

Standard Reference Material<sup>®</sup> 1974c  
Organics in Mussel Tissue (*Mytilus edulis*)

**CERTIFICATE OF ANALYSIS**

**Purpose:** The certified values delivered by this Standard Reference Material (SRM) are intended for use in evaluating analytical methods for the determination of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, polybrominated diphenyl ether (PBDE) congeners, mercury and tin species, proximates, calories, fatty acids, vitamins, amino acids, and elements in marine bivalve mollusk tissue and similar matrices.

**Description:** A unit of SRM 1974c consists of five jars each containing approximately 10 g (wet basis) of frozen tissue homogenate.

**Certified Values:** Certified mass fraction values are provided for PAHs (Table 1), PCB congeners (Table 2), chlorinated pesticides (Table 3), PBDE congeners (Table 4), and mercury and tin species (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.

**Non-Certified Values:** Non-certified values are provided in Appendix A.

**Additional Information:** Additional information is provided in Appendix B.

**Period of Validity:** The certified values delivered by **SRM 1974c** are valid within the measurement uncertainty specified until **30 September 2032**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

**Maintenance of Certified Values:** NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (<https://www.nist.gov/srm>) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

Table 1. Certified Mass Fraction Values for PAHs in SRM 1974c

	Mass Fraction <sup>(a,b,c)</sup> ( $\mu\text{g}/\text{kg}$ )		<i>k</i>		
	Wet-Mass Basis			Dry-Mass Basis	
Fluorene	2.31	$\pm$ 0.04	22.6	$\pm$ 0.4	1.97
Dibenzothiophene	1.53	$\pm$ 0.02	15.0	$\pm$ 0.2	1.99
Phenanthrene	19.6	$\pm$ 0.4	191	$\pm$ 4	1.96
Anthracene	1.17	$\pm$ 0.08	11.4	$\pm$ 0.8	1.97
1-Methylphenanthrene	3.07	$\pm$ 0.11	30.0	$\pm$ 1.1	1.97
2-Methylphenanthrene	4.56	$\pm$ 0.04	44.5	$\pm$ 0.5	1.97
3-Methylphenanthrene	4.09	$\pm$ 0.03	39.9	$\pm$ 0.4	1.97
9-Methylphenanthrene	2.46	$\pm$ 0.02	24.0	$\pm$ 0.3	1.97
2-Methylantracene	0.951	$\pm$ 0.007	9.3	$\pm$ 0.1	1.97
Fluoranthene	45.3	$\pm$ 0.8	442	$\pm$ 8	1.97
Pyrene	23.9	$\pm$ 1.6	233	$\pm$ 15	1.97
Benzo[ghi]fluoranthene	3.03	$\pm$ 0.09	29.5	$\pm$ 0.9	1.96
Benzo[c]phenanthrene	1.99	$\pm$ 0.04	19.4	$\pm$ 0.4	1.97
Benzo[a]anthracene	5.69	$\pm$ 0.11	55.5	$\pm$ 1.1	1.96
Benzo[k]fluoranthene	2.75	$\pm$ 0.02	26.8	$\pm$ 0.3	2.04
Benzo[a]fluoranthene	0.543	$\pm$ 0.006	5.30	$\pm$ 0.07	1.97
Benzo[e]pyrene	7.34	$\pm$ 0.05	71.6	$\pm$ 0.7	1.98
Benzo[a]pyrene	2.32	$\pm$ 0.03	22.6	$\pm$ 0.4	1.96
Perylene	5.60	$\pm$ 0.22	54.6	$\pm$ 2.2	1.97
Benzo[ghi]perylene	2.82	$\pm$ 0.05	27.6	$\pm$ 0.5	1.97
Benzo[b]chrysene	0.694	$\pm$ 0.013	6.77	$\pm$ 0.14	1.97
Picene	1.36	$\pm$ 0.08	13.2	$\pm$ 0.8	1.97

<sup>(a)</sup> Mass fractions are reported on both a wet- and dry-mass basis; material as received contains 89.75 %  $\pm$  0.08 % (95 % confidence level) moisture.

<sup>(b)</sup> The certified value reported on a wet-mass basis is a weighted mean of average mass fractions, with one average each from two analytical methods [3,4]. The expanded uncertainty is the half width of a symmetric 95 % parametric bootstrap confidence interval [5], which is consistent with the JCGM Guide [6,7]. The effective coverage factor *k* is included in the table for each PAH. The measurands are the total mass fraction values of the PAHs in mussel tissue listed above. The certified values are metrologically traceable to the SI unit for mass, expressed as micrograms per kilogram.

<sup>(c)</sup> GC/MS (Ia) using SPE clean-up followed by analysis on a DB-17MS column using EI-MS and GC/MS (IIa) using SPE and SEC clean-up followed by analysis on a DB-XLB column using EI-MS.

Table 2. Certified Mass Fraction Values for PCB Congeners<sup>(a)</sup> in SRM 1974c

			Mass Fraction <sup>(b,c,d)</sup> ( $\mu\text{g}/\text{kg}$ )		<i>k</i>
			Wet-Mass Basis	Dry-Mass Basis	
PCB	8	(2,4'-Dichlorobiphenyl)	0.191 ± 0.003	1.86 ± 0.03	1.97
PCB	18	(2,2',5-Trichlorobiphenyl)	0.589 ± 0.007	5.75 ± 0.08	1.97
PCB	28	(2,4,4'-Trichlorobiphenyl)	1.48 ± 0.02	14.4 ± 0.3	1.97
PCB	31	(2,4',5-Trichlorobiphenyl)	1.16 ± 0.06	11.3 ± 0.6	1.97
PCB	44	(2,2',3,5'-Tetrachlorobiphenyl)	1.54 ± 0.08	15.1 ± 0.8	1.97
PCB	45	(2,2',3,6-Tetrachlorobiphenyl)	0.214 ± 0.019	2.09 ± 0.18	1.96
PCB	49	(2,2',4,5'-Tetrachlorobiphenyl)	1.76 ± 0.02	17.1 ± 0.3	1.97
PCB	52	(2,2',5,5'-Tetrachlorobiphenyl)	2.49 ± 0.06	24.3 ± 0.6	1.97
PCB	56	(2,3,3',4'-Tetrachlorobiphenyl)	0.663 ± 0.008	6.46 ± 0.09	1.98
PCB	63	(2,3,4',5-Tetrachlorobiphenyl)	0.137 ± 0.013	1.34 ± 0.12	1.97
PCB	66	(2,3',4,4'-Tetrachlorobiphenyl)	1.65 ± 0.02	16.1 ± 0.2	2.05
PCB	70	(2,3',4',5-Tetrachlorobiphenyl)	1.57 ± 0.05	15.3 ± 0.5	1.97
PCB	74	(2,4,4',5-Tetrachlorobiphenyl)	0.850 ± 0.011	8.29 ± 0.12	1.96
PCB	82	(2,2',3,3',4-Pentachlorobiphenyl)	0.507 ± 0.008	4.94 ± 0.09	1.97
PCB	87	(2,2',3,4,5'-Pentachlorobiphenyl)	2.08 ± 0.02	20.3 ± 0.2	2.01
PCB	92	(2,2',3,5,5'-Pentachlorobiphenyl)	1.06 ± 0.02	10.4 ± 0.2	1.97
PCB	95	(2,2',3,5',6-Pentachlorobiphenyl)	1.82 ± 0.02	17.8 ± 0.3	2.15
PCB	99	(2,2',4,4',5-Pentachlorobiphenyl)	3.55 ± 0.05	34.7 ± 0.6	1.97
PCB	101	(2,2',4,5,5'-Pentachlorobiphenyl)	6.67 ± 0.05	65.1 ± 0.7	1.97
PCB	105	(2,3,3',4,4'-Pentachlorobiphenyl)	1.57 ± 0.03	15.3 ± 0.3	1.97
PCB	110	(2,3,3',4',6-Pentachlorobiphenyl)	5.47 ± 0.06	53.4 ± 0.7	1.97
PCB	118	(2,3',4,4',5-Pentachlorobiphenyl)	4.08 ± 0.09	39.8 ± 0.9	1.97
PCB	128	(2,2',3,3',4,4'-Hexachlorobiphenyl)	0.801 ± 0.011	7.81 ± 0.12	1.97
PCB	138	(2,2',3,4,4',5'-Hexachlorobiphenyl)	4.39 ± 0.04	42.9 ± 0.5	1.97
PCB	146	(2,2',3,4',5,5'-Hexachlorobiphenyl)	0.904 ± 0.005	8.82 ± 0.08	1.97
PCB	149	(2,2',3,4',5',6-Hexachlorobiphenyl)	3.97 ± 0.04	38.8 ± 0.5	1.97
PCB	151	(2,2',3,5,5',6-Hexachlorobiphenyl)	1.13 ± 0.03	11.0 ± 0.3	1.96
PCB	153	(2,2',4,4',5,5'-Hexachlorobiphenyl) <sup>(a)</sup>	6.76 ± 0.12	66.0 ± 1.3	1.97
PCB	132	(2,2',3,3',4,6'-Hexachlorobiphenyl)			
PCB	156	(2,3,3',4,4',5-Hexachlorobiphenyl)	0.253 ± 0.005	2.47 ± 0.05	1.96
PCB	158	(2,3,3',4,4',6-Hexachlorobiphenyl)	0.443 ± 0.003	4.33 ± 0.04	1.98
PCB	163	(2,3,3',4',5,6-Hexachlorobiphenyl)	1.10 ± 0.09	10.8 ± 0.9	1.96
PCB	170	(2,2',3,3',4,4',5-Heptachlorobiphenyl)	0.105 ± 0.009	1.03 ± 0.09	1.97
PCB	177	(2,2',3,3',4',5,6-Heptachlorobiphenyl)	0.696 ± 0.011	6.79 ± 0.12	1.97
PCB	178	(2,2',3,3',5,5',6-Heptachlorobiphenyl)	0.350 ± 0.011	3.42 ± 0.11	1.97
PCB	180	(2,2',3,4,4',5,5'-Heptachlorobiphenyl)	0.594 ± 0.008	5.79 ± 0.09	1.97
PCB	183	(2,2',3,4,4',5',6-Heptachlorobiphenyl)	0.848 ± 0.006	8.27 ± 0.09	1.98
PCB	187	(2,2',3,4',5,5',6-Heptachlorobiphenyl)	2.09 ± 0.05	20.4 ± 0.5	1.97

<sup>(a)</sup> PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [8] and later revised by Schulte and Malisch [9] to conform with IUPAC rules; for the specific congeners mentioned in this table, 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.

<sup>(b)</sup> Mass fractions are reported on both a wet- and dry-mass basis; material as received contains 89.75 % ± 0.08 % (95 % confidence level) moisture.

<sup>(c)</sup> The certified value reported on a wet-mass basis is a weighted mean of average mass fractions, with one average each from two analytical methods [3,4]. The expanded uncertainty is the half width of a symmetric 95 % parametric bootstrap confidence interval [5], which is consistent with the JCGM Guide [6,7]. The effective coverage factor *k* is included in the table for each PCB congener. The measurands are the total mass fractions of PCB congeners. The certified values are metrologically traceable to the SI unit for mass, expressed as micrograms per kilogram.

<sup>(d)</sup> GC/MS (Ia) using SPE clean-up followed by analysis on a DB-17MS column using EI-MS and GC/MS (IIa) using SPE and SEC clean-up followed by analysis on a DB-XLB column using EI-MS.

Table 3. Certified Mass Fraction Values for Chlorinated Pesticides in SRM 1974c

	Mass Fraction <sup>(a,b)</sup> ( $\mu\text{g}/\text{kg}$ )		<i>k</i>
	Wet-Mass Basis	Dry-Mass Basis	
Heptachlor <sup>(c)</sup>	0.132 $\pm$ 0.006	1.29 $\pm$ 0.06	1.97
<i>cis</i> -Chlordane <sup>(d)</sup>	1.20 $\pm$ 0.05	11.7 $\pm$ 0.5	1.97
<i>trans</i> -Chlordane <sup>(d)</sup>	0.741 $\pm$ 0.013	7.23 $\pm$ 0.14	1.97
<i>cis</i> -Nonachlor <sup>(d)</sup>	0.286 $\pm$ 0.005	2.79 $\pm$ 0.06	1.98
<i>trans</i> -Nonachlor <sup>(d)</sup>	0.742 $\pm$ 0.005	7.24 $\pm$ 0.08	1.97
Dieldrin <sup>(d)</sup>	0.285 $\pm$ 0.021	2.78 $\pm$ 0.20	1.97
2,4'-DDE <sup>(c)</sup>	0.346 $\pm$ 0.002	3.38 $\pm$ 0.04	1.98
4,4'-DDE <sup>(c)</sup>	1.85 $\pm$ 0.02	18.1 $\pm$ 0.2	1.99
2,4'-DDD <sup>(c)</sup>	0.398 $\pm$ 0.004	3.88 $\pm$ 0.05	1.96
4,4'-DDD <sup>(c)</sup>	1.30 $\pm$ 0.09	12.7 $\pm$ 0.8	1.97
2,4'-DDT <sup>(c)</sup>	0.942 $\pm$ 0.027	9.19 $\pm$ 0.27	1.97

<sup>(a)</sup> Mass fractions are reported on both a wet- and dry-mass basis; material as received contains 89.75 %  $\pm$  0.08 % (95 % confidence level) moisture.

<sup>(b)</sup> The certified value reported on a wet-mass basis is a weighted mean of average mass fractions, with one average each from two analytical methods [3,4]. The expanded uncertainty is the half width of a symmetric 95 % parametric bootstrap confidence interval [5], which is consistent with the JCGM Guide [6,7]. The effective coverage factor *k* is included in the table for each chlorinated pesticide. The measurands are the total mass fractions of chlorinated pesticides. The certified values are metrologically traceable to the SI unit for mass, expressed as nanograms per kilogram.

<sup>(c)</sup> GC/MS (Ia) using SPE clean-up followed by analysis on a DB-17MS column using EI-MS and GC/MS (IIa) using SPE and SEC clean-up followed by analysis on a DB-XLB column using EI-MS.

<sup>(d)</sup> GC/MS (Ia) using SPE clean-up followed by analysis on a DB-17MS column using EI-MS and GC/MS (IIb) using SPE and SEC clean-up followed by analysis on a DB-XLB column using NCI-MS.

Table 4. Certified Mass Fraction Values for PBDE Congeners<sup>(a)</sup> in SRM 1974c

	Mass Fraction <sup>(b,c,d)</sup> ( $\mu\text{g}/\text{kg}$ )		<i>k</i>
	Wet-Mass Basis	Dry-Mass Basis	
PBDE 17 (2,2',4-Tribromodiphenyl ether)	0.078 $\pm$ 0.003	0.761 $\pm$ 0.032	1.97
PBDE 25 (2,3',4-Tribromodiphenyl ether)	0.103 $\pm$ 0.005	1.00 $\pm$ 0.05	1.97
PBDE 47 (2,2',4,4'-Tetrabromodiphenyl ether)	0.939 $\pm$ 0.017	9.16 $\pm$ 0.18	1.96
PBDE 49 (2,2',4,4'-Tetrabromodiphenyl ether)	0.140 $\pm$ 0.005	1.37 $\pm$ 0.05	1.97
PBDE 99 (2,2',4,4',5-Pentabromodiphenyl ether)	0.375 $\pm$ 0.004	3.66 $\pm$ 0.05	1.97

<sup>(a)</sup> PBDE congeners are numbered according to IUPAC rules.

<sup>(b)</sup> Mass fractions are reported on both a wet- and dry-mass basis; material as received contains 89.75 %  $\pm$  0.08 % (95 % confidence level) moisture.

<sup>(c)</sup> The certified value reported on a wet-mass basis is a weighted mean of average mass fractions, with one average each from two analytical methods [3,4]. The expanded uncertainty is the half width of a symmetric 95 % parametric bootstrap confidence interval [5], which is consistent with the JCGM Guide [6,7]. The effective coverage factor *k* is included in the table for each PBDE congener. The measurands are the total mass fractions of PBDE Congeners. The certified values are metrologically traceable to the SI unit for mass, expressed as micrograms per kilogram.

<sup>(d)</sup> GC/MS (Ib) using SPE clean-up followed by analysis on a DB-17MS column using NCI-MS and GC/MS (IIa) using SPE and SEC clean-up followed by analysis on a DB-XLB column using EI-MS.

Table 5. Certified Mass Fraction Values<sup>(a)</sup> for Mercury and Tin Species in SRM 1974c

	Mass Fraction, Wet-Mass Basis		<i>k</i>
	(μg/kg)		
Mercury (Hg) <sup>(b)</sup>	19.7	± 0.62	1.96
Inorganic Mercury (iHg) <sup>(c)</sup>	9.9	± 3.0	2.01
Methylmercury (MeHg) <sup>(c)</sup>	8.2	± 1.4	2.12
Monobutyltin (MBT) <sup>(c)</sup>	1.06	± 0.28	1.99
Dibutyltin (DBT) <sup>(c)</sup>	2.98	± 0.67	2.02
Tributyltin (TBT) <sup>(c)</sup>	5.14	± 0.68	2.10

<sup>(a)</sup> The measurands are the total mass fraction values of mercury and tin species. The certified values are metrologically traceable to the International System of Units (SI) unit for mass, expressed as micrograms per kilogram.

<sup>(b)</sup> The certified value reported on a wet-mass basis results from measurements obtained using one ICP-MS primary analytical technique. The expanded uncertainty, *U*, is calculated as  $U = ku_c$ , in a manner consistent with the JCGM Guide [6,7]. The quantity *u<sub>c</sub>* represents, at the level of one standard deviation, the estimated uncertainty in the mass fraction for the mean of all bottles of SRM 1974c, because the underlying mass fraction is assumed to be the same for each bottle. The quantity, *k*, is the coverage factor used to obtain an expanded uncertainty that provides a symmetric approximately 95 % coverage interval.

<sup>(c)</sup> Each certified value reported on a wet-mass basis results from combining measurements obtained using two analytical techniques based on ICP-MS. The expanded uncertainty, *U*, is calculated as  $U = ku_c$ , in a manner consistent with the JCGM Guide [6,7]. The quantity *u<sub>c</sub>* represents, at the level of one standard deviation, the estimated uncertainty in the mass fraction for single bottles of SRM 1974c, because the underlying mass fraction is assumed to vary from bottle to bottle. The quantity, *k*, is the coverage factor used to obtain an expanded uncertainty that provides a symmetric approximately 95 % coverage interval.

**Safety:** SRM 1974c IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION.

**Storage:** The SRM should be stored at –80 °C (or lower). The tissue homogenate will lose its powder-like form, if allowed to warm or with extended storage at temperatures of –25 °C or higher.

**Handling:** For the handling of this material during sample preparation, the following procedures and precautions are recommended:

- If weighing relatively large quantities (≥3 g), remove a portion from the jar and reweigh the jar to determine the weight of the subsample. (Avoid heavy frost buildup by handling the jars rapidly and wiping them prior to weighing.)
- For weighing smaller quantities, transfer subsamples to a pre-cooled thick-walled glass container rather than a thin-walled plastic container to minimize heat transfer to the sample.
- If possible, use a cold workspace, e.g., an insulated container with dry ice or liquid nitrogen coolant on the bottom and pre-cooled implements, such as Teflon-coated spatulas, for transferring the powder.
- If the material has been previously thawed and is no longer powder-like, allow the sample to completely thaw, stir well, and use the contents of the entire jar for analysis.

**Use:** Subsamples of this SRM for analysis (minimum of 3 g) should be withdrawn from the jar immediately after opening and used without delay for the certified values listed in Tables 1 to 4 to be valid within the stated uncertainties. The mass fractions of PAHs, PCB congeners, chlorinated pesticides, and PBDE congeners in SRM 1974c are reported on both a wet-mass and a dry-mass basis for user convenience. The SRM tissue homogenate, as received, contains approximately 90 % moisture. A separate subsample of the SRM should be removed from the jar at the time of analysis and dried to determine the concentration on a dry-mass basis (see “Conversion to Dry-Mass Basis”).

## REFERENCES

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**Certificate Revision History:** **12 September 2022** (Change of period of validity; updated format; editorial changes); **01 May 2019** (Corrected certified value (Wet-Mass) of Benzo[e]pyrene and PCB 28; corrected certified value (Dry-Mass) of PCB 82; corrected certified and uncertainty values (Wet-Mass and Dry-Mass) of Perylene; corrected uncertainty values (Dry-Mass) for Fluoranthene, Benzo[a]pyrene, Benzo[b]chrysene, *trans*-Nonachlor, and PCBs 28, 49, 63, 95, 128, 146; corrected uncertainty value (Wet-Mass) of *cis*-Nonachlor; editorial revisions); **01 May 2018** (Addition of certified values for mercury and tin species; addition of reference values for proximates, calories, fatty acids, vitamins, amino acids, and elements; editorial changes); **05 November 2015** (Correction of PBDE 49 and PCB 109 names; editorial changes); **18 September 2012** (Original certificate date).

*Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.*

*Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or the Internet at <https://www.nist.gov/srm>.*

\* \* \* \* \* End of Certificate of Analysis \* \* \* \* \*

# APPENDIX A

**Non-Certified Values:** Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the SI or other higher-order reference system. Non-certified mass fraction values are provided below.

Table A1. Non-Certified Mass Fraction Values for PAHs in SRM 1974c

	Mass Fraction <sup>(a)</sup> ( $\mu\text{g}/\text{kg}$ )		<i>k</i>
	Wet-Mass Basis	Dry-Mass Basis	
Naphthalene <sup>(b,c)</sup>	0.990 $\pm$ 0.039	9.66 $\pm$ 0.39	1.96
1-Methylnaphthalene <sup>(b,c)</sup>	1.41 $\pm$ 0.03	13.7 $\pm$ 0.3	1.97
2-Methylnaphthalene <sup>(b,c)</sup>	1.50 $\pm$ 0.06	14.6 $\pm$ 0.6	1.97
1,2-Dimethylnaphthalene <sup>(b,c)</sup>	0.913 $\pm$ 0.007	8.91 $\pm$ 0.10	2.09
1,6-Dimethylnaphthalene <sup>(b,c)</sup>	1.19 $\pm$ 0.02	11.6 $\pm$ 0.2	1.97
2,6-Dimethylnaphthalene <sup>(b,c)</sup>	0.206 $\pm$ 0.006	2.01 $\pm$ 0.06	1.97
Biphenyl <sup>(b,c)</sup>	0.860 $\pm$ 0.008	8.39 $\pm$ 0.10	1.97
Acenaphthylene <sup>(b,c)</sup>	0.523 $\pm$ 0.007	5.11 $\pm$ 0.08	1.96
Acenaphthene <sup>(b,c)</sup>	0.343 $\pm$ 0.019	3.35 $\pm$ 0.18	1.97
1-Methylfluoranthene <sup>(d,e)</sup>	0.451 $\pm$ 0.014	4.40 $\pm$ 0.14	2.57
3-Methylfluoranthene <sup>(d,e)</sup>	1.32 $\pm$ 0.02	12.9 $\pm$ 0.2	2.57
Chrysene <sup>(d,e)</sup>	19.2 $\pm$ 0.5	187 $\pm$ 5	2.09
Triphenylene <sup>(d,e)</sup>	10.1 $\pm$ 0.1	98.5 $\pm$ 1.6	2.09
4-H-Cyclopenta[ <i>def</i> ]phenanthrene <sup>(d,e)</sup>	2.02 $\pm$ 0.04	19.7 $\pm$ 0.4	2.57
Benzo[ <i>b</i> ]fluoranthene <sup>(d,e)</sup>	5.95 $\pm$ 0.05	58.0 $\pm$ 0.7	2.09
Benzo[ <i>j</i> ]fluoranthene <sup>(d,e)</sup>	2.07 $\pm$ 0.01	20.2 $\pm$ 0.2	2.09
Dibenz[ <i>a,c+a,h</i> ]anthracene <sup>(d,e)</sup>	0.100 $\pm$ 0.001	0.976 $\pm$ 0.016	2.57
Dibenzo[ <i>b,k</i> ]fluoranthene <sup>(d,e)</sup>	0.490 $\pm$ 0.010	4.78 $\pm$ 0.11	2.57

<sup>(a)</sup> Mass fractions are reported on both a wet- and dry-mass basis; material as received contains 89.75 %  $\pm$  0.08 % (95 % confidence level) moisture. The measurand is the mass fraction as determined by the indicated method.

<sup>(b)</sup> GC/MS (Ia) using SPE clean-up followed by analysis on a DB-17MS column using EI-MS and GC/MS (IIa) using SPE and SEC clean-up followed by analysis on a DB-XLB column using EI-MS.

<sup>(c)</sup> The non-certified value reported on a wet-mass basis is a weighted mean of average mass fractions, with one average each from two analytical methods [3,4]. The expanded uncertainty is the half width of a symmetric 95 % parametric bootstrap confidence interval [5], which is consistent with the JCGM Guide [6,7]. The effective coverage factor *k* is included in the table for each PAH.

<sup>(d)</sup> GC/MS (IIa) using SPE and SEC clean-up followed by analysis on a DB-XLB column using EI-MS.

<sup>(e)</sup> The non-certified value reported on a wet-mass basis is the mean of results obtained 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.

Table A2. Non-Certified Mass Fraction Values for PCB Congeners<sup>(a)</sup> in SRM 1974c

			Mass Fraction <sup>(b,c,d)</sup> ( $\mu\text{g}/\text{kg}$ )		<i>k</i>
			Wet-Mass Basis	Dry-Mass Basis	
PCB	29	(2,4,5-Trichlorobiphenyl)	0.131 $\pm$ 0.003	1.28 $\pm$ 0.03	2.57
PCB	109	(2,3,3',4',5-Pentachlorobiphenyl)	0.451 $\pm$ 0.004	4.40 $\pm$ 0.06	2.57
PCB	114	(2,3,4,4',5-Pentachlorobiphenyl)	0.155 $\pm$ 0.005	1.51 $\pm$ 0.05	2.57
PCB	119	(2,3',4,4',6-Pentachlorobiphenyl)	0.341 $\pm$ 0.004	3.33 $\pm$ 0.05	2.57
PCB	130	(2,2',3,3',4,5'-Hexachlorobiphenyl)	0.356 $\pm$ 0.008	3.47 $\pm$ 0.08	2.57
PCB	137	(2,2',3,4,4',5-Hexachlorobiphenyl)	0.095 $\pm$ 0.001	0.924 $\pm$ 0.014	2.57
PCB	154	(2,2',4,4',5,6'-Hexachlorobiphenyl)	0.990 $\pm$ 0.020	9.66 $\pm$ 0.22	2.57
PCB	157	(2,3,3',4,4',5'-Hexachlorobiphenyl)	0.086 $\pm$ 0.003	0.840 $\pm$ 0.026	2.57
PCB	165	(2,3,3',5,5',6-Hexachlorobiphenyl)	1.56 $\pm$ 0.02	15.2 $\pm$ 0.3	2.57
PCB	166	(2,3,4,4',5,6-Hexachlorobiphenyl)	0.020 $\pm$ 0.001	0.192 $\pm$ 0.010	2.57
PCB	167	(2,3',4,4',5,5'-Hexachlorobiphenyl)	0.305 $\pm$ 0.004	2.98 $\pm$ 0.05	2.57
PCB	175	(2,2',3,3',4,5',6-Heptachlorobiphenyl)	0.139 $\pm$ 0.002	1.36 $\pm$ 0.03	2.57
PCB	176	(2,2',3,3',4,6',6'-Heptachlorobiphenyl)	0.165 $\pm$ 0.004	1.61 $\pm$ 0.04	2.57
PCB	202	(2,2',3,3',5,5',6,6'-Octachlorobiphenyl)	0.214 $\pm$ 0.003	2.09 $\pm$ 0.04	2.57

<sup>(a)</sup> PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [8] and later revised by Schulte and Malisch [9] to conform with IUPAC rules; IUPAC PCB 109 is BZ#107.

<sup>(b)</sup> Mass fractions are reported on both a wet- and dry-mass basis; material as received contains 89.75 %  $\pm$  0.08 % (95 % confidence level) moisture.

<sup>(c)</sup> The non-certified value reported on a wet-mass basis is the mean of results obtained using one analytical technique. The expanded uncertainty,  $U$ , is calculated as  $U = k u_c$ , 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 [6,7]. The measurand is the mass fraction as determined by the indicated method.

<sup>(d)</sup> GC/MS (IIa) using SPE and SEC clean-up followed by analysis on a DB-XLB column using EI-MS.

Table A3. Non-Certified Mass Fraction Values for Chlorinated Pesticides in SRM 1974c

	Mass Fraction <sup>(a,b,c)</sup> ( $\mu\text{g}/\text{kg}$ )		<i>k</i>
	Wet-Mass Basis	Dry-Mass Basis	
Hexachlorobenzene	0.021 $\pm$ 0.001	0.205 $\pm$ 0.014	2.57
Mirex	0.164 $\pm$ 0.005	1.60 $\pm$ 0.05	2.57

<sup>(a)</sup> Mass fractions are reported on both a wet- and dry-mass basis; material as received contains 89.75 %  $\pm$  0.08 % (95 % confidence level) moisture.

<sup>(b)</sup> The non-certified value reported on a wet-mass basis is the mean of results obtained using one analytical technique. The expanded uncertainty,  $U$ , is calculated as  $U = k u_c$ , 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 [6,7]. The measurand is the mass fraction as determined by the indicated method.

<sup>(c)</sup> GC/MS (IIb) using SPE and SEC clean-up followed by analysis on a DB-XLB column using NCI-MS.



Table A4. Non-Certified Mass Fraction Values for Proximates and Calories in SRM 1974c

	Mass Fraction, Wet-Mass Basis <sup>(a)</sup> (%)	<i>k</i>
Ash <sup>(b)</sup>	1.64 ± 0.05	2.16
Protein <sup>(c)</sup>	5.86 ± 0.16	2.14
Carbohydrates	1.95 ± 0.34	2.23
Fat (as the sum of fatty acids as triglycerides)	0.410 ± 0.041	2.20
	Energy (kcal per 100 g)	<i>k</i>
Calories <sup>(d)</sup>	35.0 ± 1.2	2.20

<sup>(a)</sup> Each non-certified mass fraction value is the median of the means of results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  represents the combined uncertainty consistent with the JCGM Guide and  $k$  is a coverage factor corresponding to approximately 95 % confidence [6,7]. The measurands are the mass fractions of the proximates and caloric content in mussel tissue as determined by the collaborating laboratories and the methods they used. For proximates, metrologically traceable is to the mass fraction (expressed as grams per 100 grams) as realized by the methods used. For calories, the measurand is the caloric content (expressed as kilocalories per 100 grams), as determined by the method indicated.

<sup>(b)</sup> The method reported by collaborating laboratories was weight loss after ignition in a muffle furnace.

<sup>(c)</sup> A factor of 6.25 was used to convert nitrogen results to protein. The method reported by collaborating laboratories was Kjeldahl.

<sup>(d)</sup> The non-certified value for calories is the median of lab mean caloric calculations from the interlaboratory comparison exercise. If the mean proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (as the sum of fatty acids), protein, and carbohydrate, respectively, the mean caloric content is 34.97 kcal per 100 grams.

Table A5. Non-Certified Mass Fraction Values for Fatty Acids (as Free Fatty Acids) in SRM 1974c

	Common Name	Mass Fraction, Wet-Mass Basis <sup>(a,b)</sup> (g/100 g)	<i>k</i>
Tetradecanoic Acid (C14:0)	Myristic Acid	0.0135 ± 0.0019	2.23
Pentadecanoic Acid (C15:0)		0.0025 ± 0.0012	2.78
Hexadecanoic Acid (C16:0)	Palmitic Acid	0.090 ± 0.012	2.23
(Z)-9-Hexadecenoic Acid (C16:1 n-7)	Palmitoleic Acid	0.0397 ± 0.0027	2.26
Heptadecanoic Acid (C17:0)	Margaric Acid	0.0030 ± 0.0017	2.45
Octadecanoic Acid (C18:0)	Stearic Acid	0.0180 ± 0.0027	2.31
(Z)-9-Octadecenoic Acid (C18:1 n-9)	Oleic Acid	0.0120 ± 0.0029	2.31
(Z)-11-Octadecenoic Acid (C18:1 n-7)	Vaccenic Acid	0.0135 ± 0.0043	2.45
Total <i>cis</i> -C18:1 Fatty Acids		0.0225 ± 0.0050	2.26
Total <i>cis</i> -C18:2 Fatty Acids		0.0160 ± 0.0054	2.57
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)	$\alpha$ -Linolenic Acid	0.0075 ± 0.0040	2.45
(Z,Z,Z,Z)-6,9,12,15-Octadecatetraenoic Acid (C18:4 n-3)	Stearidonic Acid	0.0185 ± 0.0027	2.57
Total <i>cis</i> -C20:1 Fatty Acids		0.0158 ± 0.0078	2.31
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6)	Arachidonic Acid	0.0100 ± 0.0014	2.31
(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic Acid (C20:5 n-3)	EPA	0.0890 ± 0.0083	2.26
Total <i>cis</i> -C22:5 Fatty Acids		0.0060 ± 0.0023	2.78
Total <i>cis</i> -C22:6 Fatty Acids		0.0380 ± 0.0031	2.23
Saturated Fatty Acids		0.128 ± 0.010	2.26
<i>cis</i> -Monounsaturated Fatty Acids		0.080 ± 0.012	2.23
<i>cis</i> -Polyunsaturated Fatty Acids		0.161 ± 0.035	2.23
Total Omega-3 Fatty Acids		0.140 ± 0.022	2.23
Total Omega-6 Fatty Acids		0.028 ± 0.014	2.26

<sup>(a)</sup> Each non-certified mass fraction value is the median of the means of results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  represents the combined uncertainty consistent with the JCGM Guide and  $k$  is a coverage factor corresponding to approximately 95 % confidence [6,7]. The measurands are the mass fractions of the fatty acids in mussel tissue as determined by the collaborating laboratories and the methods they used.

<sup>(b)</sup> The method reported by collaborating laboratories was GC/FID.

Table A6. Non-Certified Mass Fraction Values for Vitamins in SRM 1974c

	Mass Fraction, Wet-Mass Basis <sup>(a)</sup> (mg/kg)	<i>k</i>
Riboflavin <sup>(b)</sup>	1.62 ± 0.15	2.57
Niacin <sup>(c)</sup>	9.55 ± 2.19	2.78

<sup>(a)</sup> Each non-certified mass fraction value is the median of the means of results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  represents the combined uncertainty consistent with the JCGM Guide and  $k$  is a coverage factor corresponding to approximately 95 % confidence [6,7]. The measurands are the mass fractions of the vitamins in mussel tissue as determined by the collaborating laboratories and the methods they used.

<sup>(b)</sup> The methods reported by collaborating laboratories were microbiological assay and extraction with LC-fluorescence.

<sup>(c)</sup> The method reported by collaborating laboratories was microbiological assay.

Table A7. Non-Certified Mass Fraction Values for Amino Acids in SRM 1974c

	Mass Fraction, Wet-Mass Basis <sup>(a,b)</sup> (mg/kg)	<i>k</i>
Alanine	0.230 ± 0.030	3.18
Arginine	0.31 ± 0.18	3.18
Aspartic Acid	0.472 ± 0.094	3.18
Cystine	0.080 ± 0.011	4.30
Glutamic Acid	0.58 ± 0.14	3.18
Glycine	0.368 ± 0.015	3.18
Histidine	0.0957 ± 0.0074	3.18
Isoleucine	0.205 ± 0.022	3.18
Leucine	0.302 ± 0.051	3.18
Lysine	0.314 ± 0.074	3.18
Methionine	0.105 ± 0.034	3.18
Phenylalanine	0.160 ± 0.055	3.18
Proline	0.216 ± 0.020	3.18
Serine	0.277 ± 0.022	3.18
Tyrosine	0.175 ± 0.048	3.18
Valine	0.222 ± 0.017	3.18

<sup>(a)</sup> Each non-certified mass fraction value is the median of the means of results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  represents the combined uncertainty consistent with the JCGM Guide and  $k$  is a coverage factor corresponding to approximately 95 % confidence [6,7]. The measurands are the mass fractions of the amino acids in mussel tissue as determined by the collaborating laboratories and the methods they used.

<sup>(b)</sup> The method reported by collaborating laboratories was hydrolysis and derivatization followed by LC.

Table A8. Non-Certified Mass Fraction Values for Elements in SRM 1974c

	Mass Fraction, Wet-Mass Basis ( $\mu\text{g}/\text{kg}$ )		$k$
Arsenic (As) <sup>(a)</sup>	1180	$\pm$ 118	2.19
Cadmium (Cd) <sup>(b)</sup>	157	$\pm$ 4.9	1.94
Copper (Cu) <sup>(c)</sup>	1120	$\pm$ 140	2.20
Lead (Pb) <sup>(b)</sup>	800	$\pm$ 50	2.01
Manganese (Mn) <sup>(c)</sup>	1610	$\pm$ 130	2.23
Selenium (Se) <sup>(c)</sup>	358	$\pm$ 47	2.36
Silver (Ag) <sup>(b)</sup>	17.7	$\pm$ 1.4	2.03
Tin (Sn) <sup>(b)</sup>	28.5	$\pm$ 6.0	1.99
	Mass Fraction, Wet-Mass Basis ( $\text{mg}/\text{kg}$ )		$k$
Calcium (Ca) <sup>(c)</sup>	650	$\pm$ 48	2.16
Iron (Fe) <sup>(c)</sup>	57.0	$\pm$ 4.2	2.14
Magnesium (Mg) <sup>(c)</sup>	537	$\pm$ 15	2.16
Phosphorus (P) <sup>(c)</sup>	898	$\pm$ 26	2.16
Potassium (K) <sup>(c)</sup>	1418	$\pm$ 45	2.18
Sodium (Na) <sup>(c)</sup>	3550	$\pm$ 75	2.16
Zinc (Zn) <sup>(c)</sup>	12.4	$\pm$ 0.73	2.18

- <sup>(a)</sup> The non-certified mass fraction value is the combination of two analytical techniques utilizing ICP-MS. The expanded uncertainty,  $U$ , is calculated as  $U = ku_c$ , in a manner consistent with the JCGM Guide [6,7]. The quantity  $u_c$  represents, at the level of one standard deviation, the estimated uncertainty in the mass fraction for the mean of all bottles of SRM 1974c, because the underlying mass fraction is assumed to be the same for each bottle. The quantity,  $k$ , is the coverage factor used to obtain an expanded uncertainty that provides a symmetric approximately 95 % coverage interval.
- <sup>(b)</sup> Each non-certified mass fraction value is the mean of results obtained using one ICP-MS analytical technique. The expanded uncertainty,  $U$ , is calculated as  $U = ku_c$ , in a manner consistent with the JCGM Guide [6,7]. The quantity  $u_c$  represents, at the level of one standard deviation, the estimated uncertainty in the mass fraction for the mean of all bottles of SRM 1974c, because the underlying mass fraction is assumed to be the same for each bottle. The quantity,  $k$ , is the coverage factor used to obtain an expanded uncertainty that provides a symmetric approximately 95 % coverage interval.
- <sup>(c)</sup> Each non-certified mass fraction value is the median of the means of results provided by collaborating laboratories using atomic absorption spectroscopy, inductively-coupled plasma atomic emission spectroscopy, inductively-coupled plasma mass spectrometry, and/or colorimetry. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  represents the combined uncertainty consistent with the JCGM Guide and  $k$  is a coverage factor corresponding to approximately 95 % confidence [6,7]. The measurands are the mass fractions of the selected elements in mussel tissue as determined by the collaborating laboratories and the methods they used.

**Maintenance of Non-Certified Value:** NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix. Before making use of any of the values delivered by this material, users should obtain the most recent version of this documentation, available free of charge through the <https://www.nist.gov/srm> website.

\* \* \* \* \* End of Appendix A \* \* \* \* \*

# APPENDIX B

**Sample Collection and Preparation:** The mussels (*Mytilus edulis*) used for the preparation of SRM 1974c were collected from Dorchester Bay, MA in 2004 by TDI-Brooks International (College Station, TX). The mussels were frozen and delivered to NIST (Hollings Marine Laboratory, Charleston, SC) where they were stored in a liquid nitrogen (LN<sub>2</sub>) vapor-phase freezer at -150 °C. For processing, the mussels were allowed to warm to approximately 0 °C, shells were opened, and the tissue removed using titanium knives. Approximately 70 kg of mussel tissue was stored in fluorinated ethylene propylene (FEP) bags in an LN<sub>2</sub> vapor-phase freezer (-150 °C) until homogenization. For homogenization, the frozen mussel material was removed from the FEP bags, placed in a pre-frozen polytetrafluorethylene (PTFE) smasher, and crushed into smaller pieces using a manual smashing device and/or a compressed-air smashing device. The frozen, crushed mussel material was then immediately placed back in an LN<sub>2</sub> vapor-phase freezer (-150 °C) and divided among four stainless steel buckets within the freezer. The Palla VM-KT Vibrating Cryomill (KHD Humboldt Wedag GmbH, Cologne, Germany) was cooled allowing LN<sub>2</sub> to flow through the mill until a temperature of -180 °C was reached. The LN<sub>2</sub> was shut off and the crushed mussel tissue from all four buckets was processed through the cryomill until a fresh, frozen powder was created. This procedure was repeated four times prior to bottling to ensure the mussel material was completely blended. Subsamples (approximately 10 g) of the frozen mussel powder homogenate were aliquoted into cleaned, pre-cooled glass jars within an LN<sub>2</sub> vapor-phase freezer (-150 °C) and the glass jars were then stored in -80 °C upright mechanical freezers.

**Conversion to Dry-Mass Basis:** Sixteen samples were analyzed for moisture using an automated moisture/solids microwave analysis system (CEM, Matthews, NC). Each sample was approximately 1 g of material; the automated moisture determination temperature maximum was set to 105 °C and the power was set to 100 %. A sample was determined to have reached dry mass when the mass of the sample had not changed more than 0.1 mg in 10 s. The moisture content at the time of the certification analyses was 89.75 % ± 0.08 % (expanded uncertainty at a 95 % confidence level for 16 samples with a standard deviation of 0.0015). Analytical results for the constituents were determined on a wet-mass basis and then converted to a dry-mass basis by dividing by the conversion factor of 0.1025 (grams dry mass per gram wet mass). The uncertainty component for the conversion factor obtained from the moisture measurements is incorporated in the uncertainties of the certified and non-certified values using the methods of reference 6, reported on a dry-mass basis, that are provided in this certificate.

**PAHs, PCBs, Chlorinated Pesticides, and PBDEs:** Value assignment for the PAHs, PCBs, chlorinated pesticides, and PBDEs in SRM 1974c consisted of combining results from analyses using various combinations of different extraction techniques, cleanup/isolation procedures, and chromatographic separation and detection techniques. Two sets of gas chromatography/mass spectrometry (GC/MS) analysis methods, designated as GC/MS (I) and GC/MS (II), were used at NIST.

For GC/MS (I) analyses, duplicate test portions of approximately 3 g from each of 10 jars of SRM 1974c were mixed with diatomaceous earth (Hydromatrix, Restek, Bellefonte, PA) in glass extraction thimbles. The mixtures were extracted using Soxhlet extraction with hexane:acetone (1:1 volume fraction) for 20 h. The extract was fractionated using two aminopropyl solid-phase extraction (SPE) columns to isolate the compounds 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-17MS, Agilent Technologies, Wilmington, DE). The PAHs, PCBs, and pesticides were analyzed on the DB-17MS column using electron impact MS (EI-MS), method GC/MS (Ia). The PBDEs were analyzed on a 0.25 mm × 15 m fused silica capillary column containing a 5 % phenyl methylsubstituted polysiloxane phase (Restek), 0.25 µm film thickness using negative chemical ionization MS (NCI-MS), method GC/MS (Ib).

For the GC/MS (II) analyses, a 9 g sample from each of six jars was extracted using pressurized-fluid extraction (PFE) with dichloromethane (DCM). The fraction of interest was first isolated using an alumina (5 % deactivated) SPE column. Size exclusion chromatography (SEC) on a divinylbenzene-polystyrene column (10 µm particle size, 10 nm (100 Å) pore size, 7.5 mm × 300 mm i.d PLGel column, Polymer Labs, Inc., Amherst, MA) was used to remove the majority of the remaining lipid and biogenic material. The processed extract was then analyzed by GC/MS using a 0.18 mm i.d. × 30 m fused silica capillary column with a low-bleed, low-polarity phase (0.18 µm film thickness; DB-XLB, Agilent Technologies, Santa Clara, CA). The PAHs, PCBs, PBDEs, and certain pesticides were analyzed on the DB-XLB column using EI-MS, method GC/MS (IIa). The remaining pesticides were analyzed on the same capillary column using NCI-MS, method GC/MS (IIb). For the methods described above, selected perdeuterated PAHs, carbon-13 labeled PCB congeners, chlorinated pesticides, PBDE congeners, and fluorinated PBDE congeners were added to the mussel tissue prior to extraction for use as internal standards for quantification purposes.

**Homogeneity Assessment for PAHs, PCBs, Chlorinated Pesticides, and PBDEs:** The homogeneity of SRM 1974c was assessed by analyzing duplicate test portions of 3 g from 10 jars selected by stratified random sampling. Test portions were processed and analyzed as described above for GC/MS (I). No differences among jars were observed for the PAHs, PCBs, chlorinated pesticides, or PBDEs for a 3 g test portion size.

**Arsenic:** Value assignment of arsenic (As) in SRM 1974c was determined by combining two sets of inductively coupled plasma-mass spectrometry (ICP-MS) analyses at NIST. The first method sampled duplicate test portions of 5 g from six jars of SRM 1974c. The aliquots were mixed with Optima grade nitric acid (HNO<sub>3</sub>) and Optima grade hydrofluoric acid (HF) and extracted using a microwave (CEM MARS, Matthews, NC). After microwave digestion, a 1 g aliquot of a solution containing 350 ng/g rhodium (Rh) was added to each bottle, and the contents were diluted with water. Measurements of <sup>75</sup>As and the internal standard, <sup>103</sup>Rh, were made in “H2” quantitative analysis mode of an ICP-MS (Agilent 7500cs). The second method is as described below.

**Arsenic, Cadmium, Lead, Silver, and Tin:** Value assignment for arsenic (As), cadmium (Cd), lead (Pb), silver (Ag), and tin (Sn), was based on the analysis of 1 g aliquots from six jars of SRM 1974c spiked with an internal standard solution of germanium (Ge), selenium (Se), and yttrium (Y), and duplicate 1 g aliquots from the same six jars spiked with both the internal standard solution and the primary calibration solution with a spike factor of approximately 2.5. Ultra-high purity (UHP) HNO<sub>3</sub> was added prior to digestion in a microwave (Anton Paar Multiwave 3000, Graz, Austria). Following digestion, the samples were cooled to room temperature, and diluted with high-purity de-ionized water prior to analysis by ICP-MS (Thermo Electron X-Series II, Waltham, MA).

**Mercury:** Value assignment of mercury (Hg) in SRM 1974c was based on analysis of 0.25 g aliquots from seven jars selected by stratified random sampling. The aliquots were spiked with a known amount of <sup>201</sup>Hg internal standard prior to digestion with HNO<sub>3</sub> and hydrochloric acid (HCl) in a microwave (Anton Paar Multiwave 3000). The digested contents were diluted with high-purity de-ionized water. The following day, the samples were further diluted with high-purity de-ionized water to a concentration of approximately 0.1 ng/g <sup>201</sup>Hg before analysis by cold-vapor ICP-MS (Thermo X7).

**Mercury Species:** Value assignment of methylmercury (MeHg) and inorganic mercury (iHg) in SRM 1974c consisted of combining results from analyses at NIST and the University of Oviedo, Spain. The NIST analysis was conducted using 1 g aliquots from six jars of SRM 1974c selected by stratified random sampling. The aliquots were spiked with a known amount of IRMM-670, <sup>202</sup>Hg enriched MeHg CRM solution, and inorganic <sup>198</sup>Hg prepared in high purity water and 25 % mass fraction tetramethylammonium hydroxide (TMAH) prior to digestion in a microwave (Explorer, CEM, Matthews, NC). After cooling to room temperature, the digested extracts were buffered with 1 M sodium acetate and UHP HNO<sub>3</sub> to approximately pH 5 before adding 20 % mass fraction sodium tetraethylborate and shaking for 5 min to ensure complete derivatization of the mercury species. Hexane was added to the derivatized solutions and the samples were again vortexed to ensure complete extraction of the derivatized species into the organic layer. The tubes were centrifuged to facilitate separation of the organic and aqueous phases. The organic phase of the extracts was further cleaned up with 5 % (mass fraction) water-deactivated alumina SPE cartridges (Rapidtrace, Caliper Life Science, Hopkinton, MA). The final extracts were concentrated to approximately 0.2 mL under a stream of nitrogen gas (Turbovap, Caliper Life Science) before analysis by GC-ICP-MS (Thermo X7).

Analysis at the University of Oviedo consisted of sampling duplicate 0.15 g aliquots from four jars of SRM 1974c selected by stratified random sampling. The aliquots were spiked with <sup>201</sup>Hg-enriched MeHg solution in a 3:1 mixture of acetic acid and methanol and a <sup>199</sup>Hg-enriched Hg (II) solution in with 2 % sub-boiled HCl in 18 MΩ·cm water. The aliquots were digested with 25 % TMAH using a microwave. Interconversion reactions occurring between both species were corrected by double spiking IDMS. A 1 mL portion of the extract was diluted in 4 mL of acetic acid/sodium acetate buffer and sub-boiled HCl to pH 4 before adding 0.3 mL of 2 % w/v sodium tetraethylborate in 18 MΩ·cm water and 1 mL of hexane prior to manual shaking and then centrifuging. Most of the organic layer was concentrated to approximately 20 μL under nitrogen gas prior to analysis with GC-ICP-MS.

**Tin Species:** Value assignment of monobutyltin (MBT), dibutyltin (DBT), and tributyltin (TBT) in SRM 1974c consisted of combining results from analyses at NIST and the University of Oviedo, Spain. The NIST analysis was conducted using 1 g aliquots from six jars of SRM 1974c selected by stratified random sampling. The methods of triple-spiked isotope dilution were utilized. Briefly, the aliquots were spiked with a known amount of butyltin prepared in high purity water and 25 % mass fraction TMAH prior to digestion in a microwave (Explorer, CEM, Matthews, NC). After cooling to room temperature, the digested extracts were buffered with 1 M sodium acetate and UHP HNO<sub>3</sub> to approximately pH 5 before adding 20 % mass fraction sodium tetraethylborate and shaking for 5 min to ensure complete derivatization of the butyltin species. Hexane was added to the derivatized solutions and the samples were again vortexed to ensure complete extraction of the derivatized species into the organic layer. The tubes were centrifuged to facilitate separation of the organic and aqueous phases. The organic phase of the extracts was further cleaned up with 5 % (mass fraction) water-deactivated alumina SPE cartridges (Rapidtrace, Caliper Life

Science, Hopkinton, MA). The final extracts were concentrated to approximately 0.2 mL under a stream of nitrogen gas (Turbovap, Caliper Life Science) before analysis by GC-ICP-MS (Thermo X7).

Analysis at the University of Oviedo consisted of sampling duplicate 0.15 g aliquots from four jars of SRM 1974c selected by stratified random sampling. The aliquots were spiked with a known amount of mono-, di-, and tri-butyltin enriched in <sup>119</sup>Sn diluted in a 3:1 mixture of acetic acid and methanol buffered to pH 4.9 with sodium acetate in 18 MΩ·cm water and glacial acetic acid. The aliquots were capped and placed in a water bath for 2 h with manual shaking every 30 min. A 1 mL portion of the aliquot was diluted in 4 mL of acetic acid/sodium acetate buffer (pH 4.9) before adding 0.5 mL of 2 % w/v sodium tetraethylborate in 18 MΩ·cm water and 1 mL of hexane prior to manual shaking and then centrifuging. Most of the organic layer was concentrated to approximately 20 μL under nitrogen gas prior to analysis with GC-ICP-MS.

**Collaborating Laboratories' Analyses:** The GMA FIACC laboratories were asked to use their usual methods to make single measurements of proximates, calories, fatty acids, vitamins, elements, and amino acids on test portions taken from each of two jars of SRM 1974c. Because of variability among data provided by laboratories participating in an interlaboratory comparison exercise, the median of laboratory means is used, with the uncertainty estimated using a function of the median absolute deviation (MAD) [6–7].

Overall direction and coordination of technical measurements leading to certification were performed by M.M. Schantz, and L.C. Sander formerly of the NIST Chemical Sciences Division, K.E. Sharpless of the NIST Special Programs Office, and W. Koschute of the Grocery Manufacturers Association (GMA, Washington, DC).

Preparation of the material was performed by W.C. Davis, J.C. Hoguet, A.J. Moors, and R.S. Pugh of the NIST Chemical Sciences Division; and G. Ballihaut, P.R. Becker, M.B. Ellisor, B.J. Porter, L.B. Rust, and J.M. Yordy formerly of NIST.

Statistical analyses of the certification data were performed by N.A. Heckert, A.L. Pintar, and J.H. Yen of the NIST Statistical Engineering Division.

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Analytical measurements at NIST were performed by C.E. Bryan, B.L. Catron, W.C. Davis, M.B. Ellisor, S.E. Long, M.M. Schantz, S.S. Schuur, L.J. Wood, and L.L. Yu of the Chemical Sciences Division. Analyses for value assignment for mercury and tin species were performed by University of Oviedo (Oviedo, Spain). Analysis for value assignment were also provided by analysts participating in a GMA Food Industry Analytical Chemists Committee (FIACC) interlaboratory comparison exercise: Campbell Soup Company (Camden, NJ); ConAgra Foods (Omaha, NE); Covance Laboratories, Inc. (Battle Creek, MI); Covance Laboratories, Inc. (Madison, WI); Del Monte Foods (Walnut Creek, CA); Eurofins Central Analytical Laboratories (Metairie, LA); Eurofins Chemical Control SRL (Cuneo, Italy); Eurofins Scientific (Des Moines, IA); Eurofins Steins Laboratorium (Vejen, Denmark); General Mills, Inc. (Golden Valley, MN); Krueger Food Laboratories (Billerica, MA); Land O'Lakes (Arden Hills, MN); Schwan Food Company (Salina, KS); Silliker Illinois Analytical Laboratory (Crete, IL); and The National Food Laboratory (Livermore, CA).

\* \* \* \* \* End of Appendix B \* \* \* \* \*