National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2974a

Organics in Freeze-Dried Mussel Tissue (*Mytilus edulis*)

This Standard Reference Material (SRM) is intended for use in evaluating analytical methods for the determination of selected polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, polybrominated diphenyl ether (PBDE) congeners, hexabromocyclododecane (HBCD) isomers, methylmercury, inorganic mercury, and total mercury in marine bivalve mollusk tissue and similar matrices. All of the constituents for which certified and reference values are provided are naturally present in the freeze-dried mussel tissue. A unit of SRM 2974a consists of one bottle containing approximately 5 g of freeze-dried mussel tissue.

Certified Mass Fraction Values: Certified values, expressed as mass fractions, for 22 PAHs (some in combination), 29 PCB congeners (some in combination), eight chlorinated pesticides, and six PBDE congeners (some in combination) are provided in Tables 1 through 4, respectively. Certified values for methylmercury, inorganic mercury, and total mercury are provided in Table 5. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified values are based on the agreement of results obtained at NIST using multiple analytical techniques.

Reference Mass Fraction Values: Reference values, expressed as mass fractions, are provided in Table 6 for eight additional PAHs, in Table 7 for 14 additional PCB congeners (some in combination), and in Table 8 for 8 additional PBDE congeners and for 2 HBCD isomers. Reference values are noncertified values that are estimates of the true value; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1].

Expiration of Certification: The certification of **SRM 2974a** is valid, within the measurement uncertainty specified, until **31 July 2026**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Handling, Storage, and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Overall direction and coordination of technical measurements leading to certification were performed by M.M. Schantz and L.C. Sander of the NIST Chemical Sciences Division.

The mussels were collected by TDI-Brooks International (College Station, TX). Preparation of the freeze-dried material was performed by the NIST Office of Reference Materials and M.B. Ellisor, A.J. Moors, B.J. Porter, and R.S. Pugh of the NIST Chemical Sciences Division.

Analytical measurements at NIST were performed by W.C. Davis, J.M. Lynch, J.R. Kucklick, B.J. Porter, M.M. Schantz, and S.S. Schuur of the NIST Chemical Sciences Division.

Statistical consultation was provided by S.D. Leigh, formerly of the NIST Statistical Engineering Division.

Carlos A. Gonzalez, Chief
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Gaithersburg, MD 20899
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Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Handling: SRM 2974a IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION. Normal biohazard safety precautions for the handling of biological tissues should be exercised.

Storage: SRM 2974a is provided as a freeze-dried tissue homogenate in amber glass bottles. The tissue material should be stored at room temperature or below.

Use: Prior to removal of test portions for analysis, the contents of the bottle should be mixed. The mass fraction for each constituent in SRM 2974a is reported on a dry-mass basis. The freeze-dried mussel tissue homogenate is hygroscopic and as received contains approximately 3 % (mass fraction) residual moisture. The mussel tissue sample should be dried to a constant mass before weighing for analysis, or if the constituents of interest are volatile, a separate test portion of the mussel tissue should be removed from the bottle at the time of analysis and dried to determine the mass fraction on a dry-mass basis.

PREPARATION AND ANALYSIS[1]

Sample Collection and Preparation: The mussels (*Mytilus edulis*) used for the preparation of SRM 2974a were collected in Dorchester Bay, MA in 2004. The mussels were frozen and trucked to NIST (Hollings Marine Laboratory, Charleston, SC) where they were stored in a liquid nitrogen vapor freezer. For processing, the mussels were allowed to warm to approximately 0 °C, shells were opened, and the tissue removed using titanium knives. The tissue was stored in a liquid nitrogen vapor freezer until the next step. The tissue was allowed to thaw, was blended into a puree form, and was poured into aluminum trays and frozen. The material was then freeze-dried with a starting temperature of –40 °C and slowly warmed to a temperature of 10 °C. The dry material was shipped frozen to NIST in Gaithersburg, MD. Once received, it was thawed and broken into smaller chunks, then jet-milled to produce a fine powder. The powder was blended for homogeneity by processing through the jet mill twice. The material was radiation sterilized (30 kGy of ^{60}Co) and aliquoted into jars (≈ 5 g each).

PAHs, PCBs, Chlorinated Pesticides, and PBDEs: The general approach used for value assignment of the PAHs, PCBs, chlorinated pesticides, and PBDEs in SRM 2974a was similar to that reported for the certification of several environmental matrix SRMs [2] and consisted of combining results from analyses using various combinations of different extraction techniques, cleanup/isolation procedures, and chromatographic separation and detection techniques.

Three sets of gas chromatography/mass spectrometry (GC/MS) results, designated as GC/MS (I), GC/MS (II), and GC/MS (III) were obtained at NIST. For GC/MS (I) analyses, single test portions of between 1 g and 2 g from nine bottles of SRM 2974a were extracted using Soxhlet extraction with hexane:acetone (1+1 volume fraction). The extract was fractionated using an aminopropyl solid-phase extraction (SPE) column to isolate the fraction of interest. The processed extract was then analyzed by GC/MS using a 0.25 mm i.d. × 60 m fused silica capillary column with a 50 % (mole fraction) phenyl methylpolysiloxane phase (0.25 µm film thickness; DB-17, Agilent Technologies, Wilmington, DE) and a 0.25 mm i.d. × 15 m fused silica capillary column with a 50 % (mole fraction) liquid crystal polysiloxane phase (0.15 µm film thickness; LC-50, J&K Scientific, Milton, Ontario, Canada). The PAHs, PCBs, and pesticides were analyzed on the DB-17 column using electron impact MS (EI-MS), method GC/MS (Ia). The PAHs were also analyzed on the LC-50 column using EI-MS, method GC/MS (Ib). The PBDEs were analyzed on the LC-50 column using electron-capture negative chemical ionization MS (NCI-MS), method GC/MS (Ic).

For the GC/MS (II) determination of the PAHs, one test portion (3 g) from each of six bottles was extracted using pressurized-fluid extraction (PFE) with dichloromethane (DCM). Size exclusion chromatography (SEC) on a preparative-scale divinylbenzene-polystyrene column (10 µm particle size, 10 nm (100 Å) pore size, 2.5 cm i.d. × 60 cm, PL-Gel, Agilent Technologies, Santa Clara, CA) was used to remove the majority of the lipid and biogenic material. The fraction of interest was further isolated using an alumina column (5 % deactivated) SPE column. The isolated fraction was then analyzed by GC/MS using a 0.25 mm i.d. × 60 m fused silica capillary column with a 50 % phenyl methylpolysiloxane phase (0.25 µm film thickness; DB-17MS, Agilent Technologies).

[1] Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify 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.

The GC/MS (III) analyses focused on the PCBs, pesticides, and PBDEs. Seven samples (between 1 g and 3 g) were extracted using PFE with DCM. The extracts were cleaned up using an alumina column (5 % deactivated) SPE column followed by an acidified silica SPE column. Two fractions were collected from the acidified silica column. SEC on a preparative-scale divinylbenzene-polystyrene column (see above) was then used to remove the remainder of the lipid and biogenic material from both fractions. The PCBs, some pesticides, and the PBDEs were quantified using GC/EI-MS on a 0.18 mm i.d. × 30 m fused silica capillary column with a 5 % (mole fraction) phenyl methylpolysiloxane phase (0.18 µm film thickness; DB-5MS, Agilent Technologies). The same column was used for GC/NCI-MS to quantify additional pesticides. The PBDEs were also quantified using GC/NCI-MS on a 0.18 mm i.d. × 10 m fused silica capillary column with a 5 % (mole fraction) phenyl methylpolysiloxane phase (0.18 µm film thickness; DB-5MS, Agilent Technologies).

For the methods described above, selected perdeuterated PAHs, ^{13}C-labeled PCBs, perdeuterated pesticides, and fluorinated and ^{13}C-labeled PBDEs were added to the mussel tissue prior to solvent extraction for use as internal standards for quantification purposes.

Homogeneity Assessment for PAHs, PCBs, Chlorinated Pesticides, and PBDEs: The homogeneity of SRM 2974a was assessed by analyzing duplicate 1 g to 2 g samples from nine bottles selected by stratified random sampling using Method GC/MS (I) above. No statistically significant differences among bottles were observed for the four classes of compounds at the 2 g sample size.

HBCDs: The second fraction from the acidified silica SPE clean-up in Method GC/MS (III) above was analyzed by LC/MS/MS for the HBCDs using both electrospray ionization (ESI), Method LC/MS/MS IIId, and atmospheric pressurized photoionization (APPI), Method LC/MS/MS IIIe. A C_{18} column (3.0 mm × 150 mm × 3.5 µm column, Eclipse Plus, Agilent Technologies) was used with a solvent gradient using 2.5 mmol/L ammonium acetate in 12.5 % water in methanol (volume fraction) and acetonitrile at a flow rate of 0.3 mL/min. ^{13}C-labeled HBCDs were added to the mussel tissue prior to solvent extraction for use as internal standards for quantification purposes.

Methylmercury and Inorganic Mercury: For the determination of methylmercury and inorganic mercury, SRM 2974a was analyzed at NIST using double-spike speciated isotope dilution GC/inductively coupled plasma mass spectrometry (GC/ICPMS). For the speciated isotope dilution GC/ICPMS analyses, approximately 0.5 g test portions were spiked with an appropriately diluted sample of IRMM-670 ^{202}Hg-enriched methylmercury isotopic CRM (Institute for Reference Materials and Measurements, Geel, Belgium) and subjected to an alkaline microwave digestion (using 25 % volume fraction tetraammonium hydroxide in water). Sodium tetraethylborate was used for ethylation. The derivatized methylmercury was back-extracted into hexane and injected into the GC/ICPMS. The GC analysis was performed using a 30 m ×0.25 mm column with a 5 % phenyl methylpolysiloxane phase (0.25 µm film thickness; DB-5MS+DG, Agilent Technologies) [3].

Methylmercury was also determined using GC/EI-MS. Duplicate test portions (1 g to 2 g) from nine bottles of SRM 2974a were spiked with an *n*-propylmercuric chloride solution. Potassium hydroxide solution was then added and the mixture sonicated. Water and sodium acetate buffer were then added to the supernatant followed by sodium tetraphenylborate. A solid-phase microextraction (SPME) fiber was then immersed in the mixture. GC analysis was performed on a 100 % dimethylpolysiloxane column, 25 m × 0.32 mm, 0.17 µm film thickness (HP-1, Agilent Technologies).

Total Mercury: Approximately 0.5 g test portions from six bottles of SRM 2974a were spiked with a ^{201}Hg isotopic solution. Nitric acid was then added and the samples were microwave digested. After cooling to room temperature, the extracts were diluted with water and analyzed using cold-vapor mercury generation coupled with inductively coupled plasma mass spectrometry (ICP-MS) [1]. The mercury vapor was generated using tin (II) chloride reductant and separated from the liquid phase using a commercial gas liquid separator.

Table 1. Certified Mass Fraction Values for Selected PAHs in SRM 2974a

	Mass Fraction[a] µg/kg (dry-mass basis)		
Phenanthrene[b,c,d]	74.4	±	4.7
1-Methylphenanthrene[b,d]	17.6	±	1.6
2-Methylphenanthrene[b,d]	28.2	±	2.6
3-Methylphenanthrene[b,d]	24.1	±	1.4
9-Methylphenanthrene[b,d]	15.9	±	1.3
4-*H*-Cyclopenta[*def*]phenanthrene[b,d]	13.15	±	0.71
Fluoranthene[b,c,d]	287	±	34
Pyrene[b,c,d]	166	±	21
1-Methylpyrene[b,d]	10.69	±	0.83
4-Methylpyrene[b,d]	19.77	±	0.89
Benzo[*ghi*]fluoranthene[b,c,d]	18.7	±	1.7
Benzo[*c*]phenanthrene[b,c,d]	23.2	±	1.9
Benz[*a*]anthracene[b,c,d]	31.1	±	3.9
Chrysene and Triphenylene[b,d]	123.6	±	2.9
Benzo[*b*]fluoranthene[b,d]	41.5	±	2.6
Benzo[*j*]fluoranthene[b,d]	21.4	±	1.1
Benzo[*k*]fluoranthene[b,d]	18.95	±	0.54
Benzo[*e*]pyrene[b,c,d]	58.9	±	2.9
Benzo[*a*]pyrene[b,c,d]	9.73	±	0.43
Perylene[b,c]	6.80	±	0.34
Benzo[*ghi*]perylene[b,c,d]	23.7	±	2.2

[a] The certified value is an unweighted mean of the results from two or three analytical methods. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6]. The measurand is the total mass fraction on a dry-mass basis for each selected PAH. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).

[b] GC/EI-MS Ia using Soxhlet extraction followed by analysis on a DB-17 column.

[c] GC/EI-MS Ib using Soxhlet extraction followed by analysis on a LC-50 column.

[d] GC/EI-MS II using PFE followed by analysis on a DB-17MS column.

Table 2. Certified Mass Fraction Values for Selected PCB Congeners[a] in SRM 2974a

			Mass Fraction[b] µg/kg (dry mass basis)		
PCB	8	(2,4'-Dichlorobiphenyl)[c,d]	2.01	±	0.08
PCB	18	(2,2',5-Trichlorobiphenyl)[c,d]	4.03	±	0.22
PCB	44	(2,2'3,5'-Tetrachlorobiphenyl)[c,d]	16.24	±	0.71
PCB	49	(2,2',4,5'-Tetrachlorobiphenyl)[c,d]	17.1	±	1.2
PCB	52	(2,2',5,5'-Tetrachlorobiphenyl)[c,d]	22.42	±	0.92
PCB	66	(2,3',4,4'-Tetrachlorobiphenyl)[c,d]	20.6	±	1.1
PCB	70	(2,3',4',5-Tetrachlorobiphenyl)[c,d]	15.45	±	0.64
PCB	74	(2,4,4',5-Tetrachlorobiphenyl)[c,d]	9.02	±	0.37
PCB	87	(2,2',3,4,5'-Pentachlorobiphenyl)[c,d]	14.36	±	0.56
PCB	95	(2,2',3,5',6-Pentachlorobiphenyl)[c,d]	23.72	±	0.49
PCB	99	(2,2',4,4'5-Pentachlorobiphenyl)[c,d]	24.51	±	0.54
PCB	101	(2,2',4,5,5'-Pentachlorobiphenyl)[c,d]	39.84	±	0.96
PCB	105	(2,3,3',4,4'-Pentachlorobiphenyl)[c,d]	16.47	±	0.43
PCB	110	(2,3,3',4',6-Pentachlorobiphenyl)[c,d]	35.88	±	0.87
PCB	118	(2,3',4,4',5-Pentachlorobiphenyl)[c,d]	42.9	±	2.1
PCB	128	(2,2',3,3',4,4'-Hexachlorobiphenyl)[c,d]	8.24	±	0.33
PCB	138	(2,2',3,4,4',5'-Hexachlorobiphenyl)[c,d]	61.5	±	2.3
PCB	146	(2,2',3,4',5,5'-Hexachlorobiphenyl)[c,d]	8.07	±	0.40
PCB	149	(2,2',3,4',5',6-Hexachlorobiphenyl)[c,d]	31.77	±	0.95
PCB	151	(2,2',3,5,5',6-Hexachlorobiphenyl)[c,d]	5.99	±	0.20
PCB	153	(2,2',4,4',5,5'-Hexachlorobiphenyl)[c,d]	78.8	±	2.5
PCB	156	(2,3,3',4,4',5-Hexachlorobiphenyl)[c,d]	5.80	±	0.25
PCB	170	(2,2',3,3',4,4',5-Heptachlorobiphenyl)[c,d]	2.04	±	0.08
PCB	177	(2,2'3,3',4',5,6-Heptachlorobiphenyl)[c,d]	5.48	±	0.20
PCB	180	(2,2',3,4,4',5,5'-Heptachlorobiphenyl)[c,d]	5.31	±	0.16
	193	(2,3,3',4',5,5',6-Heptachlorobiphenyl)			
PCB	183	(2,2',3,4,4'5',6-Heptachlorobiphenyl)[c,d]	7.06	±	0.26
PCB	187	(2,2',3,4',5,5'6-Heptachlorobiphenyl)[c,d]	15.52	±	0.48
PCB	194	(2,2',3,3',4,4',5,5'-Octachlorobiphenyl)[c,d]	0.485	±	0.040

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [7] and later revised by Schulte and Malisch [8] to conform with IUPAC rules; for the specific congeners mentioned in this SRM, the Ballschmiter-Zell numbers correspond to those of Schulte and Malisch. When two or more congeners are known to coelute under the GC analysis conditions used, the PCB congener listed first is the major component and the additional congeners may be present as minor components. The quantitative results are based on the response of the congener listed first.

[b] The certified value is an unweighted mean of the results from two analytical methods. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6]. The measurand is the total mass fraction on a dry-mass basis for each selected PCB Congener. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).

[c] GC/EI-MS Ia using Soxhlet extraction followed by analysis on a DB-17 column.

[d] GC/EI-MS IIIa using PFE followed by analysis on a DB-5 column (30 m).

Table 3. Certified Mass Fraction Values for Selected Chlorinated Pesticides in SRM 2974a

	Mass Fraction[a] µg/kg (dry-mass basis)
Hexachlorobenzene[b,c]	0.113 ± 0.007
4,4'-DDE[b,c]	17.37 ± 0.82
4,4'-DDD[b,c]	13.56 ± 0.58
cis-Chlordane[b,d]	8.54 ± 0.17
trans-Chlordane[b,d]	7.12 ± 0.15
cis-Nonachlor[b,d]	1.91 ± 0.10
trans-Nonachlor[b,d]	5.60 ± 0.39

[a] The certified value is an unweighted mean of the results from two analytical methods. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6]. The measurand is the total mass fraction on a dry-mass basis for each selected chlorinated pesticide. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).
[b] GC/EI-MS Ia using Soxhlet extraction followed by analysis on a DB-17 column.
[c] GC/EI-MS IIIa using PFE followed by analysis on a DB-5 column (30 m).
[d] GC/NCI-MS IIIb using PFE followed by analysis on a DB-5 column (30 m).

Table 4. Certified Mass Fraction Values for Selected PBDE Congeners[a] in SRM 2974a

			Mass Fraction[b] µg/kg (dry-mass basis)
PBDE	28	(2,4,4'-Tribromodiphenyl ether)[c,d]	0.905 ± 0.051
	33	(2',3,4-Tribromodiphenyl ether)	
PBDE	49	(2,2',4,5'-Tetrabromodiphenyl ether)[c,d,e]	1.36 ± 0.06
PBDE	99	(2,2',4,4',5-Pentabromodiphenyl ether)[c,d,e]	4.78 ± 0.24
PBDE	153	(2,2',4,4',5,5'-Hexabromodiphenyl ether)[c,d]	0.201 ± 0.014
PBDE	209	(Decabromodiphenyl ether)[c,d]	1.99 ± 0.11

[a] PBDE congeners are numbered according to IUPAC rules. When two or more congeners are known to coelute under the GC analysis conditions used, the BDE congener listed first is the major component and the additional congeners may be present as minor components. The quantitative results are based on the response of the congener listed first.
[b] The certified value is an unweighted mean of the results from two or three analytical methods. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6]. The measurand is the total mass fraction on a dry-mass basis for each selected PBDE congener. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).
[c] GC/NCI-MS Ic using Soxhlet extraction followed by analysis on a LC-50 column.
[d] GC/NCI-MS IIIc using PFE followed by analysis on a DB-5 column (10 m).
[e] GC/EI-MS IIIa using PFE followed by analysis on a DB-5 column (30 m).

Table 5. Certified Mass Fraction Values for Methylmercury, Inorganic Mercury, and Total Mercury in SRM 2974a

	Mass Fraction µg/kg (dry-mass basis)
Methylmercury	69.06 ± 0.81[a,b]
Inorganic Mercury	122 ± 3[c]
Total Mercury	195 ± 3[c]

[a] Results for methylmercury are reported as micrograms per kilogram mercury.

[b] The certified value is an unweighted mean of the results from two or three analytical methods. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6]. The measurand is the total mass fraction on a dry-mass basis for methylmercury. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).

[c] The certified values are the means of results using one definitive analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte. The measurand is the total mass fraction on a dry-mass basis for inorganic and total mercury. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).

Table 6. Reference Mass Fraction Values for Selected PAHs in SRM 2974a

	Mass Fraction µg/kg (dry-mass basis)
Naphthalene[a]	9.68 ± 0.67[b]
1-Methylnaphthalene[a,c]	5.8 ± 1.5[d]
2-Methylnaphthalene[a,c]	8.1 ± 1.9[d]
1,6-Dimethylnaphthalene[c]	7.50 ± 0.26[b]
2,6-Dimethylnaphthalene[c]	3.70 ± 0.10[b]
Biphenyl[a]	3.56 ± 0.14[b]
Anthracene[a]	2.46 ± 0.10[b]
2-Methylanthracene[c]	3.00 ± 0.11[b]
1,7-Dimethylphenanthrene[c]	16.7 ± 0.4[b]
1-Methylfluoranthene[c]	9.50 ± 0.29[b]
3-Methylfluoranthene[c]	15.5 ± 0.3[b]
Chrysene[e]	85.1 ± 1.1[b]
Triphenylene[e]	42.9 ± 1.0[b]
3-Methylchrysene[c]	3.88 ± 0.11[b]
6-Methylchrysene[c]	3.42 ± 0.13[b]
Benzo[*a*]fluoranthene[a]	3.58 ± 0.17[b]
Indeno[1,2,3-*cd*]pyrene[a,c,e]	14.9 ± 4.5[d]
Coronene[c]	13.6 ± 0.3[b]

[a] GC/EI-MS Ia using Soxhlet extraction followed by analysis on a DB-17 column.

[b] The reference values are the means of results using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte. The measurand is the total mass fraction on a dry-mass basis for each selected PAH as determined by the indicated method. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).

[c] GC/EI-MS II using PFE followed by analysis on a DB-17MS column.

[d] The reference value is an unweighted mean of the results from two analytical methods. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6]. The measurand is the total mass fraction on a dry-mass basis for each selected PAH as determined by the indicated methods. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).

[e] GC/EI-MS Ib using Soxhlet extraction followed by analysis on a LC-50 column.

Table 7. Reference Mass Fractions for Selected PCB Congeners[a] in SRM 2974a

			Mass Fraction[b] µg/kg (dry-mass basis)		
PCB	28	(2,4,4'-Trichlorobiphenyl)[c]	12.4	±	0.2
PCB	31	(2,4',5-Trichlorobiphenyl)[c]	9.24	±	0.13
PCB	45	(2,2',3,6-Tetrachlorobiphenyl)[d]	1.02	±	0.06
PCB	56	(2,3,3',4'-Tetrachlorobiphenyl)[d]	10.2	±	0.6
	60	(2,3,4,4'-Tetrachlorobiphenyl)			
PCB	82	(2,2',3,3',4-Pentachlorobiphenyl)[d]	4.56	±	0.21
PCB	92	(2,2',3,5,5'-Pentachlorobiphenyl)[d]	11.9	±	1.0
	84	(2,2',3,3',6-Pentachlorobiphenyl)			
	89	(2,2',3,4,6'-Pentachlorobiphenyl)			
PCB	130	(2,2',3,3',4,5'-Hexachlorobiphenyl)[d]	3.05	±	0.14
PCB	157	(2,3,3',4,4',5'-Hexachlorobiphenyl)[d]	0.952	±	0.059
PCB	167	(2,3',4,4',5,5'-Hexachlorobiphenyl)[d]	2.12	±	0.14
PCB	178	(2,2',3,3',5,5',6-Heptachlorobiphenyl)[d]	2.40	±	0.13
PCB	202	(2,2',3,3',5,5',6,6'-Octachlorobiphenyl)[d]	1.57	±	0.07

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [7] and later revised by Schulte and Malisch [8] to conform with IUPAC rules; for the specific congeners mentioned in this SRM, the Ballschmiter-Zell numbers correspond to those of Schulte and Malisch. When two or more congeners are known to coelute under the GC analysis conditions used, the PCB congener listed first is the major component and the additional congeners may be present as minor components. The quantitative results are based on the response of the congener listed first.

[b] The reference values are the means of results using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte. The measurand is the total mass fraction on a dry-mass basis for each selected PCB congener as determined by the indicated method. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).

[c] GC/EI-MS Ia using Soxhlet extraction followed by analysis on a DB-17 column.

[d] GC/EI-MS IIIa using PFE followed by analysis on a DB-5 column (30 m).

Table 8. Reference Mass Fraction Values for Selected PBDE Congeners[a] and HBCDs in SRM 2974a

			Mass Fraction µg/kg (dry-mass basis)		
PBDE	17	(2,2',4-Tribromodiphenyl ether)[b,c,d]	0.534	±	0.062[e]
PBDE	47	(2,2',4,4'-Tetrabromodiphenyl ether)[b,c,d]	14.3	±	2.8[e]
PBDE	66	(2,3',4,4'-Tetrabromodiphenyl ether)[b,c,d]	0.34	±	0.14[e]
PBDE	75	(2,4,4',6-Tetrabromodiphenyl ether)[d]	0.237	±	0.017[f]
PBDE	85	(2,2',3,4,4'-Pentabromodiphenyl ether)[d]	0.358	±	0.054[f]
PBDE	100	(2,2',4,4',6-Pentabromodiphenyl ether)[b,c,d]	2.83	±	0.56[e]
PBDE	154	(2,2',4,4',5,6'-Hexabromodiphenyl ether)[b,c]	0.297	±	0.014[e,g]
PBDE	155	(2,2',4,4',6,6'-Hexabromodiphenyl ether)[d]	0.104	±	0.036[f]
alpha-HBCD[h]			0.555	±	0.074[e]
beta-HBCD[h]			0.063	±	0.020[e]

[a] BDE congeners are numbered according to IUPAC rules. When two or more congeners are known to coelute under the GC analysis conditions used, the BDE congener listed first is the major component and the additional congeners may be present as minor components. The quantitative results are based on the response of the congener listed first.

[b] GC/NCI-MS Ic using Soxhlet extraction followed by analysis on a LC-50 column.

[c] GC/NCI-MS IIIc using PFE followed by analysis on a DB-5 column (10 m).

[d] GC/EI-MS IIIa using PFE followed by analysis on a DB-5 column (30 m).

[e] The reference value is an unweighted mean of the results from two or three analytical methods. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [5] with a pooled, within-method variance following the ISO/JCGM Guide [6]. The measurand is the total mass fraction on a dry-mass basis for each selected PBDE congener as determined by the indicated methods. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).

[f] The reference values are the means of results using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte. The measurand is the total mass fraction on a dry-mass basis for each selected PBDE congener as determined by the indicated method. Metrological traceability is to the SI derived unit for mass faction (expressed as micrograms per kilogram).

[g] The reference value for PBDE 154 includes a contribution from PBB 153 (2,2',4,4',5,5'-hexabromobipenyl).

[h] Methods LC/MS/MS IIId (electrospray ionization) and LC/MS/MS IIIe (atmospheric pressurized photoionization).

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