National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1946

Lake Superior Fish Tissue

This Standard Reference Material (SRM) is a frozen fish tissue homogenate that was prepared from lake trout (*Salvelinus namaycush namaycush*) collected near the Apostle Islands in Lake Superior (U.S./Canada), and is intended primarily for use in evaluating analytical methods for the determination of polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, polybrominated diphenyl ether (PBDE) congeners, perfluorooctanesulfonic acid (PFOS), fatty acids (including omega-3 fatty acids), extractable fat, methylmercury, total mercury, proximates, α-hexabromocyclododecane (α-HBCD), and selected trace elements in fish tissue and similar matrices. All of the constituents for which certified, reference, and information mass fraction values are provided are naturally present in the fish tissue homogenate. A unit of SRM 1946 consists of five bottles, each containing approximately 7 g to 9 g (wet basis) of frozen tissue homogenate.

Certified Mass Fraction Values: Certified mass fraction values are provided in Tables 1, 2, and 3 for selected PCB congeners, chlorinated pesticides, and PBDE congeners. The certified values for PCBs, chlorinated pesticides, and PBDEs are based on results obtained from two or more independent analytical techniques. Certified values are provided in Table 4 for extractable fat and individual fatty acids. The certified values for fat and fatty acids are based on measurements made by NIST and by collaborating laboratories. Certified values for methylmercury, total mercury, arsenic, and iron are provided in Table 5. The certified values for methylmercury and these elements are based on results from two or more independent analytical techniques performed at NIST and collaborating laboratories. 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].

Reference Mass Fraction Values: Reference mass fraction values for selected PCB congeners, chlorinated pesticides, PBDE congeners, PFOS, fatty acids, proximates, caloric content, and elements are provided in Tables 6 through 9. Reference values are noncertified values that represent the best estimate of the true values based on available data; however, the values do not meet the NIST criteria for certification [1] 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.

Information Mass Fraction Values: Information mass fraction values are provided for carbohydrates, two additional trace elements, four additional fatty acids, and α-HBCD in Table 10. An information value is a value that may be of use to the SRM user, but insufficient information is available to assess adequately the uncertainty associated with the value. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 1946** is valid, within the measurement uncertainty specified, until **31 December 2026**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for 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.

Coordination of the technical measurements leading to the certification of this SRM was performed by S.A. Wise of the NIST Chemical Sciences Division and M.M. Schantz formerly of NIST.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 28 October 2014
Certificate Revision History on Page 15

Robert L. Watters, Jr., Director
Office of Reference Materials

Analytical measurements at NIST were performed by S.J. Christopher, J.M. Keller, J.R. Kucklick, S.E. Long, D.L. Poster, and J.L. Reiner of the NIST Chemical Sciences Division; E.A. Mackey of the Materials Measurement Laboratory; and W.W. Brubaker, Jr., B.J. Porter, M.S. Rearick, M.M. Schantz, C.S. Phinney, and H.M. Stapleton formerly of NIST. Additional PBDE measurements were provided by R.A. Hites and Y.L. Zhu of Indiana University (Bloomington, IN). Measurements from the NIST Intercomparison Exercise Program for Organic Contaminants in the Marine Environment were coordinated by M.M. Schantz; see Appendix A for participating laboratories. Measurements by the Grocery Manufacturers Association (GMA) Food Industry Analytical Chemists were coordinated by K.E. Sharpless of the NIST Chemical Sciences Division and H.B. Chin and D.W. Howell of the GMA (Dublin, CA and Washington, DC, respectively); see Appendix B for participating laboratories. Measurements from an informal interlaboratory comparison study for PFOS in a variety of matrices were coordinated by J.M. Keller and J.L. Reiner; see Appendix C for participating laboratories. Analytical measurements for mercury and methylmercury were also performed at the Institute of Applied Physical Chemistry, Research Centre Jülich (Jülich, Germany) by H. Emons and at the Jožef Stefan Institute (Lubljana, Slovenia) by M. Horvat and D. Gibičar. Selected trace elements in SRM 1946 were analyzed by NIST, U.S. Department of Agriculture (USDA) Food Composition Laboratory (Beltsville, MD), and one laboratory from the GMA interlaboratory exercise.

Fish used for SRM 1946 were collected with the assistance of S. Schram and T. Gerrard of the Wisconsin Department of Natural Resources, G. Cholwak of the U.S. Geological Service, and J. Bodine and T. Chaney of the Bodine Fish House, Bayfield, WI. The coordination for the collection, field preparation of the fish fillets, and cryogenic homogenization of the fish tissue were performed by J.R. Kucklick, B.J. Porter, R.S. Pugh, and D.J. Struntz of the NIST Chemical Sciences Division, and M.P. Cronise and C.N. Fales of the NIST Office of Reference Materials.

Statistical analysis was provided by S.D. Leigh and B. Toman of the NIST Statistical Engineering Division.

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

NOTICE AND WARNING TO USERS

WARNING: FOR LABORATORY USE ONLY; NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: SRM 1946 is packaged as a frozen tissue homogenate in glass bottles. The tissue homogenate should not be allowed to thaw prior to subsampling for analysis. This material has been stored at NIST at −80 °C (or lower) since it was prepared and should be stored by the user at this temperature for the certified values to be valid within the stated uncertainties.

Use: This material is a frozen tissue homogenate. After extended storage at temperatures of −25 °C or higher, or if it is allowed to warm, the tissue homogenate will lose its powder-like form. For the handling of this material during sample preparation, the following procedures and precautions are recommended. If weighing relatively large quantities, remove a portion from the bottle and reweigh the bottle to determine the mass of the subsample. Avoid heavy frost buildup by handling the bottles quickly and wiping them prior to weighing. For weighing, 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 work space, (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). Standard biohazard safety practices and precautions for the handling of biological tissues should be exercised. Subsamples of this SRM for analysis (minimum sample size of 1 g) should be withdrawn from the bottle immediately after opening and used without delay for the certified values listed in Tables 1 through 5 to be valid within the stated uncertainties. The mass fractions of constituents in SRM 1946 are reported on a wet-mass basis. The SRM tissue homogenate, as received, contains approximately 71 % moisture.

PREPARATION AND ANALYSIS[1]

Sample Collection: SRM 1946 was prepared from fillets from adult lake trout (*Salvelinus namaycush namaycush*) collected near the Apostle Islands in Lake Superior in October 1997. The fillets were removed from the fish using stainless steel knives and placed in Teflon bags. The tissue was placed on wet ice and transported to NIST where it was stored in liquid nitrogen vapor freezers (−120 °C) until processed and bottled. A total of 78 kg of fillets was obtained from approximately 70 fish. The frozen fillets were pulverized in batches of approximately 350 g using the cryogenic procedure described previously [2]. The pulverized fish tissue was then homogenized in an aluminum mixing drum in two batches of approximately 40 kg each [3]. The mixing drum was designed to fit inside a liquid nitrogen vapor freezer and to rotate in the freezer thereby mixing the frozen tissue powder. After mixing for 2 h, subsamples of approximately 10 g of fish tissue homogenate were aliquoted into pre-cooled glass bottles.

Moisture Content: The moisture content of the fish tissue homogenate was determined by measuring the mass loss from freeze drying. Twelve bottles (six from each batch) of SRM 1946 were selected according to a stratified randomization scheme for the drying study. The entire contents of each glass bottle were transferred to a Teflon bottle and dried for 8 days at 1 Pa with a −10 °C shelf temperature and a −50 °C condenser temperature. Based on these studies, the mean moisture content of SRM 1946 is 71.4 % ± 0.1 % (mass fraction expressed as percent ± expanded uncertainty with k = 2, approximately 95 % confidence). The mass fraction values are reported on a wet-mass (as-received) basis. If necessary, the results can be converted to a dry-mass basis by dividing by the conversion factor of 0.2863 (grams dry mass per gram wet mass). An uncertainty component for the conversion factor (0.41 %) obtained from the moisture measurement should be incorporated in the uncertainties of the values provided on this certificate if comparing on a dry-mass basis.

PCBs and Chlorinated Pesticides: The general approach used for the value assignment of mass fractions for PCBs and chlorinated pesticides in SRM 1946 was similar to that reported for the recent certification of several environmental matrix SRMs [4] and consisted of combining results from analyses at NIST using a variety of extraction techniques and solvents, cleanup/isolation procedures, and chromatographic separation and detection techniques. This approach consisted of Soxhlet extraction and pressurized fluid extraction (PFE) using dichloromethane (DCM) or a hexane/acetone mixture; cleanup/isolation using solid-phase extraction (SPE), size-exclusion chromatography (SEC), or normal-phase liquid chromatography (LC); followed by analysis using gas chromatography with electron capture detection (GC-ECD) or gas chromatography with mass spectrometric detection (GC/MS) on two columns with different selectivity for the separation of PCBs and chlorinated pesticides.

Three sets of results were obtained by GC-ECD and are designated as GC-ECD (I), GC-ECD (IIA), and GC-ECD (IIB). For the GC-ECD (I) analyses, duplicate subsamples of 1 g from 10 bottles of SRM 1946 were extracted using PFE with DCM. SEC was used to remove the majority of the lipid material. The concentrated eluant was then fractionated on a semi-preparative aminopropylsilane column to isolate two fractions containing: (1) the PCBs and the less polar pesticides and (2) the more polar pesticides. GC-ECD analyses of the two fractions were performed on a 0.25 mm i.d. × 60 m fused silica capillary column with a 5 % (mole fraction) phenyl methylpolysiloxane phase (0.25 µm film thickness) (DB-5, J&W Scientific, Folsom, CA). For GC-ECD (IIA) and GC-ECD (IIB), 4 g subsamples from each of six bottles were extracted using PFE with DCM. The SEC and normal-phase LC cleanup steps were the same as for GC-ECD (I). GC-ECD (IIA) analyses were performed on a 5 % phenyl methylpolysiloxane phase as described above, and GC-ECD (IIB) analyses were on a 0.25 mm × 60 m fused silica capillary column with nonpolar proprietary phase (0.25 µm film thickness) (DB-XLB, J&W Scientific). For both GC-ECD analyses, two PCB congeners that are not significantly present in the fish extract (PCB 103 and PCB 198), and 4,4'-DDT-d_8, 4,4'-DDE-d_8, 4,4'-DDD-d_8, and endosulfan I-d_4 were added to the fish tissue prior to extraction for use as internal standards for quantification purposes.

Three sets of results were obtained by GC/MS. For GC/MS (I) and GC/MS (II), 3 g subsamples from six bottles were mixed with 50 g of sodium sulfate and Soxhlet extracted for 20 h with a mixture of hexane:acetone (1:1 volume fraction). The concentrated extract was treated with concentrated sulfuric acid to remove the majority of the lipid material, followed by additional cleanup on a silica solid-phase extraction cartridge with 10 % (volume fraction) DCM in hexane. The extract was then analyzed by GC/MS using the two different columns described above and using different ionization modes for the mass spectrometric detection. GC/MS (I) was performed using the nonpolar proprietary phase (DB-XLB) with electron impact ionization (EI) and GC/MS (II) was performed using the 5 % phenyl methylpolysiloxane phase with negative ion chemical ionization (NICI). For the GC/MS analyses, PCB 103,

[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.

PCB 198, and ^{13}C-labeled 4,4'-DDT, lindane, PCB 28, PCB 101, PCB 118, PCB 138, PCB 153, and PCB 169 were added to the fish tissue prior to extraction for use as internal standards for quantification purposes.

For GC/MS (III) analyses, 1.5 g subsamples from three bottles of SRM 1946 were mixed with sodium sulfate and Soxhlet extracted with DCM for 16 h. The concentrated extract was subjected to SEC to remove lipid material, followed by additional cleanup on a silica SPE cartridge with 10 % DCM in hexane. The GC/MS (III) analyses were performed using the same column and EI MS detection as in GC/MS (I). PCB 103, PCB 198, and 4,4'-DDT-d_8 were added to the fish tissue prior to extraction for use as internal standards for quantification purposes.

In addition to the analyses performed at NIST, SRM 1946 was used in an interlaboratory comparison exercise in 1999 as part of the NIST Intercomparison Exercise Program for Organic Contaminants in the Marine Environment [5]. Results from 30 laboratories that participated in this exercise (see Appendix A) were used as the seventh data set in the determination of the certified values for PCB congeners and chlorinated pesticides in SRM 1946. The laboratories participating in this exercise used the analytical procedures routinely used in their laboratories to measure these analytes.

Non-*Ortho*-Substituted PCBs (NOPCBs): Three sets of results for NOPCBs (PCB 77, PCB 126, and PCB 169) were obtained using GC/MS after LC isolation of the NOPCB fraction [6]. For GC/MS (IV) and GC/MS (V), 1 g subsamples from nine bottles of SRM 1946 were mixed with sodium sulfate and extracted using PFE with DCM. The extracts were subjected to SEC to remove lipids followed by normal-phase LC on a semi-preparative aminopropylsilane column with hexane as the mobile phase to isolate the PCB fraction. The PCB fraction was then separated into a *ortho*-substitued PCB fraction and a NOPCB fraction using a 2-(pyrenyl)ethyldimethylsilylated silica (PYE) column (4.6 mm i.d. × 25 cm, 5 μm Comosil-PYE, Nacalai Tesque, Kyoto, Japan) with hexane as the mobile phase. The NOPCB fraction was then analyzed by GC/MS using NICI on a 0.25 mm i.d. × 30 m fused silica capillary column containing a 5 % (mole fraction) diphenyl dimethylpolysiloxane phase (HP-5, 0.25 μm film thickness, Hewlett-Packard, Palo Alto, CA) [denoted as GC/MS (IV)]. The same samples were also analyzed by GC with high resolution EI MS on a 0.25 mm i.d. × 30 m fused silica capillary column containing a 5 % phenyl methylpolysiloxane phase (DB-5MS, 0.25 μm film thickness, J&W Scientific) [denoted as GC/MS (V)]. For GC/MS (VI) subsamples of 5 g from three bottles of SRM 1946 were extracted and the NOPCB fraction isolated as described above for GC/MS (IV) and (V). The NOPCB fractions were analyzed by GC/MS with NICI on a 0.25 mm i.d. × 60 m fused silica capillary column with a 5 % phenyl methylpolysiloxane phase (DB-5MS, 0.25 μm film thickness).

Homogeneity Assessment for PCB Congeners and Chlorinated Pesticides: The homogeneity of SRM 1946 was assessed by analyzing duplicate samples of 1 g from 10 bottles selected by stratified random sampling. Samples were extracted, processed, and analyzed as described above for GC-ECD (I). No statistically significant differences among bottles were observed for the PCB congeners and chlorinated pesticides at the 1 g sample size.

Polybrominated Diphenyl Ethers: Value assignment of mass fractions for PBDE congeners was based on four sets of data (three sets from NIST and one set from a collaborating laboratory) using a variety of extraction, cleanup, and quantification methods. All measurements were performed by using GC/MS operated in either electron impact (GC/EI-MS) or negative chemical ionization (GC/NCI-MS) mode.

For two of the NIST data sets, 1 g to 2 g subsamples of tissue from each of five bottles were extracted using PFE with DCM. The concentrated extract was subjected to SEC to remove the majority of the lipids, followed by an additional cleanup step employing silica SPE cartridges. The extracts were analyzed by using both GC/EI-MS and GC/NCI-MS on a 0.25 mm × 15 m fused silica capillary column with a 5 % (mole fraction) phenyl methylpolysiloxane phase (DB-5, 0.25 μm film thickness). For both methods ^{13}C-labeled 4,4'-dibromodiphenyl ether (PBDE 15) and ^{13}C-labeled 2,2',3,4,5-pentachlorodiphenyl ether (CDE 86) were added to the tissue sample prior to extraction for use as internal standards for quantification purposes.

For the third NIST data set, 3 g to 4 g subsamples of tissue from each of six bottles were extracted using PFE with DCM. The extracts were processed as above using SEC followed by a second cleanup step using a 5 % deactivated alumina SPE column. The extracts were analyzed by using GC/EI-MS on a 0.25 mm × 60 m fused silica capillary column with a 5 % phenyl methylpolysiloxane phase (0.25 μm film thickness) (DB-5MS). ^{13}C-labeled 2,2,4,4',5-pentabromodiphenyl ether (PBDE 99) was added to the tissue samples prior to extraction for use as an internal standard for quantification of the PBDEs.

For the measurements from the collaborating laboratory (Indiana University), four subsamples of 8 g were Soxhlet-extracted using hexane:acetone (1:1, volume fraction) after spiking with two internal standards, ^{13}C-labeled 2,3,3',4,4',5-hexachlorodiphenyl ether (CDE 156) and ^{13}C-labeled 2,2',3,3',4,4',5,5'-octachlorodiphenyl ether (CDE 194). Lipids were removed by adding concentrated H_2SO_4 and shaking; the organic phase was collected and the extracts were further cleaned using a 3 % deactivated silica column and an alumina column in series. The extracts

were analyzed by using GC/NCI-MS on a 0.25 mm × 60 m fused silica capillary column with a 5 % phenyl methylpolysiloxane phase (0.25 μm film thickness) (DB-5). Details of the analyses by the collaborating laboratory are presented by Zhu and Hites [7].

Perfluorooctanesulfonic Acid: Value assignment of mass fractions for PFOS was based on three sets of data (two sets from NIST and one set from an interlaboratory study) using a variety of extraction, cleanup, and quantification methods. All measurements were performed by using liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS).

For NIST PFOS method 1, a known amount of internal standard solution (containing ^{13}C-labeled PFOS) was added to a fish tissue sample (approximately 0.5 g), vortexed, and 0.5 mL of HPLC-grade water was added to the sample. Three milliliters of 0.01 mol/L of potassium hydroxide in methanol was added to the samples and the samples were then sonicated for 30 min. The supernatant was removed, evaporated to 1 mL, filtered using a Whatman UniPrep 0.2 μm filter (Stanford, ME), and poured into a clean polypropylene tube. Ten milliliters of 50 % (volume fraction) formic acid in water was added to each extract. Samples were loaded onto Oasis WAX SPE columns (3 mL, 60 mg, 30 μm; Waters, Milford, MA). Compounds of interest were eluted off the columns using methanol followed by 1 % (volume fraction) ammonium hydroxide in methanol. Following concentration, samples were analyzed using LC-MS/MS with a C_8 column (Agilent Zorbex Eclipse Plus C_8, 100 mm × 2.1 mm × 3.5 μm, Agilent Technologies, Santa Clara, CA) and a pentafluorophenyl (PFP) column (Phenomenex Kinetex PFP, 50 mm × 3.0 mm × 2.6 μm, Phenomenex, Torrance, CA) using both a methanol-ammonium acetate in water gradient method and a formic acid in acetonitrile-formic acid in water gradient method.

For NIST PFOS method 2, a known amount of internal standard solution (containing ^{13}C-PFOS) was added to a fish tissue sample (approximately 0.5 g), vortexed, and 0.5 mL of HPLC-grade water was added to the sample. Three milliliters of acetonitrile was added to the samples and the samples were then sonicated for 10 min. The supernatant was removed and poured into a clean polypropylene tube. Samples were solvent exchanged into methanol and then loaded onto Supelco Supelclean ENVI-Carb SPE columns (3 mL, 250 mg 120 to 400 mesh; Bellefonte, PA). Compounds of interest were eluted off the columns using methanol. Following concentration, samples were analyzed using LC-MS/MS with a C_8 column (Agilent Zorbex Eclipse Plus C_8, 100 mm × 2.1 mm × 3.5 μm, Agilent Technologies, Santa Clara, CA) and a PFP column (Phenomenex Kinetex PFP, 50 mm × 3.0 mm × 2.6 μm, Phenomenex, Torrance, CA) using a methanol-ammonium acetate in water gradient method.

The laboratories participating in the interlaboratory study (see Appendix C) used the analytical methods typically used in their laboratories to measure PFOS.

α-Hexabromocyclododecane: Three sets of results were combined for the information value of **α**-HBCD. In all three methods, a known amount of internal standard (^{13}C-labeled α-HBCD) was added to replicates of approximately 3 g subsamples. Samples were extracted with PFE using DCM, cleanup/isolation was accomplished with SEC followed by SPE. Extracts were analyzed by LC-MS/MS using negative electrospray ionization with separation on either an Agilent Eclipse Plus C18 (3.0 mm × 150 mm × 3.5 mm) analytical column (NIST HBCD methods 1 and 2) or a Waters YMC Carotenoid S5 C30 (4.6 mm × 250 mm × 5 mm) column (NIST HBCD method 3).

GMA Interlaboratory Comparison Exercise: Results for proximates, extractable fat, fatty acids, and selected trace elements were obtained from an interlaboratory comparison exercise organized in 1999 by the GMA Food Industry Analytical Chemists (FIAC; 11 participating laboratories, listed in Appendix B). The laboratories listed in Appendix B were asked to use AOAC methods or their equivalent, to make single measurements from each of two bottles, and to report the analytical method that was used. A summary of the methodological information and the number of laboratories using a particular analytical technique is provided in Appendix D. The methods used by NIST for these analytes are also included in this listing.

Extractable Fat Determination: The certified value for extractable fat was determined from the combination of results from analyses performed at NIST and the results from the GMA interlaboratory comparison exercise as for previous food-matrix SRMs [8]. Two sets of results were obtained at NIST. Six samples were extracted with DCM using PFE and three samples were extracted with DCM using Soxhlet extraction. For both extraction sets, the extract was evaporatively concentrated to approximately 20 mL (known mass) and an aliquot of 90 μL was placed on an aluminum pan. The extract on the pan was air dried, and the mass of the dried extract determined. For the GMA study, most of the laboratories used an acid digestion and ether extraction to obtain the extract and then determined the extractable fat by drying the extract and determining the mass of the remaining residue (see Appendix D).

Fatty Acids: The approach for value assignment of mass fractions of individual fatty acids in SRM 1946 was similar to that reported for the recent certification of several food-matrix SRMs [8] and consisted of combining results from analyses at NIST using gas chromatography with flame ionization detection (GC-FID) with results from the GMA interlaboratory comparison exercise.

For the NIST analyses, duplicate subsamples of approximately 2.5 g from each of nine bottles of SRM 1946 were analyzed in three sets of six samples over a three-day period. The fish tissue samples were mixed with diatomaceous earth and Soxhlet extracted for 18 h to 22 h with a mixture of 1:1 hexane:acetone. Prior to extraction a recovery standard, triheneicosanoin (C21 triglyceride), was added to the sample. Two fatty acid methyl esters (FAMEs), methyltridecanoate (C13:0 FAME) and methyltricosanoate (C23:0 FAME), were added to the extract for use as internal standards for quantification. The extract was then subjected to a two-step process employing methanolic sodium hydroxide and boron trifluoride to convert the fatty acids to their methyl esters (FAMEs). FAMEs were extracted into hexane, and analyzed by GC-FID on a 0.25 mm i.d. × 30 m fused capillary column with a 100 % poly(bis cyanopropylsiloxane) phase (SP-2340, 25 μm film thickness, Supelco, Bellefonte, PA).

Proximates: Results for proximates (solids, ash, protein, and fat) were obtained from the GMA interlaboratory comparison exercise described above.

Methylmercury and Total Mercury: The general approach for the assignment of values for methylmercury and total mercury was similar to that used for these analytes in recent marine tissue SRMs [9]. The certified values for methylmercury and total mercury are based on results of analyses of SRM 1946 at NIST and two collaborating laboratories: the Institute of Applied Physical Chemistry, Research Centre Jülich (Jülich, Germany) and the Jožef Stefan Institute (Ljubljana, Slovenia). For the determination of methylmercury, SRM 1946 was analyzed at NIST using microwave digestion under acidic conditions, derivatization (phenylation), and preconcentration using solid-phase microextraction (SPME) followed by GC with atomic emission detection (GC-AED) [9,10]. The GC-AED analyses were performed using a nonpolar 0.32 mm × 25 m fused silica capillary column with a polydimethylsiloxane phase (0.17 μm film thickness) (HP-1, Hewlett Packard, Wilmington, DE). For detection, the emission lines of mercury at 254 nm and carbon at 264 nm were used. A total of 13 subsamples (0.5 g to 1 g) from 6 bottles of SRM 1946 were analyzed at NIST. At the Research Centre of Jülich the analytical procedure for methylmercury consisted of water steam distillation under acid conditions, anion exchange chromatographic separation of inorganic mercury and methylmercury, followed by cold vapor atomic absorption spectrometric (CVAAS) detection before and after ultraviolet radiation [11-13]. Triplicate subsamples (250 mg to 450 mg) from two bottles of SRM 1946 were analyzed. At the Jožef Stefan Institute, duplicate subsamples (≈500 mg) from six bottles of SRM 1946 were analyzed using solid-liquid extraction into toluene followed by GC-ECD [14,15].

For total mercury measurements at NIST, subsamples (300 mg to 500 mg) from six bottles of SRM 1946 were analyzed. The analytical procedure consisted of spiking with ^{201}Hg as an internal standard, microwave-assisted acid digestion of the tissue, followed by cold vapor generation coupled with inductively coupled plasma mass spectrometry (CV-ICP-MS) isotope ratio measurements as described by Christopher et al. [16]. For mercury determination at the Research Centre Jülich, triplicate subsamples of 350 mg to 600 mg from two bottles of SRM 1946 were digested with concentrated nitric acid in heated quartz vessels closed with a cap and then analyzed by CVAAS) [17]. At the Jožef Stefan Institute, duplicate subsamples (≈300 mg) from six bottles of SRM 1946 were digested with acid and analyzed by CVAAS [18,19].

Additional Trace Element Analyses: Value assignment of the mass fractions of selected trace elements was accomplished by combining results of the analyses of SRM 1946 at NIST, U.S. Department of Agriculture (USDA) Food Composition Laboratory (Beltsville, MD), and one laboratory from the GMA interlaboratory exercise. Analyses were performed at NIST using ICP-MS (cadmium, copper, iron, and selenium) and instrumental neutron activation analysis (INAA) (arsenic, iron, selenium, and zinc). For ICP-MS analyses, six subsamples (1 g) from one bottle were digested in 5 mL of concentrated nitric acid in closed vessels in a microwave oven. The digest was then analyzed by ICP-MS with rhodium as an internal standard. For INAA analyses, the contents of eight bottles of SRM 1946 were freeze-dried and ten subsamples (≈200 mg) were pelletized and analyzed as described previously [20].

USDA used inductively coupled plasma-optical emission spectrometry (ICP-OES) to determine calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc. One laboratory from the GMA study provided results using ICP-OES (calcium, magnesium, and sodium) and flame atomic absorption spectrometry (FAAS) (copper, iron, manganese, potassium, and zinc).

Table 1. Certified Mass Fractions (Wet-Mass Basis) for Selected PCB Congeners in SRM 1946

PCB Congener[a]			Mass Fraction[b] (μg/kg)		
PCB	44	(2,2',3,5'-Tetrachlorobiphenyl)[c,d,e,f,g,h]	4.66	±	0.86
PCB	49	(2,2',4,5'-Tetrachlorobiphenyl)[c,d,e,f,g]	3.80	±	0.39
PCB	52	(2,2',5,5'-Tetrachlorobiphenyl)[c,d,e,f,g,h]	8.1	±	1.0
PCB	66	(2,3',4,4'-Tetrachlorobiphenyl)[f,g,h,i]	10.8	±	1.9
PCB	70	(2,3',4',5-Tetrachlorobiphenyl)[c,e,f,i]	14.9	±	0.6
PCB	74	(2,4,4',5-Tetrachlorobiphenyl)[c,e,f,i]	4.83	±	0.51
PCB	77	(3,3',4,4'-Tetrachlorobiphenyl)[j,k,l]	0.327	±	0.025[m]
PCB	87	(2,2',3,4,5'-Pentachlorobiphenyl)[c,d,f,g,i]	9.4	±	1.4
PCB	95	(2,2',3,5',6-Pentachlorobiphenyl)[e,f,g,h]	11.4	±	1.3
PCB	99	(2,2',4,4',5-Pentachlorobiphenyl)[c,d,e,f,g,i]	25.6	±	2.3
PCB	101	(2,2',4,5,5'-Pentachlorobiphenyl)[c,d,f,g,h,i]	34.6	±	2.6
PCB	105	(2,3,3',4,4'-Pentachlorobiphenyl)[c,d,e,f,g,h,i]	19.9	±	0.9
PCB	110	(2,3,3',4',6-Pentachlorobiphenyl)[e,f,g,i]	22.8	±	2.0
PCB	118	(2,3',4,4',5-Pentachlorobiphenyl)[c,d,e,f,g,h,i]	52.1	±	1.0
PCB	126	(3,3',4,4',5-Pentachlorobiphenyl)[j,k,l]	0.380	±	0.017[m]
PCB	128	(2,2',3,3',4,4'-Hexachlorobiphenyl)[c,e,f,g,h,i]	22.8	±	1.9
PCB	138	(2,2',3,4,4',5'-Hexachlorobiphenyl)[d,f,g]	115	±	13
PCB	146	(2,2',3,4',5,5'-Hexachlorobiphenyl)[c,d,e,f,i]	30.1	±	3.5
PCB	149	(2,2',3,4',5',6-Hexachlorobiphenyl)[c,d,e,f,g,i]	26.3	±	1.3
PCB	153	(2,2',4,4',5,5'-Hexachlorobiphenyl)[c,d,e,f,g,h,i]	170	±	9
PCB	156	(2,3,3',4,4',5-Hexachlorobiphenyl)[c,e,f,g,i]	9.52	±	0.51
PCB	169	(2,2',3,4,4',5'-Hexachlorobiphenyl)[j,k,l]	0.106	±	0.014[m]
PCB	170	(2,2',3,3',4,4',5-Heptachlorobiphenyl)[c,d,e,f,g,h,i]	25.2	±	2.2
PCB	180	(2,2',3,4,4',5,5'-Heptachlorobiphenyl)[c,d,e,f,g,h,i]	74.4	±	4.0
PCB	183	(2,2',3,4,4',5',6-Heptachlorobiphenyl)[c,d,f,g,i]	21.9	±	2.5
PCB	187	(2,2',3,4',5,5',6-Heptachlorobiphenyl)[c,d,f,g,h,i]	55.2	±	2.1
PCB	194	(2,2',3,3',4,4',5,5'-Octachlorobiphenyl)[c,d,e,f,i]	13.0	±	1.3
PCB	195	(2,2',3,3',4,4',5,6-Octachlorobiphenyl)[c,d,e,f,g,h,i]	5.30	±	0.45
PCB	206	(2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl)[c,d,e,f,g,h,i]	5.40	±	0.43
PCB	209	(Decachlorobiphenyl)[c,d,e,f,g,h,i]	1.30	±	0.21

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [21] and later revised by Schulte and Malisch [22] to conform with IUPAC rules; for the specific congeners listed in this table the Ballschmiter-Zell numbers correspond to those of Schulte and Malisch.

[b] Unless otherwise noted, the certified values are a weighted mean of the results from four to seven 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 [23] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurands are the total mass fractions of selected PCB congeners on a wet-mass basis. The certified values are metrologically traceable to the SI unit of mass, expressed as micrograms per kilogram.

[c] GC-ECD (I) on 5 % phenyl methylpolysiloxane phase after PFE with DCM.

[d] GC-ECD (IIB) on a proprietary nonpolar phase; same extracts analyzed as GC-ECD (IIA).

[e] GC-ECD (IIA) on 5 % phenyl methylpolysiloxane phase after PFE with DCM.

[f] GC/MS (I) on a proprietary nonpolar phase after Soxhlet extraction with hexane/acetone mixture.

[g] GC/MS (III) on a proprietary nonpolar phase after Soxhlet extraction with DCM.

[h] Results from up to 30 laboratories participating in an interlaboratory comparison exercise.

[i] GC/MS (II) on a 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC/MS (I).

[j] GC/MS (IV) with NICI on 5 % diphenyl dimethylpolysiloxane phase.

[k] GC/HRMS (V) with EI on a 5 % phenyl methylpolysiloxane phase.

[l] GC/MS (VI) with NICI on a 5 % phenyl methylpolysiloxane phase.

[m] The certified value is an unweighted mean of the results from three analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [26] with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurand is the total mass fraction of the selected PCB congener on a wet-mass basis. The certified value is metrologically traceable to the SI unit of mass, expressed as micrograms per kilogram.

Table 2. Certified Mass Fractions (Wet-Mass Basis) for Selected Chlorinated Pesticides in SRM 1946

Chlorinated Pesticides	Mass Fraction[a] (μg/kg)
Hexachlorobenzene[b,d,e,f,g,h]	7.25 ± 0.83
α-HCH[b,c,e,f,g]	5.72 ± 0.65[h]
γ-HCH[b,c,f,g]	1.14 ± 0.18
Heptachlor epoxide[b,c,e,f,g,i]	5.50 ± 0.23
Oxychlordane[b,d,e,f,g,i]	18.9 ± 1.5
cis-Chlordane (α-Chlordane)[b,c,e,f,g,i]	32.5 ± 1.8
trans-Chlordane[b,c,e,f,g,i]	8.36 ± 0.91
cis-Nonachlor[b,c,e,f,g,i]	59.1 ± 3.6
trans-Nonachlor[b,c,e,f,g,i]	99.6 ± 7.6
Dieldrin[b,c,f,g]	32.5 ± 3.5
Mirex[b,d,e,f,g]	6.47 ± 0.77
4,4'-DDE[b,c,e,f,g]	373 ± 48
2,4'-DDD[b,c,e,f,g]	2.20 ± 0.25
4,4'-DDD[b,c,e,f,g]	17.7 ± 2.8
4,4'-DDT[d,e,f,g]	37.2 ± 3.5

[a] Unless otherwise noted, the certified values are a weighted mean of the results from four to six 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 [23] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurands are the total mass fractions of selected chlorinated pesticides on a wet-mass basis. The certified values are metrologically traceable to the SI unit of mass, expressed as micrograms per kilogram.

[b] GC-ECD (I) on 5 % phenyl methylpolysiloxane phase after PFE with DCM.

[c] GC-ECD (IIB) on a proprietary nonpolar phase; same extracts analyzed as GC-ECD (IIA).

[d] GC-ECD (IIA) on 5 % phenyl methylpolysiloxane phase after PFE with DCM.

[e] GC/MS (I) on a proprietary nonpolar phase after Soxhlet extraction with hexane/acetone mixture.

[f] GC/MS (III) on a proprietary nonpolar phase after Soxhlet extraction with DCM.

[g] Results from up to 30 laboratories participating in an interlaboratory comparison exercise.

[h] The certified value is an unweighted mean of the results from five analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [26] with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurand is the total mass fraction of the selected chlorinated pesticide on a wet-mass basis. The certified value is metrologically traceable to the SI unit of mass, expressed as micrograms per kilogram.

[i] GC/MS (II) on a 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC/MS (I).

Table 3. Certified Mass Fractions (Wet-Mass Basis) for Selected PBDE Congeners in SRM 1946

PBDE Congener[a]			Mass Fraction (µg/kg)		
PBDE	28 33	(2,4,4'-Tribromodiphenyl ether)[b,c,d,e] (2',3,4-Tribromodiphenyl ether)	0.742	±	0.027[f]
PBDE	47	(2,2',4,4'-Tetrabromodiphenyl ether)[b,c,d,e]	29.9	±	2.3[f]
PBDE	66	(2,3',4,4'-Tetrabromodiphenyl ether)[b,c,d,e]	1.35	±	0.16[f]
PBDE	99	(2,2',4,4',5-Pentabromodiphenyl ether)[b,c,d,e]	18.5	±	2.1[f]
PBDE	100	(2,2',4,4',6-Pentabromodiphenyl ether)[b,c,d,e]	8.57	±	0.52[f]
PBDE	153	(2,2',4,4',5,5'-Hexabromodiphenyl ether)[b,c,d,e]	2.81	±	0.41[f]
PBDE	154	(2,2',4,4',5,6'-Hexabromodiphenyl ether)[c,d,e]	5.77	±	0.80[g]

[a] PBDE congeners are numbered according to IUPAC rules.
[b] GC/NCI-MS on a 15 m 5 % phenyl methylpolysiloxane phase.
[c] GC/EI-MS (I) on a 15 m 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC/NCI-MS.
[d] GC/NCI-MS results reported by Zhu and Hites [7].
[e] GC/EI-MS (II) on a 60 m 5 % phenyl methylpolysiloxane phase.
[f] The certified value is a weighted mean of the results from four analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence) calculated by combining a between-method variance [23] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurand is the total mass fraction of the selected PBDE congener on a wet-mass basis. The certified value is metrologically traceable to the SI unit of mass, expressed as micrograms per kilogram.
[g] The certified value is an unweighted mean of the results from three analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [26] with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurand is the total mass fraction of the selected PBDE congener on a wet-mass basis. The certified value is metrologically traceable to the SI unit of mass, expressed as micrograms per kilogram.

Table 4. Certified Mass Fractions (Wet-Mass Basis) for Fat and Selected Fatty Acids (as the Triglyceride) in SRM 1946

Fat	Mass Fraction[a] (%)		
Fat (Extractable)	10.17	±	0.48
Fat (Sum of Fatty Acids)[b]	8.76	±	0.17
Selected Fatty Acids (as the triglyceride)			
Hexadecanoic Acid (C16:0) (Palmitic Acid)	1.22	±	0.04
Octadecanoic Acid (C18:0) (Stearic Acid)	0.263	±	0.011
(Z,Z)-9,12-Octadecadienoic Acid (C18:2) (Linoleic Acid)	0.348	±	0.023
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3) (Linolenic Acid)	0.221	±	0.025
Eicosanoic Acid (C20:0) (Arachidic Acid)	0.0100	±	0.0012
(Z,Z)-11,14-Eicosadienoic Acid (C20:2)	0.0990	±	0.0043
(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic Acid (C20:5) (EPA)	0.296	±	0.019
(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic Acid (C22:5) (DPA)	0.335	±	0.026
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6) (DHA)	0.92	±	0.10

[a] The certified values are the unweighted mean of the mean of the average of results provided by laboratories listed in Appendix B and the mean of the NIST measurements. 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 [26] with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurands are the total mass fractions of fat and selected fatty acids (as the triglyceride) on a wet-mass basis. The certified values are metrologically traceable to the SI unit of mass, expressed as a percent.
[b] Fat as the sum of the fatty acids represents the sum of individual fatty acid mass fractions reported in Tables 3, 7, and 9.

Table 5. Certified Mass Fractions (Wet-Mass Basis) of Methylmercury, Total Mercury, Arsenic, and Iron in SRM 1946

	Mass Fraction[a] (mg/kg)
Methylmercury[b]	0.394 ± 0.015
Mercury (Total)	0.433 ± 0.009
Arsenic	0.277 ± 0.010
Iron	4.00 ± 0.32

[a] The certified values are an unweighted mean of the results from two or more 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 [26] with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurands are the total mass fractions of total mercury, arsenic, and iron on a wet-mass basis. The certified values are metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram.

[b] The measurand is the total mass faction of methylmercury on a wet-mass basis. The certified value is metrologically traceable to the SI unit of mass, expressed as milligrams of mercury per kilogram.

Table 6. Reference Mass Fractions (Wet-Mass Basis) for Selected PCB Congeners, Pesticides, PBDE Congeners, and PFOS in SRM 1946

	Mass Fraction[b] (μg/kg)
PCB Congeners[a]	
PCB 18 (2,2',5-Trichlorobiphenyl)[c,d]	0.84 ± 0.11
PCB 28 (2,4,4'-Trichlorobiphenyl)[c,d,f,g,h]	2.00 ± 0.24
PCB 31 (2,4',5-Trichlorobiphenyl)[c,e,f,h]	1.46 ± 0.20[i]
PCB 56 (2,3,3',4'-Tetrachlorobiphenyl)[c,e,h,j]	5.77 ± 0.93
PCB 63 (2,3,4',5-Tetrachlorobiphenyl)[d,e,h,j]	1.28 ± 0.19
PCB 107 (2,3,3',4',5-Pentachlorobiphenyl)[c,d,e,h,j]	8.86 ± 0.20
PCB 132 (2,2',3,3',4,6'-Hexachlorobiphenyl)[c,e,h,j]	5.83 ± 0.76
PCB 158 (2,3,3',4,4',6-Hexachlorobiphenyl)[c,e,h,j]	7.66 ± 0.88
PCB 163 (2,3,3',4',5,6-Hexachlorobiphenyl)[d,e,j]	31.8 ± 0.8[i]
PCB 174 (2,2',3,3',4,5,6'-Heptachlorobiphenyl)[c,d,e,h,j]	9.3 ± 1.3
PCB 193 (2,3',3,4',5,5',6-Heptachlorobiphenyl)[c,d,e,h,j]	5.78 ± 0.72
PCB 201 (2,2',3,3',4,5,5',6'-Octachlorobiphenyl)[e,j]	2.83 ± 0.13
Pesticides	
2,4'-DDE[e,f,g,h,j]	1.04 ± 0.29
2,4'-DDT[e,f,g]	22.3 ± 3.2
PBDE Congener[k]	
PBDE 49 (2,2',4,5'-Tetrabromodiphenyl ether)[l,m,n,o]	1.10 ± 0.23
PBDE 155 (2,2',4,4',6,6'-Hexabromodiphenyl ether)[l,m,o]	0.51 ± 0.11[i]
PBDE 183 (2,2',3,4,4',5',6-Heptabromodiphenyl ether)[l,n]	0.235 ± 0.033[i]
Perfluorooctanesulfonic Acid (PFOS)[p]	2.19 ± 0.08[q]

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [21] and later revised by Schulte and Malisch [22] to conform with IUPAC rules; for the specific congeners listed in this table, only PCB 107 and PCB 201 are different in the numbering systems. Under the Ballschmiter and Zell numbering system, the IUPAC PCB 107 is listed as PCB 108 and the IUPAC PCB 201 is listed as PCB 200.

[b] Unless otherwise noted, the reference values are the weighted mean of the results from two to five 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 [23] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurands are the mass fraction listed as determined by the methods indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as micrograms per kilogram on a wet-mass basis.

[c] GC-ECD (IIA) on 5 % phenyl methylpolysiloxane phase after PFE with DCM.

[d] GC-ECD (I) on 5 % phenyl methylpolysiloxane phase after PFE with DCM.

[e] GC/MS (I) on a proprietary nonpolar phase after Soxhlet extraction with hexane/acetone mixture.

[f] GC/MS (III) on a proprietary nonpolar phase after Soxhlet extraction with DCM.
[g] Results from up to 32 laboratories participating in an interlaboratory comparison exercise.
[h] GC-ECD (IIB) on a proprietary nonpolar phase; same extracts analyzed as GC-ECD (IIA).
[i] The reference value is the unweighted mean of the results from two to four analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [26] with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurand is the mass fraction listed as determined by the methods indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as micrograms per kilogram on a wet-mass basis.
[j] GC/MS (II) on a 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC/MS (I).
[k] PBDE congeners are numbered according to IUPAC rules.
[l] GC/NCI-MS on a 15 m 5 % phenyl methylpolysiloxane phase.
[m] GC/EI-MS (I) on a 15 m 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC/NCI-MS.
[n] GC/NCI-MS results reported by Zhu and Hites [7].
[o] GC/EI-MS (II) on a 60 m 5 % phenyl methylpolysiloxane phase.
[p] LC/MS/MS results from two NIST methods and an interlaboratory study.
[q] The reference value is the weighted mean of the results from three analytical methods [23,27]. The uncertainty listed with the value is an expanded uncertainty about the mean with coverage factor, $k = 2$, calculated by combining a pooled within method variance with a between method variance [29] following the ISO/JCGM Guide [24,25]. The measurand is the mass fraction listed as determined by the methods indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as micrograms per kilogram on a wet-mass basis.

Table 7. Reference Mass Fraction Values (Wet-Mass Basis) for Fatty Acids (as the Triglyceride) in SRM 1946

Fatty Acids (as the triglyceride)	Mass Fraction (%)
Dodecanoic Acid (C12:0) (Lauric Acid)	0.00555 ± 0.00051[a]
Pentadecanoic Acid (C15:0)	0.0285 ± 0.0016[b]
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.0225 ± 0.0023[b]
(E)-9-Octadecenoic Acid (C18:1) (Elaidic Acid)	0.0098 ± 0.0010[c]
(Z)-11-Octadecenoic Acid (C18:1) (Vaccenic Acid)	0.373 ± 0.005[b]
(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (C18:3) (gamma-linolenic Acid)	0.0149 ± 0.0031[b]
(Z,Z,Z,Z,)-6,9,12,15-Octadecatetraenoic Acid (C18:4) (Stearidonic Acid)	0.106 ± 0.013[b]
(Z,Z,Z)-11,14,17-Eicosatrienoic Acid (C20:3)	0.109 ± 0.018[b]
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4) (Arachidonic Acid)	0.212 ± 0.019[b]
(Z)-13-Docosenoic Acid (C22:1) (Erucic Acid)	0.0266 ± 0.0060[c]
(Z,Z)-13,16-Docosadienoic Acid (C22:2)	0.0369 ± 0.0011[b]
(Z)-15-Tetracosenoic Acid (C24:1) (Nervonic Acid)	0.0429 ± 0.0028[b]

[a] The reference value is the unweighted mean of the mean of the average of results provided by laboratories listed in Appendix B and the mean of the NIST measurements. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [26] with a pooled, within method variance following the ISO/JCGM Guide [24,25]. The measurand is the mass fraction listed as determined by the methods indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as a percent on a wet-mass basis.
[b] The reference value is the weighted mean of the results provided by three to nine laboratories in Appendix B [26]. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [23] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurand is the mass fraction listed as determined by the methods indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as a percent on a wet-mass basis.
[c] The reference value is the unweighted mean of the results from three laboratories in Appendix B. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), calculated by combining a between-method variance [23] with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurand is the mass fraction listed as determined by the methods indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as a percent on a wet-mass basis.

Table 8. Reference Mass Fraction Values (Wet-Mass Basis) for Proximates and Caloric Content of SRM 1946

	Mass Fraction[a] (%)
Solids	28.6 ± 0.1
Ash	1.10 ± 0.04
Protein	17.8 ± 0.2
Fat	(see Table 3)
Carbohydrates	(see Table 10)
Caloric Content	(kcal/100 g)
Calories[b]	159 ± 4

[a] Unless otherwise noted, the reference values are the weighted mean of the results provided by the laboratories in Appendix B [26]. 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 [23] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurands are the mass fraction for proximates as determined by the methods indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as a percent on a wet-mass basis. The measurand is the mass fraction for the caloric content and the value listed is metrologically traceable to the SI unit of calories, expressed as a kcal per 100 g on a wet-mass basis.

[b] The value for caloric content is the mean of individual caloric calculations from the laboratories listed in Appendix B. If the proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (as the sum of the fatty acids), protein, and carbohydrate, respectively, the mean caloric content is 154 kcal/100 g.

Table 9. Reference Mass Fraction Values (Wet-Mass Basis) for Elements in SRM 1946

Elements	Mass Fraction[a] (mg/kg)
Cadmium	0.00208 ± 0.00026[b]
Calcium	59.1 ± 1.5
Copper	0.476 ± 0.060
Magnesium	226 ± 18
Phosphorus	1980 ± 40
Potassium	3330 ± 180
Selenium	0.491 ± 0.043
Sodium	458 ± 25
Zinc	3.10 ± 0.18

[a] Unless otherwise noted, the reference values are the unweighted mean of the results from two or more 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 [26] with a pooled, within-method variance following the ISO/JCGM Guide [24,25]. The measurands are the mass fraction listed as determined by the methods indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram on a wet-mass basis.

[b] The reference value for cadmium is the mean of results obtained by NIST using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined standard uncertainty calculated according to the ISO/JCGM Guide [24,25]. The coverage factor, k, is determined from the Student's t-distribution for the appropriate degrees of freedom to yield 95 % confidence. The measurand is the mass fraction listed as determined by the methods indicted and the value listed is metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram on a wet-mass basis.

Table 10. Information Mass Fraction Values (Wet-Mass Basis) for Carbohydrates, Fatty Acids (as the Triglyceride), Elements, and α-HBCD in SRM 1946

	Mass Fraction[(a)] (%)
Carbohydrates	0.93

Fatty Acids (as the triglyceride)	Mass Fraction[(a)] (%)
Hexadecadienoic Acid (C16:2)	0.032
(E)-9-Hexadecenoic Acid (C16:1) (Palmitelaidic Acid)	0.066
Heptadecenoic Acid (C17:1)	0.041
(E,E)-9,12-Octadecadienoic Acid (C18:2) (Linoelaidic Acid)	0.011

Elements	Mass Fraction[(a)] (mg/kg)
Lead	0.7
Manganese	0.07

	Mass Fraction[(a)] (µg/kg)
α-Hexabromocyclododecane (α-HBCD)	5.76

[(a)] Information values are typically provided with no uncertainty because of the lack of sufficient information to assess adequately the uncertainty associated with the value. It may be assumed that the uncertainty is relatively large.

REFERENCES

[1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at: http://www.nist.gov/srm/publications.cfm (accessed Aug 2014).

[2] Zeisler, R.; Langland, J.K.; Harrison, S.H.; *Cryogenic Homogenization of Biological Tissues*; Anal. Chem., Vol. 55, pp. 2431–2434 (1983).

[3] Wise, S.A.; Christensen, R.G.; Benner, B.A. Jr.; Koster, B.J.; Schantz, M.M.; Zeisler, R.; *Preparation and Analysis of a Frozen Mussel Tissue Reference Material for the Determination of Trace Organic Constituents;* Environ. Sci. Technol., Vol. 25, pp. 1695–1704 (1991).

[4] Wise, S.A.; Poster, D.L.; Kucklick, J.R.; Keller, J.M.; VanderPol, S.S.; Sander, L.C.; Schantz, M.M.; *Standard Reference Materials (SRMs) for Determination of Organic Contaminants in Environmental Samples*; Anal. Bioanal. Chem., Vol. 386 pp. 1153–1190 (2006).

[5] Schantz, M.M.; Parris, R.M.; Wise, S.A.; *NIST/NOAA NS&T Intercomparison Exercise Program for Organic Contaminants in the Marine Environment: Description and Results of 1999 Organic Intercomparison Exercises*; NOAA Technical Memorandum NOS ORCA 146, Silver Spring, MD (2000).

[6] Brubaker, W.W., Jr.; Schantz, M.M.; Wise, S.A.; *Determination of Non-ortho Polychlorinated Biphenyls in Environmental Standard Reference Materials*; Fresenius' J. Anal. Chem., Vol. 367, pp. 401–406 (2000).

[7] Zhu, L.Y.; Hites, R.A.; *Determination of Polybrominated Diphenyl Ethers in Environmental Standard Reference Materials*; Anal. Chem., Vol. 75, pp. 6696–6700 (2003).

[8] Welch, M.J.; Colbert, J.C.; Gill, L.M.; Phinney, C.S.; Sharpless, K.S.; Sniegoski, L.T.; Wood, L.J.; *The Certification of SRM 1546 – Meat Homogenate, A New Reference Material for Nutrients in a High Protein, High Fat Matrix*; Fresenius' J. Anal. Chem., Vol. 370, pp. 42–47 (2001).

[9] Tutschku, S.; Schantz, M.M.; Horvat, M.; Logar, M.; Akagi, H.; Emons, H.; Levenson, M.; Wise, S.A.; *Certification of the Methylmercury Content in SRM 2977 Mussel Tissue and SRM 1566b Oyster Tissue*; Fresenius' J. Anal. Chem., Vol. 369, pp. 364–369 (2001).

[10] Tutschku, S.; Schantz, M.M.; Wise, S.A.; *Determination of Methylmercury and Butyltin Compounds in Marine Biota and Sediments Using Microwave-Assisted Acid Leaching, Solid Phase Microextraction, and Gas Chromatography with Atomic Emission Detection*; Anal. Chem., Vol. 74, pp. 4694–4701 (2002).

[11] May, K.; Stoeppler, M.; Reisinger, K.; *Studies of the Ratio of Total Mercury/Methylmercury in the Aquatic Food Chain*; Toxicol. Environ. Chem., Vol. 13, pp. 153–159 (1987).

[12] Ahmed, R.; May, K.; Stoeppler, M.; *Ultratrace Analysis of Mercury and Methylmercury (MM) in Rain Water Using Cold Vapour Atomic Absorption Spectrometry*; Fresenius' J. Anal. Chem., Vol. 326, pp. 510–516 (1987).

[13] Padberg, S.; Burow, M.; Stoeppler, M.; *Methylmercury Determination in Environmental and Biological Reference and Other Materials by Quality Control with Certified Reference Materials*; Fresenius' J. Anal. Chem., Vol. 346, pp. 686–688 (1993).

[14] Horvat, M.; May, K.; Stoeppler, M.; Byrne, A.R.; *Comparative Studies of Methylmercury Determination in Biological and Environmental Samples*; Appl. Organomet. Chem., Vol. 2, pp. 850–860 (1988).

[15] Horvat, M.; Byrne, A.R.; May, K.; *Rapid Quantitative Separation and Determination of Methylmercury by Gas Chromatography*; Talanta, Vol. 37, pp. 207–212 (1989).

[16] Christopher, S.J.; Long, S.E.; Rearick, M.S.; Fassett, J.D.; *Development of Isotope Dilution Cold Vapor Inductively Coupled Plasma Mass Spectrometry and Its Application to the Certification of Mercury in NIST Standard Reference Materials*; Anal. Chem., Vol. 73, pp. 2190–2199 (2001).

[17] May, K.; Stoeppler, M.; *Pretreatment Studies with Biological and Environmental Materials, IV. Complete Wet Digestion in Partly and Completely Closed Quartz Vessels for Subsequent Trace and Ultratrace Mercury Determinations*; Fresenius' J. Anal. Chem., Vol. 317, pp. 248–251 (1984).

[18] Horvat, M.; Zvonarič, T.; Stegnar, P.; *Optimization of a Wet Digestion Method for the Determination of Mercury in Blood by Cold Vapour Absorption Spectrometry (CV AAS)*; Vestn. Slov. Kem. Drus., Vol. 33, pp. 475–486 (1986).

[19] Horvat, M.; Lupšina, V.; Pihlar, B.; *Determination of Total Mercury in Coal Fly Ash by Gold Amalgamation Cold Vapour Atomic Absorption Spectrometry*; Anal. Chim. Acta, Vol. 243, pp. 71–79 (1991).

[20] Mackey, E.A.; Demiralp, R.; Fitzpatrick, K.A.; Porter, B.J.; Wise, S.A.; Becker, P.R.; Greenberg, R.R.; *Quality Assurance in the Analysis of Cryogenically Stored Liver Tissue Specimens from the NIST National Biomonitoring Specimen Bank*; Sci. Total Environ., Vol. 226, pp. 165–176 (1999).

[21] Ballschmiter, K.; Zell, M.; *Analysis of Polychlorinated Biphenyls (PCB) by Glass Capillary Gas Chromatography - Composition of Technical Aroclor- and Clophen-PCB Mixtures*; Fresenius' J. Anal. Chem., Vol. 302, pp. 20–31 (1980).

[22] Schulte, E.; Malisch, R.; *Calculation of the Real PCB Content in Environmental Samples. I. Investigation of the Composition of Two Technical PCB Mixtures*; Fresenius' J. Anal. Chem., Vol. 314, pp. 545–551 (1983).

[23] Ruhkin, A.L.; Vangel, M.G.; *Estimation of a Common Mean and Weighted Means Statistics*; J. Am. Stat. Assoc., Vol. 93, pp. 303–308 (1998).

[24] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement*; (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Aug 2014); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://www.nist.gov/pml/pubs/index.cfm (accessed Aug 2014).
[25] JCGM 101:2008; *Evaluation of measurement data – Supplement 1 to the "Guide to the expression of uncertainty in measurement" - Propagation of distributions using a Monte Carlo method*; JCGM (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Aug 2014).
[26] Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H.-k.; Vangel, M.G.; Yen, J.H.; Zhang, N.F.; *An Approach to Combining Results from Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571–579 (2000).
[27] Dersimonian, R.; Laird, N.; *Meta-Analysis in Clinical Trials*; Control Clin. Trials, Vol. 7, pp. 177–188 (1986).
[28] Rukhin, A.L.; *Weighted Means Statistics in Interlaboratory Studies*; Metrologia, Vol. 46, pp. 323–331 (2009).
[29] Horn, R.A.; Horn, S.A.; Duncan, D.B.; *Estimating Hetroscedastic Variance in Linear Models*; J. Am. Stat. Assoc., Vol. 70, pp. 380–385 (1975).

Certificate Revision History: 28 October 2014 (Corrected revision history page number referenced on page 1); **25 August 2014** (Removal of certified values, tetradecanoic acid (C14:0), (Z)-9-Hexadecenoic Acid (C16:1), (Z)-9-octadecenoic acid (C18:1), and (Z)-11-eicosenoic acid (C20:1) in Table 4, due to recent analysis indicating bias; editorial changes); **17 September 2012** (This revision adds certified and reference values for PBDE congeners, a minimum sample size, a reference value for PFOS, and an information value for α-HBCD, and extends the expiration date; editorial changes); **29 September 2003** (Change in grams per bottle); **20 February 2003** (Original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX A

The laboratories listed below performed measurements that contributed to the value assignment for PCBs and pesticides in SRM 1946.

Arthur D. Little, Inc.; Cambridge, MA, USA
Axys Analytical Services; Sidney, BC, Canada
B & B Laboratories; College Station, TX, USA
Battelle Ocean Sciences; Duxbury, MA, USA
California Department of Fish and Game; Rancho Cordova, CA, USA
Central Contra Costa Sanitary District; Martinez, CA, USA
Chesapeake Biological Laboratory; Solomons, MD, USA
Centro de Investigaciones Energetices Medioambientales y Tecnologicas (CIEMAT); Madrid, Spain
City of Los Angeles, Environmental Monitoring Division; Playa del Rey, CA, USA
City of San Jose, Environmental Sciences Department; San Jose, CA, USA
Columbia Analytical Services; Kelso, WA
Environment Canada, Environmental Sciences Centre; Moncton, New Brunswick, Canada
U.S. Environmental Protection Agency (EPA), Atlantic Ecology Division; Narragansett, RI, USA
Florida Department of Environmental Protection; Tallahassee, FL, USA
Murray State University; Murray, KY, USA
Massachusetts Water Resources Authority Central Laboratory; Winthrop, MA, USA
National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NOAA/NMFS), Center for Coastal Environmental Health and Biomolecular Research (CCEHBR); Charleston, SC, USA
NOAA/NMFS, Sandy Hook Marine Laboratory; Highlands, NJ, USA
NOAA/NMFS, Northwest Fisheries Science Center; Seattle, WA, USA
Orange County Sanitation District; Fountain Valley, CA, USA
Philip Analytical Services; Burlington, Ontario, Canada
Serv de Hidrografia Naval; Buenos Aires, Argentina
Skidaway Institute of Technology; Savannah, GA, USA
Southwest Laboratory of Oklahoma; Broken Arrow, OK, USA
Texas A & M University, Geochemical and Environmental Research Group (GERG); College Station, TX, USA
Texas Parks and Wildlife Department; San Marcos, TX, USA
University of Connecticut, Environmental Research Institute; Storrs, CT, USA
University of Rhode Island, Graduate School of Oceanography; Narragansett, RI, USA
U.S. Geological Survey, National Water Quality Laboratory; Denver, CO, USA
Wright State University; Dayton, OH, USA

APPENDIX B

The laboratories listed below performed measurements that contributed to the value assignment for proximates, caloric content, elements, extractable fat, and fatty acids in SRM 1946.

Covance Laboratories; Madison, WI, USA
Dionex Corporation; Salt Lake City, UT, USA (extractable fat only)*
General Mills, Inc.; Minneapolis, MN, USA
Hormel Foods Corporation; Austin, MN, USA
Kraft Foods, Glenview; IL, USA
Nabisco, Inc.; East Hanover, NJ, USA
Nestlé USA; Dublin, OH, USA
Novartis Nutrition Corporation; St. Louis Park, MN, USA
Pillsbury; St. Paul, MN, USA
Ralston Purina Company; St. Louis, MO, USA
U.S. Department of Agriculture, Food Composition Laboratory; Beltsville, MD, USA
Woodson-Tenent Laboratories; Memphis, TN, USA

* Not a GMA FIAC laboratory

APPENDIX C

The laboratories listed below performed measurements that contributed to the value assignment for PFOS in SRM 1946.

3M Company; St. Paul, MN, USA
Bundesamt fuer Seeschifffahrt und Hydrographie; Hamburg, Germany
Environment Canada; Burlington, Canada
University of Toronto; Toronto, Canada
US Environmental Protection Agency; Research Triangle Park, NC, USA
Wageningen IMARES; Ijmuiden, The Netherlands

APPENDIX D

The methodological information reported by laboratories whose results were used for value assignment of proximates, caloric content, fatty acids, and trace elements is summarized below. The number of laboratories using a particular method is provided in parentheses.

Proximates, Fatty Acids, and Calories

Solids	Moisture determined by mass loss after oven-drying: Forced-air oven (3) Vacuum oven (7)
Ash	Mass loss after ignition in muffle furnace (10)
Extractable Fat	Acid digestion, ether extraction (8) Soxhlet extraction (2 + NIST) Pressurized-fluid extraction (1 + NIST)
Fatty Acids	Hydrolysis followed by gas chromatography (10 + NIST)
Nitrogen	Kjeldahl (5) Thermal conductivity (2) Pyrolysis, gas chromatography (1) Combustion (2)
Protein	Calculated; a factor of 6.25 was used to calculate protein from nitrogen results
Carbohydrates	Calculated; [solids − (protein + fat + ash)]
Calories	Calculated; [9(fat) + 4(protein) + 4(carbohydrates)]

Elements

Methods	
FAAS	Flame atomic absorption spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ID-ICP-MS	Isotope dilution inductively coupled plasma mass spectrometry
INAA	Instrumental neutron activation analysis
CVAAS	Cold vapor atomic absorption spectrometry
Arsenic	ICP-MS (NIST), INAA (NIST)
Calcium	ICP-OES (2)
Cadmium	ICP-MS (NIST)
Copper	FAAS (1), ICP-OES (1), ICP-MS (NIST)
Iron	FAAS (1), ICP-OES (1), ICP-MS (NIST), INAA (NIST)
Magnesium	ICP-OES (2)
Manganese	FAAS (1), ICP-OES (1)
Mercury	ID-ICP-MS (NIST), CVAAS (2)
Phosphorus	ICP-OES (2)
Potassium	FAAS (1), ICP-OES (1)
Selenium	ICP-MS (NIST), INAA (NIST)
Sodium	ICP-OES (2)
Zinc	FAAS (1), ICP-OES (1), ICP-MS (NIST)