National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1947

Lake Michigan Fish Tissue

This Standard Reference Material (SRM) is a frozen fish tissue homogenate that was prepared from fish collected from Lake Michigan, and is intended primarily for use in evaluating analytical methods for the determination of selected trace elements, methylmercury, total mercury, polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, polybrominated diphenyl ether (PBDE) congeners, perfluoroalkyl acids (PFAAs), proximates, α-hexabromocyclododecane (α-HBCD), caloric content, and fatty acids 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 1947 consists of five bottles, each containing approximately eight grams (wet basis) of frozen tissue homogenate.

Certified Mass Fraction Values: Certified mass fraction values are provided in Table 1 for selected trace elements including total mercury and methylmercury. Certified mass fraction values are provided in Tables 2 through 4 for selected PCB congeners, chlorinated pesticides, and PBDE congeners. The certified values for trace elements, PCBs, and chlorinated pesticides are based on results obtained from two or more independent analytical techniques. 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 Values: Reference mass fraction values are provided in Table 5 for additional PCB congeners, chlorinated pesticides, PBDE congeners, and perfluorooctanesulfonic acid (PFOS). Reference values are provided for proximates, caloric content, selected fatty acids In Tables 6 and 7. Reference Values for Hg Isotope Ratio Differences in SRM 1947 are shown in Table 8. 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 reproducibility, 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 in Table 9 for additional PFAAs and α-HBCD and in Table 10 for carbohydrates. An information value is considered to be a value that will be of interest and use to the SRM user, but insufficient information is available to assess adequately the uncertainty associated with the value or only a limited number of analyses were performed [1]. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 1947** 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 preparation and technical measurements leading to the certification of this SRM was performed by S.J. Christopher, G.C. Turk, M.M. Schantz and S.A. Wise of the NIST Chemical Sciences Division.

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Office of Reference Materials

Analytical measurements at NIST were performed by M.M. Schantz, S.J. Christopher, W.C. Davis, R.D. Day, 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; B.J. Porter, M.S. Rearick, 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 (FIAC) were coordinated by K.E. Sharpless of the NIST Office of Special Programs and H.B. Chin and D.W. Howell of the GMA (Washington, DC); see Appendix B for participating laboratories. Measurements from the 2001 NIST/NOAA Interlaboratory Comparison Exercise for Trace Elements in Marine Mammals were coordinated by S.J. Christopher of the NIST Chemical Sciences Division; see Appendix C for participating laboratories. Measurements from an informal interlaboratory comparison study for PFAAs in a variety of matrices were coordinated by J.M. Keller and J.L. Reiner of the NIST Chemical Sciences Division; see Appendix D for participating laboratories. Analytical measurements for methylmercury were also performed at the University of Pau (Pau, France) by E. Krupp, D. Point, and O. Donard. Analytical measurements of mercury stable isotopes were performed at NIST by R.D. Day of the Chemical Sciences Division and by the following interlaboratory comparison participants: J. Masbou, L. Laffont, J. Sonke, and D. Point at the Laboratoire Geosciences Environnement Toulouse Observatoire Midi-Pyrenees (GET) (Toulouse, France); J. Blum and M. Johnson from the University of Michigan (Ann Arbor, MI); H. Hintelmann and B. Dimock at Trent University, (Petersborough, Canada); S. Berail, D. Amouroux, and O.F.X. Donard at the University of Pau/Institut Pluridisciplinaire de Recherche sur l'Environnement et les Materiaux (IPREM), (Pau, France).

Fish used for SRM 1947 were collected with the assistance of J. Jonas of the Michigan Department of Natural Resources (DNR) and Captain J. Meggison, J. Ranville, and J. Harris of the Michigan DNR survey vessel *Steelhead*. The coordination for the collection was performed by J.R. Kucklick and B.J. Porter. Field collection was performed by B.J. Porter; B. Flood and J. Stevens of the Michigan DNR; and M.P. Cronise and C.N. Fales of the NIST Office of Reference Materials. Cryogenic homogenization of the fish tissue was performed by B.J. Porter and R.S. Pugh of the NIST Chemical Sciences Division and D.J. Struntz formerly of NIST.

Statistical analysis was provided by S.D. Leigh, J.H. Yen, 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 RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: SRM 1947 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, 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). 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 4 to be valid within the stated uncertainties. The mass fractions of constituents in SRM 1947 are reported on a wet-mass basis. The SRM tissue homogenate, as received, contains approximately 73 % moisture.

PREPARATION AND ANALYSIS[1]

Sample Collection: SRM 1947 was prepared from fillets from adult lake trout (*Salvelinus namaycush*) collected in October 1997 at Fisherman's Island and Big Reef near Charlevoix, MI, in northern Lake Michigan. 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 79 kg of fillets was obtained. 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 8 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 1947 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 seven days at 1 Pa with a −20 °C shelf temperature and a −50 °C condenser temperature. Based on these studies, the mean moisture content of SRM 1947 is 73.00 % ± 0.15 % (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 0.2700 (grams dry mass per gram wet mass). An uncertainty component for the conversion factor (0.46 %) 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.

Trace Elements: Trace element data are derived from three sources: (1) NIST measurements, (2) data from the 2001 NIST/NOAA Interlaboratory Comparison Exercise for Trace Elements in Marine Mammals, and (3) data obtained in 2002 from an interlaboratory comparison exercise conducted by the GMA. The NIST measurements were collected using collision cell inductively coupled plasma mass spectrometry (ICPMS) and the method of standard additions. A maximum likelihood solution algorithm was used to determine the consensus mean estimates and analytical uncertainties associated with the interlaboratory data, after rejection of outlier laboratories. This algorithm weights data based on inverse variance. Certified values for As, Cu, Fe, Mn, Rb, Se, and Zn were then assigned by combining the NIST values and interlaboratory consensus mean estimates using an equal-weighting scheme. Analytical uncertainties were calculated using the bound-on-bias method [4]. The certified value for Hg is derived from isotope dilution cold vapor inductively coupled plasma mass spectrometry (IDCV/ICPMS) measurements performed at NIST [5].

Methylmercury: The general approach for the assignment of a value for methylmercury was similar to that used in marine tissue SRMs [6,7]. The certified value for methylmercury is based on results of analyses of SRM 1947 at NIST and the University of Pau using four analytical techniques. The three NIST methods used for methylmercury measurements were based on (1) solid-phase microextraction (SPME) with gas chromatography/mass spectrometry (GC/MS), (2) SPME with standard additions quantification and GC/inductively coupled plasma mass spectrometry (GC/ICPMS) [8], and (3) microwave extraction with speciated isotope dilution and GC/ICPMS [9]. The fourth method also utilized microwave extraction with speciated isotope dilution GC/ICPMS. For the SPME GC/MS analyses, approximately 1 g subsamples of SRM 1947 were spiked with an appropriately diluted solution of IRMM-670 ^{202}Hg enriched methylmercury isotopic Certified Reference Material (CRM) (Institute of Reference Materials and Measurements [IRMM], Geel, Belgium) followed by an acidic microwave digestion using 1 mol/L hydrochloric acid. Sodium tetraphenylborate was used for phenylation. After headspace SPME sampling, the SPME fiber was desorbed using a GC injection temperature of 250 °C. The GC analysis used a 30 m × 0.25 mm column with a 5 % (mole fraction) phenyl methylpolysiloxane phase (0.25 µm film thickness) (DB-5MS, J&W Scientific, Folsom, CA). For the SPME GC/ICPMS analyses, approximately 0.5 g subsamples of SRM 1947 were subjected to an acidic microwave digestion using 1 mol/L hydrochloric acid. Sodium tetraphenylborate was used for phenylation. After headspace SPME sampling, the SPME fiber was desorbed using a GC injection temperature of 210 °C. The GC analysis used a 30 m × 0.28 mm column with a 100 % dimethylpolysiloxane phase (0.50 µm film thickness) (MXT-1, Restek, Bellefonte, PA). For the speciated isotope dilution GC/ICPMS analyses, approximately 1.0 g to 2.0 g subsamples were spiked with an appropriately diluted sample of IRMM-670 ^{202}Hg enriched methylmercury isotopic CRM and subjected to an alkaline microwave digestion (using 25 % volume fraction tetraammoniumhydroxide in water). Sodium tetraethylborate was used for ethylation. The derivatized methylmercury was back-extracted into isooctane and injected into a GC/ICPMS. The GC analysis used a 30 m × 0.32 mm column with a 100 % dimethylpolysiloxane phase (0.17 µm film thickness) (HP-1, J&W Scientific). *SRM 1946 Lake Superior Fish Tissue* was used as a control in each of the methods described.

[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.

Mercury Isotopes: Analytical measurements were made as part of an inter-laboratory comparison with five expert Hg isotope labs reporting a total of nine methods. All measurements used cold vapor multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS). NIST (Charleston, SC) achieved sample decomposition using microwave assisted acid digestion in a Discover Open-Focus Microwave (CEM, Matthews, NC). NIST's measurements were made using a Nu Plasma II (Nu Instruments, Wrexham, UK), and reported data using two different methods of instrumental mass bias correction: thallium introduced by desolvation nebulizer (Aridus II, CETAC, Omaha, NE), or Hg and standard-sample-standard bracketing. University of Michigan (Ann Arbor, MI) reported data using both thermal decomposition and microwave-assisted acid digestion, each coupled to an additional matrix separation step of bubbling, trapping, and purging in $KMnO_4$ and H_2SO_4. University of Michigan's measurements were made on a Nu Plasma with instrumental mass bias correction by thallium introduced by Aridus. Trent University (Petersborough, Canada) used a hotblock/acid decomposition method, Neptune (Thermo-Finnigan, Germany) for quantification, and Apex-q desolvation nebulizer (Elemental Scientific, Omaha, NE) for thallium introduction and mass bias correction. University of Pau/IPREM (Pau, France) decomposed the sample using a high pressure asher (Anton Paar) followed by hotblock/acid digestion. University of Pau/IPREM's measurements were made on a Nu Plasma, and mass bias corrections performed using thallium introduced by DSN-100 (Nu Instruments). Géosciences Environnement Toulouse (GET) (Toulouse, France) reported data using both a microwave assisted acid digestion (CEM Discover microwave) and hotplate/acid digestion. LMTG's measurements were made on a Neptune and instrumental mass bias correction performed using Hg and standard-sample-standard bracketing.

Notation and reporting of differences in measured isotope ratios (δ) of stable mercury isotopes in SRM 1947 follow the conventions outlined by Blum and Bergquist [10]. All δ values are reported in permil, ‰ (part per thousand, equal to 0.001), and are calculated relative to the reference standard NIST *SRM 3133 Mercury (Hg) Standard Solution (Lot No. 061204)* using the following equation:

$$\delta^{xxx}\mathrm{Hg}(‰) = [((^{xxx}\mathrm{Hg}/^{198}\mathrm{Hg})_{\mathrm{Sample}}/(^{xxx}\mathrm{Hg}/^{198}\mathrm{Hg})_{\mathrm{SRM3133}}) - 1] * 1000$$

where

xxx is the Hg isotope 199, 200, 201, 202, or 204
$(^{xxx}\mathrm{Hg}/^{198}\mathrm{Hg})_{\mathrm{Sample}}$ is the ratio of each isotope to ^{198}Hg in SRM 1947.
$(^{xxx}\mathrm{Hg}/^{198}\mathrm{Hg})_{\mathrm{SRM3133}}$ is the average ratio of the two NIST SRM 3133 standards bracketing that sample

The measurement community typically calculates mass-independent fractionation (MIF) values from these δ^{xxx}Hg values, which are reported using the capital delta notation (Δ). These values were calculated using the Blum and Bergquist [10] formulas below and are provided in this report for convenience:

$$\Delta^{199}\mathrm{Hg}(‰) = \delta^{199}\mathrm{Hg} - (\delta^{202}\mathrm{Hg} * 0.2520)$$
$$\Delta^{200}\mathrm{Hg}(‰) = \delta^{200}\mathrm{Hg} - (\delta^{202}\mathrm{Hg} * 0.5024)$$
$$\Delta^{201}\mathrm{Hg}(‰) = \delta^{201}\mathrm{Hg} - (\delta^{202}\mathrm{Hg} * 0.7520)$$

The Δ^{xxx}Hg notation describes the difference between measured δ^{xxx}Hg and the theoretically predicted δ^{xxx}Hg using mass dependent fractionation (MDF) laws.

PCBs and Chlorinated Pesticides: The general approach used for the value assignment of mass fractions for PCBs and chlorinated pesticides in SRM 1947 was similar to that reported for the recent certification of SRM 1946 [11] and consisted of combining results from analyses at NIST using various combinations of extraction techniques and solvents, cleanup/isolation procedures, and chromatographic separation and detection techniques. For SRM 1947 the 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); and finally analysis by using gas chromatography with electron capture detection (GC-ECD) or gas chromatography with mass spectrometric detection (GC/MS) on three 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, between 2 g and 3 g subsamples from each of six bottles of SRM 1947 were extracted using Soxhlet 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 % phenyl methylpolysiloxane phase (0.25 μm film thickness) (DB-5, J&W Scientific). For GC-ECD (IIA) and GC-ECD (IIB), 3.5 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 those used for GC-ECD (I). GC-ECD (IIA) analyses were performed 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), and GC-ECD (IIB)

analyses were performed on a 5 % phenyl methylpolysiloxane phase as described above. For both GC-ECD analyses, two PCB congeners that are not significantly present in the fish extract [PCB 30 and PCB 198 for GC-ECD (I) or PCB 103 and PCB 198 for GC-ECD (II)], and 4,4'-DDT-d_8, 4,4'-DDE-d_8, and 4,4'-DDD-d_8 were added to the fish tissue prior to extraction for use as internal standards for quantification purposes.

Two sets of results were obtained by GC/MS. The samples used for GC/MS (I) analyses were the same extracts as analyzed for GC-ECD (I). The GC/MS (I) analyses were performed using a DB-XLB column as described above and MS detection. For GC/MS (II) analyses, two subsamples of between 1 g and 2 g were used from three bottles of SRM 1947. The six samples were extracted using PFE with hexane:acetone (1:1, volume fraction). The concentrated extract was subjected to cleanup on a silica solid phase extraction (SPE) cartridge with 10 % DCM in hexane, followed by a second silica SPE cartridge that was used for additional sample cleanup. The GC/MS (II) analyses were performed using a 50 % (mole fraction) phenyl methylpolysiloxane phase (0.25 µm film thickness) (DB-17MS, J&W Scientific). For the GC/MS (II) analyses, PCB 103, 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.

In addition to the analyses performed at NIST, SRM 1947 was used in an interlaboratory comparison exercise in 2002 as part of the NIST Intercomparison Exercise Program for Organic Contaminants in the Marine Environment [12]. Results from 28 laboratories were used as the sixth data set in the determination of the certified values for PCB congeners and chlorinated pesticides in SRM 1947; see Appendix A for participating laboratories7. The laboratories participating in this exercise used the analytical procedures routinely used in their laboratories to measure these analytes.

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 different 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 (0.25 µm film thickness) (DB-5). For both methods, ^{13}C-labeled 4,4'-dibromodiphenyl ether (BDE 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 (BDE 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 [13].

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

For NIST PFAA method 1, a known amount of internal standard solution (containing selected ^{13}C-labeled PFAAs) 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 mL (volume fraction) 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 mL 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, Millford, MA). Compounds of interest were eluted off the columns using methanol followed by 1 % (volume fraction) ammonium hydroxide in methanol and 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 PFAA method 2, a known amount of internal standard solution (containing ^{13}C-PFAA) 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 Supelclean ENVI-Carb SPE columns (3 mL, 250 g 120–400 mesh Supelco, Bellefote, PA). Compounds of interest were eluted off the columns using methanol and 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 D) used the analytical methods typically used in their laboratories to measure PFAAs.

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 2002 by the GMA FIAC; see Appendix B for a list of participating laboratories. 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.

Extractable Fat Determination: The reference mass fraction value for extractable fat was determined from the combination of results from analyses performed at NIST and the results from the interlaboratory comparison exercise. At NIST, six samples were extracted with hexane:acetone (1;1, volume fraction) using PFE. The extracts were 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 interlaboratory study, laboratories used their typical extraction methods and then determined the extractable fat by drying the extract and determining the mass of the remaining residue.

Proximates: Results for proximates (solids, ash, protein, and fat) were obtained from the GMA interlaboratory comparison exercise described above. The value for solids was a combination of the mean of the GMA measurements and the NIST moisture determination measurements described above.

Table 1. Certified Mass Fractions (Wet-Mass Basis) for Selected Elements and Methylmercury in SRM 1947

Analyte	Mass Fraction[a] (mg/kg)
Arsenic (As)	0.732 ± 0.039
Copper (Cu)	0.411 ± 0.029
Iron (Fe)	3.79 ± 0.42
Mercury (Hg)	0.254 ± 0.005[b]
Manganese (Mn)	0.076 ± 0.004
Rubidium (Rb)	4.51 ± 0.09
Selenium (Se)	0.475 ± 0.084
Zinc (Zn)	2.66 ± 0.08

	Mass Fraction[a] (mg/kg)
Methylmercury	0.233 ± 0.010[c]

[a] Unless otherwise noted, the certified mass fractions are the unweighted mean of NIST and round-robin consensus mean results. 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 [4] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [14,15]. The measurand is the total mass fractions for selected elements on a wet-mass basis listed in Table 1. Metrological traceability to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

[b] The certified mass fraction for mercury is based solely on IDCV/ICPMS measurements performed at NIST.

[c] The certified value is the unweighted 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 [4] with a pooled, within-method variance following the ISO/JCGM Guide [14,15]. The measurand is the mass fraction for methylmercury on a wet-mass basis listed in Table 1. Metrological traceability to the SI derived unit for mass fraction (expressed as milligrams of mercury per kilogram).

Table 2. Certified Mass Fractions (Wet-Mass Basis) for Selected PCB Congeners in SRM 1947

PCB Congener[a]	Mass Fraction[b] (µg/kg)
PCB 28 (2,4,4'-Trichlorobiphenyl)[c,d,e,f,g]	14.1 ± 1.0
PCB 31 (2,4',5-Trichlorobiphenyl)[c,d,e,f,g]	10.4 ± 1.4
PCB 44 (2,2',3,5'-Tetrachlorobiphenyl)[c,d,e,f,g,h]	20.4 ± 1.7
PCB 49 (2,2',4,5'-Tetrachlorobiphenyl)[c,d,e,f,g,h]	27.3 ± 3.8
PCB 52 (2,2',5,5'-Tetrachlorobiphenyl)[c,d,e,f,g,h]	36.4 ± 4.3
PCB 63 (2,3,4',5-Tetrachlorobiphenyl)[c,d,e,f]	4.75 ± 0.60
PCB 66 (2,3',4,4'-Tetrachlorobiphenyl)[c,d,e,f,g]	69.4 ± 5.3
PCB 74 (2,4,4',5-Tetrachlorobiphenyl)[c,f,g,h]	33.7 ± 3.1
PCB 87 (2,2',3,4,5'-Pentachlorobiphenyl)[d,f,g,h]	27.9 ± 1.5
PCB 99 (2,2',4,4',5-Pentachlorobiphenyl)[c,d,e,f,g,h]	78.0 ± 6.0
PCB 101 (2,2',4,5,5'-Pentachlorobiphenyl)[d,e,f,g,h]	90.8 ± 0.3
PCB 105 (2,3,3',4,4'-Pentachlorobiphenyl)[c,d,e,f,g,h]	50.3 ± 3.7
PCB 107 (2,3,3',4',5-Pentachlorobiphenyl)[c,d,e,f]	17.1 ± 1.2
PCB 110 (2,3,3',4',6-Pentachlorobiphenyl)[c,e,f]	94.6 ± 4.3
PCB 118 (2,3',4,4',5-Pentachlorobiphenyl)[c,d,e,f,g,h]	112 ± 6
PCB 128 (2,2',3,3',4,4'-Hexachlorobiphenyl)[c,d,e,f,g]	31.6 ± 2.1
PCB 132 (2,2',3,3',4,6'-Hexachlorobiphenyl)[c,d,f,h]	20.8 ± 2.1
PCB 138 (2,2',3,4,4',5'-Hexachlorobiphenyl)[c,e,f,g]	162.0 ± 6.9
PCB 146 (2,2',3,4',5,5'-Hexachlorobiphenyl[c,d,e,f,g]	40.5 ± 2.0
PCB 149 (2,2',3,4',5',6-Hexachlorobiphenyl)[c,d,e,f,g,h]	67.1 ± 3.7
PCB 153 (2,2',4,4',5,5'-Hexachlorobiphenyl)[c,d,e,f,h]	201 ± 3
PCB 156 (2,3,3',4,4',5-Hexachlorobiphenyl)[c,d,e,f,g,h]	13.3 ± 0.9
PCB 158 (2,3,3',4,4',6-Hexachlorobiphenyl)[c,d,e,f,g]	11.3 ± 0.9
PCB 170 (2,2',3,3',4,4',5-Heptachlorobiphenyl)[c,d,e,f,g]	29.2 ± 2.4
PCB 174 (2,2',3,3',4,5,6'-Heptachlorobiphenyl)[c,d,e,f,g]	18.6 ± 1.7
PCB 180 (2,2',3,4,4',5,5'-Heptachlorobiphenyl)[c,d,e,f,g,h]	80.8 ± 5.0
PCB 183 (2,2',3,4,4',5',6-Heptachlorobiphenyl)[c,d,e,f,g]	23.3 ± 1.9
PCB 187 (2,2',3,4',5,5',6-Heptachlorobiphenyl)[c,d,e,f,g,h]	54.8 ± 2.6
PCB 193 (2,3',3,4',5,5',6-Heptachlorobiphenyl)[c,d,e,f,g]	6.04 ± 0.23
PCB 194 (2,2',3,3',4,4',5,5'-Octachlorobiphenyl)[c,d,e,f,g,h]	13.2 ± 0.9
PCB 195 (2,2',3,3',4,4',5,6-Octachlorobiphenyl)[c,d,e,f,g]	4.95 ± 0.77
PCB 206 (2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl[c,d,e,f,g,h]	6.24 ± 0.88

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [16] and later revised by Schulte and Malisch [17] 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. PCB 107 is listed in this table.

[b] Unless otherwise noted, the certified values are the weighted mean of the results from four to six 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 [18] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [14,15]. The measurand is the total mass fractions for the selected PCB Congeners on a wet-mass basis listed in Table 2. Metrological traceability to the SI derived unit for mass fraction (expressed as micrograms per kilogram).

[c] GC-ECD (IIA) on a proprietary nonpolar phase after PFE with DCM.

[d] GC-ECD (IIB) on 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC-ECD (IIA).

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

[f] GC/MS (II) on a 50 % phenyl methylpolysiloxane phase after PFE with hexane/acetone mixture.

[g] Results from up to 28 laboratories participating in an interlaboratory comparison exercise.

[h] GC-ECD (I) on 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC/MS (I).

Table 3. Certified Mass Fractions (Wet-Mass Basis) for Selected Chlorinated Pesticides in SRM 1947

Pesticide	Mass Fraction[a] (µg/kg)
Hexachlorobenzene[b,c,d,e,f,g]	7.48 ± 0.66
α-HCH[b,c,d,g]	1.06 ± 0.12
Heptachlor epoxide[b,c,d,e,f,g]	13.4 ± 0.8
Oxychlordane[b,c,e,f,g]	23.6 ± 1.5
trans-Chlordane[b,c,d,e,f,g]	12.8 ± 1.2
cis-Nonachlor[b,c,d,e,f,g]	54.1 ± 7.3
trans-Nonachlor[b,c,d,e,f,g]	127 ± 6
Dieldrin[b,c,d,e,f,g]	80.8 ± 3.8
Mirex[b,c,e,f,g]	5.09 ± 0.73
2,4'-DDE[b,c,e,f,g]	3.39 ± 0.28
4,4'-DDE[c,d,e,f,g]	720 ± 43
2,4'-DDD[c,d,e,f]	3.31 ± 0.16
4,4'-DDD[b,c,d,e,f,g]	45.9 ± 3.6
2,4'-DDT[c,e,f,g]	15.7 ± 0.89
4,4'-DDT[b,c,d,e,f,g]	59.5 ± 6.7

[a] Unless otherwise noted, the certified values are the weighted mean of the results from four to six 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 [18] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [14,15]. The measurand is the total mass fractions for selected chlorinated pesticides on a wet-mass basis listed in Table 3. Metrological traceability to the SI derived unit for mass fraction (expressed as micrograms per kilogram).
[b] GC-ECD (I) on 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC/MS (I).
[c] GC-ECD (IIA) on a proprietary nonpolar phase after PFE with DCM.
[d] GC-ECD (IIB) on 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC-ECD (IIA).
[e] GC/MS (I) on a proprietary nonpolar phase after Soxhlet extraction with DCM.
[f] GC/MS (II) on a 50 % phenyl methylpolysiloxane phase after PFE with hexane/acetone mixture.
[g] Results from up to 28 laboratories participating in an interlaboratory comparison exercise.

Table 4. Certified Mass Fractions (Wet-Mass Basis) for Selected PBDE Congeners in SRM 1947

PBDE Congener[a]			Mass Fraction[b] (µg/kg)
BDE	47	(2,2',4,4'-Tetrabromodiphenylether)[c,d,e,f]	73.3 ± 2.9
BDE	49	(2,2',4,5'-Tetrabromodiphenylether)[c,d,e,f]	4.01 ± 0.10
BDE	66	(2,3',4,4'-Tetrabromodiphenylether)[c,d,e,f]	1.85 ± 0.13
BDE	99	(2,2',4,4',5-Pentabromodiphenylether)[c,d,e,f]	19.2 ± 0.8
BDE	100	(2,2',4,4',6-Pentabromodiphenylether)[c,d,e,f]	17.1 ± 0.6
BDE	153	(2,2',4,4',5,5'-Hexabromodiphenylether)[c,d,e,f]	3.83 ± 0.04
BDE	154	(2,2',4,4',5,6'-Hexabromodiphenylether)[d,e,f]	6.88 ± 0.52

[a] PBDE congeners are numbered according to IUPAC rules.
[b] The certified values are the weighted mean of the results from three or 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 [18] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [14,15]. The measurand is the total mass fractions for selected PBDE congeners on a wet-mass basis on Table 4. Metrological traceability is to the SI derived unit for mass fraction (expressed as micrograms per kilogram).
[c] GC/NCI-MS on a 15 m 5 % phenyl methylpolysiloxane phase.
[d] GC/EI-MS (I) on a 15 m 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC/NCI-MS.
[e] GC/EI-MS (II) on a 60 m 5 % phenyl methylpolysiloxane phase.
[f] GC/NCI-MS results reported by Zhu and Hites [13].

Table 5. Reference Mass Fractions (Wet-Mass Basis) for Selected PCB Congeners, PBDE Congeners, Pesticides, and PFOS in SRM 1947

PCB Congeners[a]			Mass Fraction (µg/kg)		
PCB	18	(2,2',5-Trichlorobiphenyl)[b,c,d,e,f,g]	2.72	±	0.95[h]
PCB	45	(2,2',3,6-Tetrachlorobiphenyl)[c,d,e]	1.76	±	0.76[i]
PCB	56	(2,3,3',4'-Tetrachlorobiphenyl)[e,f]	12.8	±	0.4[i]
PCB	70	(2,3',4',5-Tetrachlorobiphenyl)[b,c,e,f]	50	±	12[h]
PCB	82	(2,2',3,3',4-Pentachlorobiphenyl)[b,d,e]	3.87	±	0.67[h]
PCB	92	(2,2',3,5,5'-Pentachlorobiphenyl)[b,d,e,f]	32.6	±	5.2[h]
PCB	95	(2,2',3,5',6-Pentachlorobiphenyl)[d,e,f,g]	33.6	±	5.1[h]
PCB	151	(2,2',3,5,5',6-Hexachlorobiphenyl)[b,c,d,e]	23.3	±	5.3[h]
PCB	154	(2,2',4,4',5,6'-Hexachlorobiphenyl)[d,e]	3.51	±	0.46[i]
PCB	157	(2,3,3',4,4',5'-Hexachlorobiphenyl)[c,e,f]	4.08	±	0.77[i]
PCB	163	(2,3,3',4',5,6-Hexachlorobiphenyl)[c,e,f]	40.0	±	5.2[i]
PCB	201	(2,2',3,3',4,5,5',6'-Octachlorobiphenyl)[e,f]	3.59	±	0.43[i]
PCB	209	(Decachlorobiphenyl)[b,c,d,e,f,g]	2.45	±	0.68[h]
PBDE Congeners[j]					
BDE	28	(2,4,4'-Tribromodiphenylether)[k,l,m,n]	2.26	±	0.46[h]
	33	(2',3,4-Tribromodiphenylether)			
BDE	155	(2,2',4,4',6,6'-Hexabromodiphenylether)[k,m]	0.45	±	0.10[i]
Pesticides					
γ-HCH[b,c,d,g]			0.355	±	0.095[h]
cis-Chlordane (α-Chlordane)[b,e,f,g]			49.0	±	5.5[h]
Perfluorooctanesulfonic acid (PFOS)[o]			5.90	±	0.39[p]

[a] PCB congeners are numbered according to the scheme proposed by Ballschmiter and Zell [16] and later revised by Schulte and Malisch [16] 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. PCB 107 is listed in this table.

[b] GC-ECD (I) on 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC/MS (I).

[c] GC-ECD (IIA) on a proprietary nonpolar phase after PFE with DCM.

[d] GC-ECD (IIB) on 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC-ECD (IIA).

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

[f] GC/MS (II) on a 50 % phenyl methylpolysiloxane phase after PFE with hexane/acetone mixture.

[g] Results from up to 28 laboratories participating in an interlaboratory comparison exercise.

[h] The reference value is the weighted mean of the results from four to six 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 [18] incorporating inter-method bias with a pooled, within-method variance following the ISO/JCGM Guide [13,14]. The measurand is the total mass fractions on a wet-mass basis for the selected PCB congeners, PBDE congeners, and pesticides listed in Table 5 as determined by the methods indicted. Metrological traceability is to the SI derived unit for mass fraction (expressed as micrograms per kilogram).

[i] The reference value is the unweighted mean of the results from two to 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 [4] with a pooled, within-method variance following the ISO/JCGM Guide [14,15]. The measurand is the total mass fractions on a wet-mass basis for the selected PCB congeners, PBDE congeners, and pesticides listed in Table 5 as determined by the methods indicted. Metrological traceability is to the SI derived unit for mass fraction (expressed as micrograms per kilogram).

[j] BDE congeners are numbered according to IUPAC rules.

[k] GC/NCI-MS on a 15 m 5 % phenyl methylpolysiloxane phase.

[l] GC/EI-MS (I) on a 15 m 5 % phenyl methylpolysiloxane phase; same extracts analyzed as GC/NCI-MS.

[m] GC/EI-MS (II) on a 60 m 5 % phenyl methylpolysiloxane phase.

[n] GC/NCI-MS results reported by Zhu and Hites [14].

[o] LC/MS/MS results from two NIST methods and an interlaboratory study.

[p] The reference value is the weighted mean of the mass fractions from three analytical methods [19,20]. 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 [21] following the ISO/JCGM Guide [14,15]. The measurand is the total mass fractions on a wet-mass basis for PFOS listed in Table 5 as determined by the methods indicted. Metrological traceability is to the SI derived unit for mass fraction (expressed as micrograms per kilogram).

Table 6. Reference Mass Fractions (Wet-Mass Basis) for Proximates and Caloric Content in SRM 1947

Proximates	Mass Fraction[a] (%)
Solids[b]	27.1 ± 0.2
Ash	1.07 ± 0.07
Protein	17.0 ± 0.5
Fat (Extractable)	10.4 ± 0.5

Caloric Content	Mass Fraction[a] (kcal/100 g)
Calories[c]	152 ± 6

[a] Unless otherwise noted, the reference values are the weighted mean of the results provided by the laboratories in Appendix B. The uncertainty listed with the value is an expanded uncertainty about the mean with coverage factor, $k = 1.97$, determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence, and calculated to include the combined effect of between-laboratory and within-laboratory components of uncertainty, following the ISO/JCGM Guide [14,15]. The measurands are the total mass fractions for ash, protein, and fat on a wet-mass basis listed in Table 6 as determined by the methods indicted. Metrological traceability is to the SI derived unit of mass faction (expressed as a percent).

[b] The reference value for solids is the unweighted mean of the mean of the average of results provide 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, $k = 1.96$, determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence, and calculated by combining a between-method variance [4] with a pooled, within-method variance following the ISO/JCGM Guide [14,15]. The measurands are the total mass fractions for solids on a wet-mass basis listed in Table 6 as determined by the methods indicted. Metrological traceability is to the SI derived unit of mass faction (expressed as a percent).

[c] 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, protein, and carbohydrates, respectively, the mean caloric content is 165 kcal/100 g. The information mass fraction value for carbohydrates is listed in Table 9. The measurand is the total mass fraction for calories on a wet-mass basis in Table 6 as determined by the methods indicted. Metrological traceability is to the SI derived unit for energy (expressed as kilocalories per 100 g).

Table 7. Reference Mass Fractions (Wet-Mass Basis) for Fat and Selected Fatty Acids in SRM 1947

Fat	Mass Fraction[a] (%)		
Fat (Sum of Fatty Acids)[b]	8.50	±	0.54
Saturated Fat	1.75	±	0.22
Monosaturated Fat	3.55	±	0.22
Polyunsaturated Fat	2.84	±	0.44
Fatty Acid (as the triglyceride)			
Pentadecanoic Acid (C15:0)	0.025	±	0.004
Hexadecanoic Acid (C16:0) (Palmitic Acid)	1.14	±	0.08
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.024	±	0.007
Octadecanoic Acid (C18:0) (Stearic Acid)	0.230	±	0.019
(Z,Z)-9,12-Octadecadienoic Acid (C18:2) (Linoleic Acid)	0.375	±	0.014
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3) (Linolenic Acid)	0.287	±	0.026
(Z,Z,Z,Z,)-6,9,12,15-Octadecatetraenoic Acid (C18:4) (Stearidonic Acid)	0.120	±	0.025
(Z)-9-Eicosenoic Acid (C20:1) (Gadoleic Acid)	0.138	±	0.028
(Z,Z)-11,14-Eicosadienoic Acid (C20:2)	0.100	±	0.015
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4) (Arachidonic Acid)	0.247	±	0.035
(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic Acid (C20:5) (EPA)	0.395	±	0.034
(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic Acid (C22:5) (DPA)	0.320	±	0.017
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6) (DHA)	0.874	±	0.059

[a] The reference values are the weighted mean of the results provided by five to ten laboratories in Appendix B. The uncertainty listed with the value is an expanded uncertainty about the mean with coverage factor, $k = 1.97$, determined from the Student's *t*-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence, and calculated to include the combined effect of between-laboratory and within-laboratory components of uncertainty, following the ISO/JCGM Guide [14,15]. The measurand is the total mass fraction for fat and selected fatty acids on a wet-mass basis listed in Table 7 as determined by the methods indicted. Metrological traceability to the SI derived unit for mass fraction (expressed as a percent).

[b] Fat as the sum of the fatty acids represents the sum of individual fatty acid concentrations reported in Table 7.

Table 8. Reference Values for Hg Isotope Ratio Differences in SRM 1947

	Isotope Ratios[a,b,c] (‰)		
$\delta^{199}Hg$	5.62	±	0.25
$\delta^{200}Hg$	0.69	±	0.09
$\delta^{201}Hg$	5.09	±	0.18
$\delta^{202}Hg$	1.20	±	0.07
$\delta^{204}Hg$	1.66	±	0.07
	Mass-Independent Fractionation Isotope Ratios[a,b,c] (‰)		
$\Delta^{199}Hg$	5.31	±	0.29
$\Delta^{200}Hg$	0.09	±	0.02
$\Delta^{201}Hg$	4.17	±	0.28
$\Delta^{204}Hg$	-0.13	±	0.02

(a) All δ values are reported in permil, ‰ (part per thousand, equal to 0.001)

(b) Relative to the isotope ratios in NIST *SRM 3133 Mercury (Hg) Standard Solution (Lot No. 061204)*

(c) Isotopic reference values are non-certified values that are the best estimate of the true value; however, the values may not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1]. These recommended isotopic reference values were determined from a consensus estimate among five expert labs reporting nine methods using a weighted means statistical model [22]. The expanded uncertainty for isotopic values were determined using a Birge ratio expansion model and a coverage factor (k) of 2.571 to provide an expanded uncertainty interval that has approximately a 95 % probability of encompassing the consensus mean [22] based on uncertainty sources evaluated by Type A methods. Type B uncertainty and uncertainty in the bias of the methods is not included. The measurands are the Hg isotope ratios as determined by the methods used. Metrological traceability is to the delta scale to report the measured isotope ratios relative to the isotope ratios in *NIST SRM 3133 Mercury (Hg) Standard Solution (Lot No. 061204)* as described in the methods used.

Table 9. Information Mass Fractions (Wet-Mass Basis) for Selected Perfluoroalkyl Acids (PFAAs) and α-Hexabromocyclododecane (α-HBCD) in SRM 1947

PFAA	Mass Fraction[a] (µg/kg)
Perfluorononanoic acid (PFNA)[b]	0.20
Perfluorodecanoic acid (PFDA)[b]	0.26
Perfluoroundecanoic acid (PFUnA)[b]	0.28
Perfluorotridecanoic acid (PFTriA)[b]	0.20
α-Hexabromocyclododecane (α-HBCD)[c]	3.39

[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.
[b] LC/MS/MS results from two NIST methods and an interlaboratory study.
[c] LC/MS/MS results from three NIST methods.

Table 10. Information Mass Fraction (Wet-Mass Basis) for Carbohydrates in SRM 1947

	Mass Fraction[a] (%)
Carbohydrates	0.9

[a] The mass fraction value for carbohydrates is provided as an information value only; 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.

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Certificate Revision History: 31 May 2017 (Addition of reference values for mercury isotopes; editorial changes); **05 January 2016** (Editorial changes); **25 August 2014** (Removal of reference values, tetradecanoic acid (C14:0), (Z)-9-Hexadecenoic Acid (C16:1), and (Z)-9-octadecenoic acid (C18:1) in Table 7, due to recent analysis indicating bias; editorial changes); **19 September 2012** (Addition of minimum sample size, a reference value for perfluorooctanesulfonic acid, and an information value for α-hexabromocyclododecane were added; editorial changes); **30 August 2007** (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 1947.

Academy of Natural Sciences; Philadelphia, PA, USA
ALS Environmental; Vancouver, BC, Canada
Arthur D. Little, Inc.; Cambridge, MA, USA
Axys Analytical Services; Sidney, BC, Canada
B & B Laboratories; College Station, TX, USA
Battelle Columbus; Columbus, OH, USA
Battelle Ocean Sciences; Duxbury, MA, USA
Bedford Institute of Oceanography; Dartmouth, NS, Canada
Chesapeake Biological Laboratory; Solomons, MD, USA
City of Los Angeles, Environmental Monitoring Division; Playa del Rey, CA, USA
East Bay Municipal Utility District; Oakland, CA, USA
EnChem, Inc.; Madison, WI, USA
Environment Canada, Environmental Sciences Centre; Moncton, New Brunswick, Canada
King County Environmental Laboratory; Seattle, WA, USA
Manchester Environmental Laboratory; Port Orchard, WA, USA
Mississippi State Chemical Laboratory; Mississippi State, MS, USA
Murray State University; Murray, KY, 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
STL Knoxville; Knoxville, TN, USA
STL Sacramento; Sacramento, CA, USA
Texas A & M University, Geochemical and Environmental Research Group (GERG); College Station, TX, USA
U.S. Geological Survey, National Water Quality Laboratory; Denver, CO, USA
Woods Hole Group Environmental Laboratory; Raynham, MA, USA

APPENDIX B

The laboratories listed below performed measurements that contributed to the value assignment for proximates, caloric content, extractable fat, fatty acids, and trace elements in SRM 1947.

Campbell Soup; Camden, NJ, USA
Covance Laboratories; Madison, WI, USA
Eurofins; Memphis, TN, USA
General Mills, Inc.; Golden Valley, MN, USA
Hormel Foods Corporation; Austin, MN, USA
Kraft Foods Inc.; Hanover, NJ, USA
Kraft Foods Inc.; Glenview, IL, USA
Krueger Food Laboratories, Inc.; Cambridge, MA, USA
Nestlé USA; Dublin, OH, USA
Novartis Nutrition Technical Center; St. Louis Park, MN, USA

APPENDIX C

The laboratories listed below performed measurements that contributed to the value assignment for trace elements in SRM 1947.

NOAA/NMFS, Northwest Fisheries Science Center; Seattle, WA, USA
USDA Beltsville Human Nutrition Center; Beltsville, MD, USA
Australian Nuclear Science and Technology Organization; Sydney, Australia
Centre for Environment, Fisheries and Aquaculture Science; Burnham-on-Crouch, Essex, UK
Curtin University Center for Excellence in Mass Spectrometry; Perth, Australia
University of Connecticut Environmental Research Institute; Storrs, CT, USA
Geological Survey of Canada; Ottawa, Canada
Hill Laboratories; Hamilton, New Zealand
University of Alaska Fairbanks, Institute of Arctic Biology; Fairbanks, Alaska, USA
Karl-Franzens Universität Graz Institute of Chemistry; Graz, Austria
Midwest Research Institute (Florida Division); Palm Bay, FL, USA
Politechnika Poznanska; Poznań, Poland
PSC Analytical; Mississauga, Canada
R.J. Reynolds Tobacco Company; Winston-Salem, NC, USA
Research Triangle Institute; Chapel Hill, NC, USA
Savannah River Ecology Laboratory; Aiken, SC, USA
Texas A&M University Trace Element Research Laboratory; College Station, TX, USA
Texas A&M University Department of Veterinary Anatomy and Public Health; College Station, TX, USA
Ultra-Traces Analyses Aquitaine, Laboratoire de Chimie Analytique Bio-Inorganique et Environnement; University of Pau, Pau, France
University of Massachusetts Department of Chemistry; Amherst, MA, USA
University of Massachusetts University Research Institute for Analytical Chemistry; Amherst, MA, USA
Universidade Da Coruna; Coruna, Spain
University of Iowa Hygienic Laboratory; Des Moines, IA, USA
University of Maine; Orono, ME, USA
University of Nevada Las Vegas; Las Vegas, NV, USA

APPENDIX D

The laboratories listed below performed measurements that contributed to the value assignment for PFAAs in SRM 1947.

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 E

The laboratories listed below performed measurements that contributed to the value assignment of mercury isotopes in SRM 1947.

University of Michigan, Ann Arbor, Michigan, USA
University of Pau, Institut Pluridisciplinaire de Recherche sur l'Environnement et les Materiaux Equipe de Chimie Analytique BioInorganique et Environnement, Pau, France
Laboratoire Geosciences Environnement Toulouse (LMTG), Toulouse, France
Trent University, Petersborough, Canada
NIST, Hollings Marine Laboratory, Charleston, South Carolina, USA