addressed by further analysis of SRMs 1944 and 1945. Our ultimate goal is to provide certified concentration values for NOPCBs

in the three SRMs discussed in this paper as well as in SRM 1974a (Organics in Mussel Tissue [*Mytilus edulis*]). Certified values at NIST are determined by two or more "chemically independent" analytical techniques. Since the measurement of organochlorine contaminants generally requires some form of gas chromatography, "independence" will be achieved by varying cleanup procedures, GC column selectivity, and detection methods (i.e., ECD and HRMS).

Acknowledgments

Lane Sander (NIST) provided technical expertise with regard to the PYE column. W.W.B. is supported by a NIST/NRC Postdoctoral Research Associateship.

References

1. Ahlborg, U.G.; Becking, G.C.; Birnbaum, L.S.; Brouwer, A.; Derks, H.J.G.M.; Feeley, M.; Golor, G.; Hanberg, A.; Larsen, J.C.; Liem, A.K.D.; Safe, S.H.; Schlatter, C.; Waern, F.; Younes, M.; Yrjanheikki, E. *Chemosphere* **1994**, *28*, 1049-1067.
2. Sander, L.C.; Parris, R.M.; Wise, S.A.; Garrigues, P. *Anal. Chem.* **1991**, *63*, 2589-2597.
3. Creaser, C.S.; Krokos, F.; Startin, J.R. *Chemosphere* **1992**, *25*, 1981-2008.
4. Huang, E.B.; Poole. G.; Chiu, C. *J. High Resol. Chromatogr.* **1997**, *20*, 1-9.
5. Ford, C.A.; Muir, D.C.G.; Norstrom, R.J.; Simon, M.; Mulvihill, M.J. *Chemosphere* **1993**, *26*, 1981-1991.
6. Bagheri, H.; Leonards, P.E.G.; Ghijsen, R.T.; Brinkman, U.A.T. *Int. J. Environ. Anal. Chem.* **1993**, *50*, 257-268.
7. Schantz, M.M.; Koster, B.J.; Oakley, L.M.; Schiller, S.B.; Wise, S.A. *Anal. Chem.* **1995**, *67*, 901-910.
8. Schantz, M.M.; Benner, B.A.; Hays, M.J.; Kelly, W.R.; Vocke, R.D.; Demiralp, R.; Greenberg, R.R.; Laurenstein, G.G.; Wise, S.A. *Fresenius J. Anal. Chem.* **1995**, *352*, 166-173.
9. Schantz, M.M.; Parris, R.M.; Wise, S.A. *Chemosphere* **1993**, *27*, 1915-1922.
10. SRM Catalog, World Wide Web: *http://ois.nist.gov/srmcatalog/*; NIST: Gaithersburg, MD, 1999.
11. Krokos, F.; Creaser, C.S.; Wright, C.; Startin, J.R. *Fresenius J. Anal. Chem.* **1997**, *357*, 732-742.
12. Muir, D., Environment Canada, personal communication, 1997.
13. Krahn, M.M.; Ylitalo, G.M.; Buzitis, J.; Sloan, C.A.; Boyd, D.T.; Chan, S.L.; Varanasi, U. *Chemosphere* **1994**, *29*, 117-139.
14. Iannuzzi, T.J.; Huntley, S.L.; Bonnevie, N.L.; Finley, B. L.; Wenning, R.J. *Arch. Environ. Contam. Toxicol.* **1997**, *28*, 108-117.
15. Wakeford, B.; Simon, M. Report CHEM-PCDD-98-1, 1998; Canadian Wildlife Service, Hull, Quebec.