

Long-term stability and temporal trends of organic contaminants in four collections of mussel tissue frozen standard reference materials

Michele M. Schantz · Rebecca S. Pugh ·
Stacy S. Vander Pol · Stephen A. Wise

Received: 24 November 2014 / Revised: 26 January 2015 / Accepted: 27 January 2015 / Published online: 25 February 2015
© Springer-Verlag Berlin Heidelberg (outside the USA) 2015

Abstract The stability of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated pesticides in frozen mussel tissue Standard Reference Materials (SRMs) stored at -80°C was assessed by analyzing samples of SRM 1974, SRM 1974a, and SRM 1974b Organics in Mussel Tissue (*Mytilus edulis*) periodically over 25 y, 20 y, and 12 y, respectively. The most recent analyses were performed during the certification of the fourth release of this material, SRM 1974c. Results indicate the concentrations of these persistent organic pollutants have not changed during storage at -80°C . In addition, brominated diphenyl ethers (BDEs) were quantified in each of the materials during this study. The stability information is important for on-going monitoring studies collecting large quantities of samples for future analyses (i.e., formally established specimen banking programs). Since all four mussel tissue SRMs were prepared from mussels collected at the same site in Dorchester Bay, MA, USA, the results provide a temporal trend study for these contaminants over a 17 year period (1987 to 2004).

Keywords Quality assurance / control · Reference materials · Organic compounds / Trace organic compounds

Published in the topical collection *Reference Materials for Chemical Analysis* with guest editors Hendrik Emons and Stephen A. Wise.

Electronic supplementary material The online version of this article (doi:10.1007/s00216-015-8524-6) contains supplementary material, which is available to authorized users.

M. M. Schantz (✉) · S. A. Wise
Chemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA
e-mail: michele.schantz@nist.gov

R. S. Pugh · S. S. V. Pol
Chemical Sciences Division, National Institute of Standards and Technology, Charleston, SC 29412, USA

Introduction

To meet the need for quality control materials in support of the National Oceanic and Atmospheric Administration's (NOAA's) Mussel Watch and National Status and Trends Programs [1], the National Institute of Standards and Technology (NIST), with support from NOAA, developed SRM 1974 Organics in Mussel Tissue (*Mytilus edulis*) in 1990 for the determination of selected polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated pesticides [2]. The mussels used to prepare this material were collected from Dorchester Bay, MA, USA in October 1987. Due to the popularity of this material, three additional collections of mussels, designated as SRM 1974a [3], SRM 1974b [4], and SRM 1974c, were made from the same location (Table 1). Over the years, NIST has archived bottles of each material stored at -80°C . Having these highly characterized, homogeneous materials available with documented storage history provides a unique opportunity to address the question of long-term stability of selected analyte classes in frozen tissue samples.

With the mussels collected from the same location over a 17 y period (Table 1), the data from these samples also provide snapshots of the environmental pollution in Dorchester Bay, MA and an evaluation of newer analytical methods. These SRMs have been used to evaluate various analytical methods at NIST, such as moving from gas chromatography with electron capture detection (GC-ECD) to GC/mass spectrometry (GC/MS) for the determination of chlorinated analytes, and used by other researchers for method development, such as extraction using QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) [5] or SPME (Solid Phase Microextraction) [6], and these SRMs have been used in method development for additional analyte classes, such as synthetic musk fragrances [7] and brominated diphenyl ethers (BDEs) [8].

Table 1 Dates of collection and certification for the SRM 1974 series materials

SRM	Date Collected	Year Certified/Issued
SRM 1974	Dec 1987	1990
SRM 1974a	Oct 1992	1995
SRM 1974b	Oct 1999	2003
SRM 1974c	Apr 2004	2012

A previous study addressed the stability of PAHs in SRM 1974 over a 10 y storage period and SRM 1974a over a 6 year storage period [9]. The PAHs were found to be stable over these time periods. Other than that study, there is little information in the literature on the long-term stability of persistent organic contaminants in frozen tissue samples. An older study looked at the stability of tributyltin in oysters, water, sediment, and cockles, but only over an 18 month period [10]. There are two studies published discussing the effects of freeze-thaw cycles on extractability of selected PAHs from soils, but no known studies for tissues [11, 12].

The current study extends the storage period investigated for frozen mussel tissue stored at $-80\text{ }^{\circ}\text{C}$ to 25 y and addresses additional classes of analytes: PCBs and chlorinated pesticides. In addition to the storage data at $-80\text{ }^{\circ}\text{C}$, additional samples of SRM 1974 that had been subjected to a thaw-freeze situation were evaluated along with additional samples of SRM 1974 stored in a liquid nitrogen vapor freezer. With the stability assessment being done in conjunction with the certification of the latest release in the series, SRM 1974c, an additional class of compounds, BDEs, was characterized in each material, and the data are reported in this paper.

Experimental

Note the following details are for the most recent set of analyses for these mussel tissue samples. For conditions used for the previous sets of analyses, see the Certificates of Analysis (COAs) available at <http://www.nist.gov/srm/> and previous publications [2–4].

Materials SRM 1491a Methyl-substituted Polycyclic Aromatic Hydrocarbons in Toluene, SRM

2257 PBDE Congeners in 2,2,4-Trimethylpentane, SRM 2258 BDE 209 in 2,2,4-Trimethylpentane, SRM 2259 80 PCB Congeners in 2,2,4-Trimethylpentane, SRM 2260a Aromatic Hydrocarbons in Toluene, SRM 2261 Chlorinated Pesticides in Hexane (Nominal Concentration $2\text{ }\mu\text{g/mL}$), and SRM 2275 Chlorinated Pesticide Solution II in Isooctane, were used to prepare the calibration solutions. SRM 2269 Perdeuterated PAH Solution I in Hexane/Toluene and SRM 2270 Perdeuterated PAH Solution II in Hexane/Toluene were

used to prepare internal standard solutions for the PAH analyses while PCB 28 $^{13}\text{C}_{12}$, PCB 101 $^{13}\text{C}_{12}$, PCB 153 $^{13}\text{C}_{12}$, PCB 180 $^{13}\text{C}_{12}$, lindane $^{13}\text{C}_6$, 4,4'-DDE $^{13}\text{C}_{12}$, 4,4'-DDT $^{13}\text{C}_{12}$, and BDE 209 $^{13}\text{C}_{12}$ (Cambridge Isotope Laboratories, Andover, MA) and 6 F-BDE 47 (Chiron AS, Trondheim, Norway) were used to prepare internal standard solutions for the chlorinated and brominated analyses.

Sample preparation For Soxhlet extraction, the three subsamples (3 g each, exact mass known) of each mussel tissue SRM were mixed with diatomaceous earth (Hydromatrix, Restek Corporation, Bellefonte, PA) in a glass extraction thimble. An internal standard solution (containing SRM 2269, 2270, 6 F-BDE 47, and the carbon-13 labelled compounds listed above diluted in iso-octane) was gravimetrically added to the top of each extraction thimble. The mixtures were Soxhlet extracted using hexane:acetone (1:1, volume fraction) for 20 h.

Following extraction, the solvent volume was reduced to 0.5 mL using an automated evaporation system and then eluted through two NH2 Plus Sep Paks (Waters Corporation, Milford, MA) in series that were preconditioned with 20 mL of 20 % (volume fraction) dichloromethane in hexane. The mussel extract was then eluted from the Sep Paks using 20 mL of the same solvent mixture. The eluents from the Sep Pak were concentrated to approximately 0.5 mL using the automated evaporation system prior to analysis using gas chromatography/mass spectrometry (GC/MS).

Instrumental method The analytes of interest were quantified using GC/MS in the electron impact (EI) mode for the PAHs, PCBs, and pesticides and in the negative chemical ionization (NCI) mode for the BDEs. The column used for the GC/MS analysis of the PAHs, PCBs, and pesticides was a $0.25\text{ mm} \times 60\text{ m}$ fused silica capillary column containing a 50 % phenyl methylsubstituted polysiloxane phase (DB 17MS, Agilent Technologies, Wilmington, DE, $0.25\text{ }\mu\text{m}$ film thickness). For the BDEs, a $0.25\text{ mm} \times 15\text{ m}$ fused silica capillary column containing a 5 % phenyl methyl-substituted polysiloxane phase (Restek Corporation, $0.25\text{ }\mu\text{m}$ film thickness) was used.

Quantitation Five calibration solutions were prepared by independent weighings of SRM 1491a, SRM 2257, SRM 2258, SRM 2259, SRM 2260a, SRM 2261, and SRM 2275. A five point calibration curve was determined for each analyte of interest by linear regression of response data from the gravimetric solutions mixed with the internal standard solution. The response curves (not forced through the origin) were used for calculation of the concentrations in the mussel samples.

Total extractable organics (TEOs) Three aliquots of each SRM (approximately 1 g, exact mass known) were extracted using pressurized liquid extraction (PLE) with dichloromethane as the extraction solvent. The extracts were weighed and

then evaporated to dryness and reweighed. The TEOs were calculated based on the residual mass relative to the starting mass of mussel tissue.

Results and discussion

As part of on-going environmental monitoring studies, samples are often archived for retrospective analysis. For example, NOAA's Mussel Watch and National Status and Trends (NS&T) programs [1] included both a real-time analysis and a specimen banking component for long-term archival of tissue and sediment specimens. Due to the number of sites and quantities of samples, the real-time analyses were handled by a number of different organizations so NOAA had the foresight to implement quality assurance (QA) requirements for the participating laboratories. One part of the QA requirement was the inclusion of SRMs as part of the routine analyses to ensure data quality within a laboratory and comparability across laboratories. SRM 1974 Organics in Mussel Tissue (*Mytilus edulis*) was developed and certified by NIST (first issued in 1990) for use in the NOAA programs. SRM 1974 and the subsequent tissues in the series (SRM 1974a, SRM

1974b, and SRM 1974c) are cryogenically homogenized [13] mussel tissue bottled with approximately 10 g of mussel tissue homogenate per glass bottle and stored at -80°C . When maintained at this storage temperature, the homogenates remain particulate in nature and are easy to subsample.

As part of the QA protocols at NIST, SRMs are analyzed with each set of analyses of similar sample types. For SRM 1974, analyses have been done periodically over 25 y (see Electronic Supplementary Material (ESM) Table S1), 20 y for SRM 1974a (ESM Table S2), and 12 y for SRM 1974b (ESM Table S3). Over these time periods, the methods at NIST have evolved. Each set of data is based on the analytical methods used at NIST at the indicated period of time [2–4].

Subsampling One of the issues with subsampling is the heterogeneity of the sample as one takes smaller and smaller sample sizes. During the certification analysis of SRM 1974, sample sizes of 13 to 24 g were used for analysis, and during the certification analysis of SRM 1974a, sample sizes of 7 to 9 g were used for analysis. The heterogeneity of SRM 1974a was later evaluated at smaller sample sizes similar to the studies reported by Lipka and Schantz [14] for air and diesel particulate SRMs. The microheterogeneity studies for SRM 1974a indicated that a 3 g subsample resulted in a

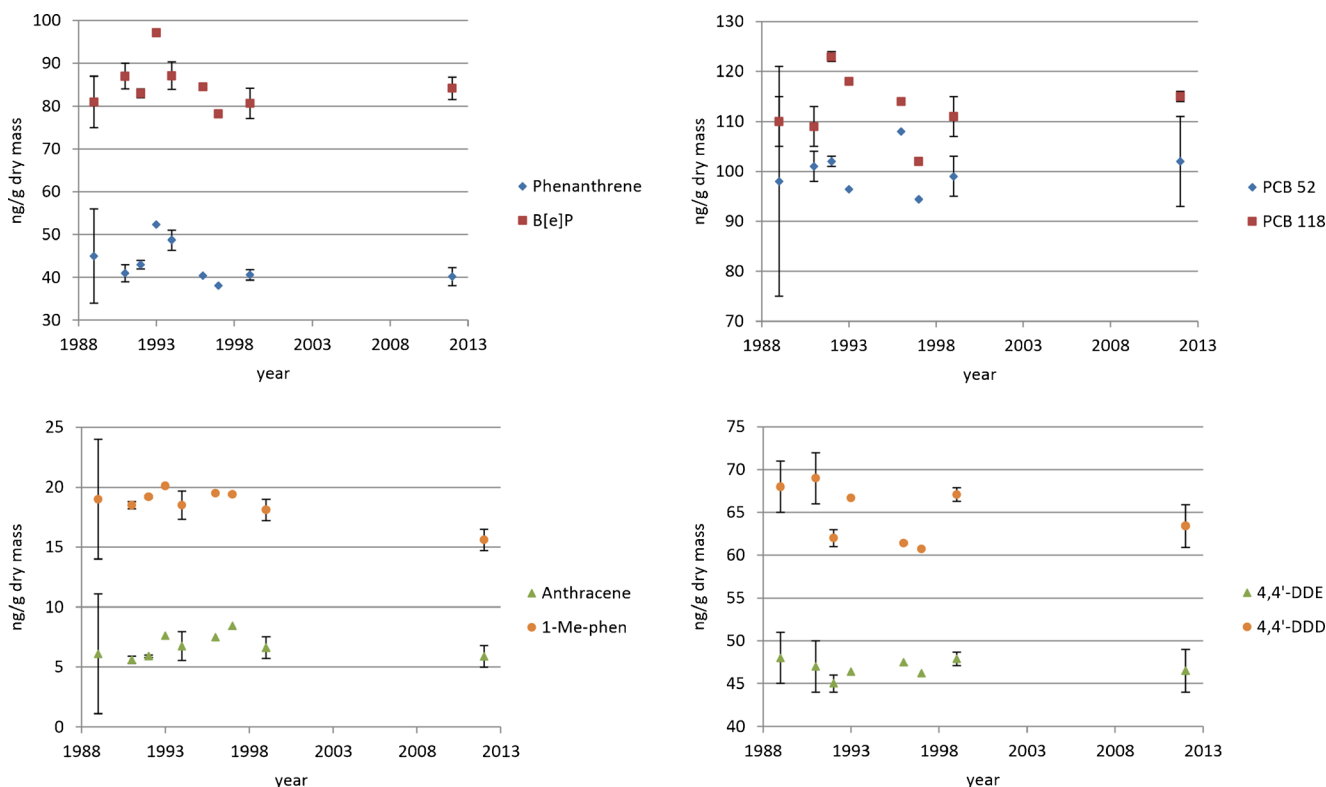


Fig. 1 Stability of selected PAHs (two graphs on left) and PCB congeners and pesticides (two graphs on right) in samples of SRM 1974 stored at -80°C . The first point on each graph is the certified value with the expanded uncertainty. The points without error bars are from the

analysis of one sample while the points (other than the first one) with error bars are the means and standard deviations from three analyses. Complete data set is shown in ESM Table S1

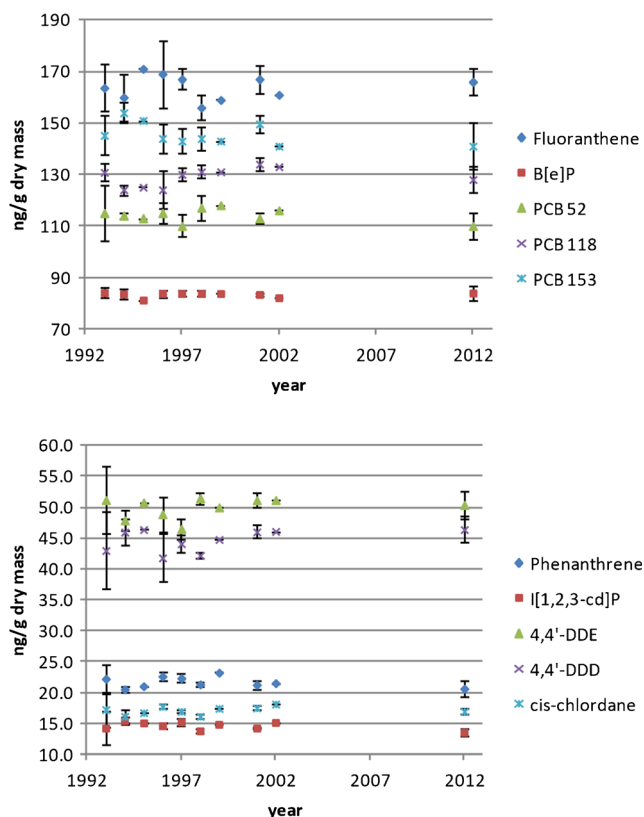


Fig. 2 Stability of selected PAHs, PCB congeners, and pesticides in samples of SRM 1974a stored at $-80\text{ }^{\circ}\text{C}$. The first point on each graph is the certified value with the expanded uncertainty. The points without error bars are from the analysis of one sample while the points (other than the first one) with error bars are the means and standard deviations from three analyses. Complete data set is shown in ESM Table S2

subsampling error of $\leq 1\%$ [15, 16]. Based on these results, 3 g were used for the certification analyses of SRM 1974b and SRM 1974c and for this storage study.

Several jars of SRM 1974 were thawed and refrozen at $-80\text{ }^{\circ}\text{C}$. The material in these jars could no longer be subsampled while frozen so they had to be thawed, thoroughly mixed before subsamples could be withdrawn for analysis. When the analytical results were corrected to a dry mass basis, they agreed within the uncertainties of the values reported on the Certificate of Analysis (COA) (ESM Table S1). In addition to these refrozen samples, some samples of SRM 1974 were

stored in a liquid nitrogen vapor freezer since preparation. The material in these jars could be subsampled while frozen, and the analytical results from the analyses corrected to a dry mass basis agreed within the uncertainties of the values reported on the COA (ESM Table S1).

Evolution of analytical methods Over the 25 y since SRM 1974 was first analyzed at NIST, a number of changes have been made to the analytical methods used. For the PCB analyses, GCECD using PCB congeners not present in the sample as the internal standards (ISs) has been replaced with GC/MS using carbon-13 labelled PCB congeners as the ISs. The change from using GC-ECD to using GC/MS also eliminated the need to do a fractionation of the PCB and lower pesticide fraction from the more polar pesticide fraction. For the PAHs, the use of carbon-13 labelled PAHs as ISs was compared to the use of deuterated PAHs as ISs during the analyses of SRM 1974a [17]. The data were comparable indicating that label on the PAH ISs did not make a difference. The data shown in ESM Tables S1 to S3 indicate the consistent quality of measurements over time even during the evolution of methods.

Long-term stability Using the current methods of analysis at each of the sampling times, the stability of the PAHs, PCBs, and pesticides was assessed in samples of SRM 1974 stored at $-80\text{ }^{\circ}\text{C}$ for 25 y (Fig. 1 and ESM Table S1), in samples of SRM 1974a stored at $-80\text{ }^{\circ}\text{C}$ for 20 y (Fig. 2 and ESM Table S2), and in samples of SRM 1974b stored at $-80\text{ }^{\circ}\text{C}$ for 12 y (ESM Table S3). As indicated in Figs. 1 and 2, the concentrations of the analytes have been stable over the storage times. All of the data have been converted to a dry mass basis. For the samples stored at $-80\text{ }^{\circ}\text{C}$ with no thawing periods, the percent water in the materials has remained constant over the storage period. However, ice crystal formation has been noted in sediment samples stored at $-20\text{ }^{\circ}\text{C}$ for multiple years hence the caution to perform a dry mass correction for each set of analyses to ensure comparability of data.

Snapshot of contaminant trends in Dorchester Bay, MA With the four collections made from the same location, Dorchester Bay, MA, USA, these materials also provide snapshots as to the environmental contaminant status of that area. Three of the

Table 2 Mass fractions (ng/g dry mass) and expanded uncertainties ($k=2$) for selected BDE Congeners and total extractable organics (TEO) (% by mass) in the SRM 1974 series collected in Dorchester Bay

BDE Congener	SRM 1974	SRM 1974a	SRM 1974b	SRM 1974c
47	4.44 \pm 0.19	3.99 \pm 0.21	3.11 \pm 0.05	9.16 \pm 0.18
49	0.719 \pm 0.028	0.684 \pm 0.033	0.712 \pm 0.025	1.37 \pm 0.05
99	1.55 \pm 0.11	1.29 \pm 0.07	1.03 \pm 0.05	3.66 \pm 0.05
TEO (% by mass)	0.81 \pm 0.05	0.79 \pm 0.01	0.60 \pm 0.02	0.76 \pm 0.01

four collections were made in the fall of the year and the fourth was made in the spring of the year (Table 1). This seasonal variation may lead to some differences in the uptake by the mussels depending on the spawning cycle. Total extractable organics (TEOs) were assessed in the four materials to quantify the difference in the lipid levels among the four collections (Table 2). SRM 1974b has the lowest TEO (0.60 % by mass) while SRM 1974 has the highest TEO (0.81 % by mass). SRM 1974c, the collection made in the spring of the year, has an intermediate TEO (0.76 % by mass) suggesting that there is not a large difference in the lipid content with season of the year at least for these collections.

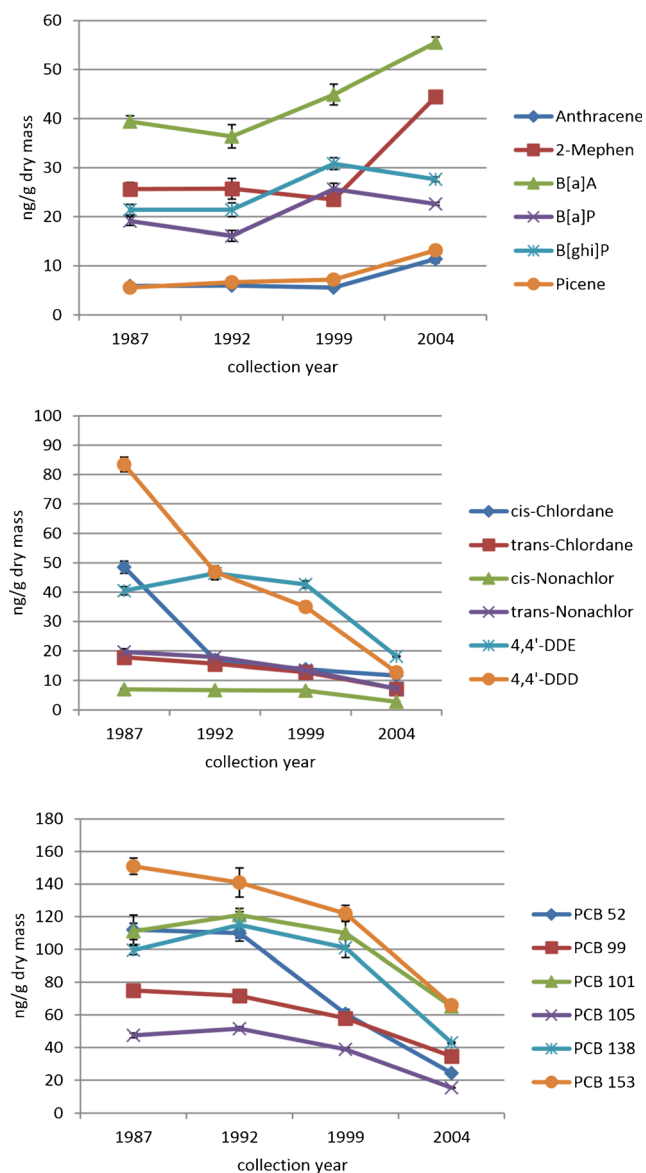


Fig. 3 Trends for selected analytes in the SRM 1974 mussels collected from Dorchester Bay in 1987 (SRM 1974), 1992 (SRM 1974a), 1999 (SRM 1974b) and 2004 (SRM 1974c). Each point is the mean value and standard deviation from three subsamples with analyses performed in conjunction with the certification of SRM 1974c

The temporal trends for selected PAHs, PCBs, and pesticides in the four collections are shown in Fig. 3. The mass fractions of the PAHs do not show any consistent trend across the four sampling periods with concentrations of some PAHs increasing and others decreasing. The mass fractions of the PCBs and pesticides show a decrease across the four sampling periods. These differences between PAHs and PCB/pesticide trends are most likely due to the more widespread sources for PAHs (most combustion processes) compared to PCBs and chlorinated pesticides, two groups of compounds that have been phased out over time.

This study was the first to quantify the BDEs, a group of flame retardants, in the four mussel tissue SRMs with the data shown in Table 2. The mass fractions for the BDEs are relatively low in the mussel tissue samples with only three congeners consistently quantified in all four materials. For those three congeners, however, the mass fractions are consistent for the 1987 to 1999 collections and then increased approximately three fold in the 2004 collection. This trend is different than that observed for BDEs in Great Lakes fish [18] where it was observed that the concentrations of penta- and hexa-BDEs started to decrease in the mid-1990s. This study, however, includes only four points in time and may not be indicative of the overall trends in Dorchester Bay, MA.

Conclusion

Persistent organic pollutants, including PAHs, PCBs, and pesticides, are stable in frozen mussel tissue samples stored at -80 °C for at least 25 y as indicated by the analysis of SRM 1974 Organics in Mussel Tissue (*Mytilus edulis*). This long-term stability information is important to large environmental monitoring studies collecting and storing large numbers of samples for future analysis, i.e., formally established specimen banking programs. The samples can undergo freeze-thawing cycles as long as the samples are well homogenized prior to subsampling and the results are reported on a dry-mass basis. In addition, having homogeneous samples available for long periods of time aids in the evaluation of new analytical methods and in the retrospective quantification of new analyte classes.

Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper 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 necessarily the best available for the purpose.

References

1. Kimbrough KL, Johnson WE, Lauenstein GG, Christensen JD, Apeti DA (2008) An Assessment of Two Decades of Contaminant Monitoring in the Nation's Coastal Zone, Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 74: 105 pp available at <http://ccma.nos.noaa.gov/publications/MWTwoDecades.pdf> (accessed 15 July 2014)
2. Wise SA, Benner BA, Christensen RG, Koster BJ, Kurz J, Schantz MM, Zeisler R (1991) *Environ Sci Technol* 25: 1695–1704
3. Schantz MM, Demiralp R, Greenberg RR, Hays MJ, Parris RM, Porter BJ, Poster DL, Sander LC, Sharpless KS, Wise SA, Schiller SB (1997) *Fresenius J Anal Chem* 358:431–440
4. Poster DL, Schantz MM, Kucklick JR, de Alda MJL, Porter BJ, Pugh R, Wise SA (2004) *Anal Bioanal Chem* 378:1213–1231
5. Johnson YS (2012) *J Food Sci* 77:T131–T137
6. Aguinaga N, Campillo N, Vinas P, Hernandez-Cordoba M (2008) *Anal Bioanal Chem* 391:1419–1424
7. Peck AM, Kucklick JR, Schantz MM (2007) *Anal Bioanal Chem* 387:2381–2388
8. Zhu LY, Hites RA (2003) *Anal Chem* 75:6696–6700
9. Schantz MM, Porter BJ, Wise SA (2000) *Polycycl Aromat Compd* 19:253–262
10. Gomez-Ariza JL, Giraldez I, Morales E, Ariese F, Cofino W, Quevauviller P (1999) *J Environ Monitor* 1:197–202
11. Zhao Q, Li PH, Stagnitti F, Ye J, Dong DB, Zhang YQ, Li P (2009) *Chemosphere* 76:447452
12. Xhao Q, Xing BS, Tai PD, Li H, Song L, Zhang LZ, Li PJ (2013) *Sci Total Environ* 444:311–319
13. Zeisler R, Langland JK, Harrison SH (1983) *Anal Chem* 55:2431–2434
14. Lippa KA, Schantz MM (2007) *Anal Bioanal Chem* 387:2389–2399
15. Ingamells CO, Switzer P (1973) *Talanta* 20:547–568
16. Ingamells CO (1974) *Talanta* 21:144–155
17. Wise SA, Phinney KW, Sander LC, Schantz MM (2012) *J Chromatogr A* 1261:3–22
18. Batterman S, Chernyak S, Gwynn E, Cantonwine D, Jia C, Begnoche L, Hickey JP (2007) *Chemosphere* 69:444–457