National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1650a

Diesel Particulate Matter

This Standard Reference Material (SRM) is intended for use in evaluating analytical methods for the determination of selected polycyclic aromatic hydrocarbons (PAHs) and nitro-substituted PAHs in diesel particulate matter and similar matrices. SRM 1650a is the same diesel particulate material that was issued previously in 1985 as SRM 1650 [1]; this material has been rebottled and reanalyzed to provide updated certified values as well as certified, reference, and information values for additional constituents. In addition to certified and reference values for PAHs and nitro-substituted PAHs, reference or information values are provided for total extractable mass, particle-size distribution, specific surface area, and mutagenic activity. All of the chemical constituents for which certified, reference, and information values are provided in SRM 1650a are naturally present in the diesel particulate material. A unit of SRM 1650a consists of a bottle containing 100 mg of diesel particulate material.

SRM 1650a complements two other diesel particulate-related SRMs available from NIST: SRM 2975 Diesel Particulate Matter (Industrial Forklift) [2] and SRM 1975 Diesel Particulate Extract [3], which is a dichloromethane extract of the same material used to prepare SRM 2975.

Certified Concentration Values: Certified values for concentrations, expressed as mass fractions, for 16 PAHs and 1-nitropyrene are provided in Table 1. 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 accounted for by NIST. The certified values for the PAHs and 1-nitropyrene are based on the agreement of results obtained at NIST from two or more independent analytical techniques.

Reference Concentration Values: Reference values for concentrations, expressed as mass fractions, are provided for 25 additional PAHs (some in combination) in Table 2. Reference values for total extractable mass and the particle size distribution are provided in Tables 3 and 4, respectively. Reference values for mutagenicity in the Salmonella plate-incorporation assay are summarized in Table 5. Reference values are noncertified values that are the best estimate of the true value; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. Explanations in support of each reference value are given as notes in Tables 2 through 5.

Information Concentration Values: Information values for concentrations, expressed as mass fractions, are provided for additional compounds in Table 6 and for specific surface area, as determined by gas adsorption, in Table 7. An information value may be of use to the SRM user, but insufficient information is available to assess the uncertainty associated with the value.

Expiration of Certification: The certification of SRM 1650a is valid until **31 December 2007**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate (See Notice and Warning to Users). However, the certification is invalid if the SRM is damaged, contaminated, or modified.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by B.S. MacDonald.

Willie E. May, Chief
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Gaithersburg, MD 20899
Certificate Issue Date: 07 November 2000

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Standard Reference Materials Program

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

The coordination of the technical measurements leading to the certification of SRM 1650a was under the leadership of S.A. Wise of the NIST Analytical Chemistry Division.

Consultation on the statistical design of the experimental work and evaluation of the data were provided by M.G. Vangel of the NIST Statistical Engineering Division.

Analytical measurements for the certification of SRM 1650a were performed by L.R. Hilpert, M. Lopez de Alda, W.A. MacCrehan, W.E. May, D.L. Poster, L.C. Sander, M.M. Schantz, and L. Walton of the NIST Analytical Chemistry Division. Mutagenicity data were provided by J. Lewtas and L.D. Claxton of the National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency (EPA), Research Triangle Park, NC. Specific surface area and porosity measurements and confirmation measurements for 1-nitropyrene were provided by P. Scheepers of the Department of Epidemiology at Katholieke Universiteit Nijmegen, Nijmegen, The Netherlands. The particle size distribution data were provided by Honeywell, Inc., Clearwater, FL.

NOTICE AND WARNING TO USERS

Storage: SRM 1650a must be stored in its original bottle at temperatures less than 30 °C away from direct sunlight.

Handling: This material is a naturally occurring diesel particulate material and contains constituents of known and unknown toxicities and mutagenicities. Therefore, appropriate caution and care should be exercised during its handling and use.

Instructions for Use: Prior to removal of subsamples for analysis, the contents of the bottle should be mixed.

PREPARATION AND ANALYSIS[1]

Sample Collection and Preparation: The diesel particulate material used for the preparation of SRM 1650a was obtained in 1983 through the Coordinating Research Council, Inc., Atlanta, GA. The particulate material was collected from the heat exchangers of a dilution tube facility, following 200 engine hours of particulate accumulation. Several direct injection four-cycle diesel engines, operating under a variety of conditions were used to generate this particulate material. Therefore, while the sample is not intended to be representative of any particular diesel engine operating under any specific conditions, it should be typical of heavy-duty diesel engine particulate emissions of the early 1980s.

Polycyclic Aromatic Hydrocarbons (Tables 1 and 2): The general approach used for the value assignment of the PAHs in SRM 1650a was similar to that reported for the recent certification of several environmental matrix SRMs [4-7] and consisted of combining results from analyses using various combinations of different 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 toluene/methanol mixture, cleanup of the extracts using solid phase extraction (SPE), followed by analysis using the following techniques: (1) reversed-phase liquid chromatography with fluorescence detection (LC-FL) analysis of isomeric PAH fractions isolated by normal-phase LC (i.e., multidimensional LC) and (2) gas chromatography/mass spectrometry (GC/MS) analysis of the PAH fraction on three stationary phases of different selectivity, i.e., a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase, a 50 % phenyl-substituted methylpolysiloxane phase, and a smectic liquid crystalline stationary phase.

Seven sets of GC/MS results, designated as GC/MS (Ia and Ib), GC/MS (II), GC/MS (III), and GC/MS (IVa, IVb, and IVc), were obtained using three columns with different selectivities for the separation of PAHs. For GC/MS (Ia and Ib) analyses, 16 subsamples of 100 mg of SRM 1650a were extracted with toluene:methanol (1:1 by volume) using PFE (excess volume in the PFE cells was filled with clean sodium sulfate) [8]. The extracts were concentrated to about 0.5 mL and placed on an aminopropylsilane SPE cartridge and eluted with 20 mL of 2 %

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

DCM in hexane. The eluant of the 16 extracts was concentrated and then analyzed by GC/MS (Ia) using a 0.25 mm i.d. x 60 m fused silica capillary column with a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase (0.25 μm film thickness) (DB-5 MS, J&W Scientific, Folsom, CA). GC/MS (Ib), a subset of 8 of the 16 extracts from GC/MS (Ia), were analyzed on a 50 % (mole fraction) phenyl-substituted methylpolysiloxane stationary phase (0.25 mm i.d. x 60 m, 0.25 μm film thickness) (DB-17 MS, J&W Scientific, Folsom, CA). For the GC/MS (II) analyses, three 100 mg samples of SRM 1650a were extracted with DCM using PFE; the extracts were processed and analyzed as described above for GC/MS (Ia). GC/MS (III) was identical to GC/MS (II) except that Soxhlet extraction with DCM for 18 hours was used instead of PFE on another set of three samples. For the GC/MS (IV) analyses, six subsamples of 40 mg to 100 mg of SRM 1650a were extracted with DCM using PFE and the extracts processed as for GC/MS (Ia and Ib). The processed extracts were then analyzed by GC/MS using three different columns: 5 % phenyl methylpolysiloxane, GC/MS (IVa); 50 % phenyl methylpolysiloxane, GC/MS (IVb); and a 0.2 mm i.d. x 25 m (0.15 μm film thickness) smectic liquid crystalline phase (SB-Smectic, Dionex, Lee Scientific Division, Salt Lake City, UT), GC/MS (IVc).

For the LC-FL results, six subsamples of approximately 200 mg of SRM 1650a were processed by Soxhlet extraction for 20 hours using 200 mL of DCM. The extracts were concentrated and processed through aminopropylsilane SPE cartridges as described above for the GC/MS analyses. The processed extract was further fractionated using normal-phase LC on a semi-preparative aminopropylsilane column (μBondapak NH_2, 9 mm i.d. x 30 cm, Waters Associates, Milford, MA) to isolate isomeric PAH fractions as described previously [9-11]. Four fractions were collected containing PAHs of specific molecular weights; 178 and 202 (fraction 1), 228 (fraction 2), 252 and 276 (fraction 3), and 278 (fraction 4). All PAH fractions were analyzed using a 5 μm particle-size polymeric octadecylsilane (C_{18}) column (4.6 mm i.d. x 25 cm, Hypersil-PAH, Keystone Scientific, Inc., Bellefonte, PA) with wavelength programmed fluorescence detection [10-12]. For all of the GC/MS and LC-FL measurements described above, selected perdeuterated PAHs were added to the diesel particulate samples prior to solvent extraction for use as internal standards for quantification purposes.

Homogeneity Assessment for PAHs: The homogeneity of SRM 1650a was assessed by analyzing duplicate samples of 100 mg from eight bottles selected by stratified random sampling. Samples were processed and analyzed as described above for GC/MS (Ia). Statistically significant differences among bottles were observed for the PAHs at the 100 mg sample size, and this source of variability has been incorporated in the calculation of the uncertainty associated with the assigned values.

Nitro-Substituted PAHs (Tables 1 and 6): The concentrations of the nitro-PAHs were value assigned based on results using LC with electrochemical and fluorescence detection and GC/MS with both electron impact and chemical ionization detection. For the LC measurements, two different LC methods were used as described in detail by MacCrehan, et al. [13] after Soxhlet extraction with DCM for 24 hours. The first method included isolation of the nitro-PAH fraction from the extract using normal-phase LC on an aminopropylsilane phase followed by reversed-phase LC separation on a 5 μm monomeric C_{18} column with wavelength programmed fluorescence detection after post-column, on-line conversion of the nitro-PAHs to the corresponding amine. The second LC method, which was used only for the measurement of 1-nitropyrene, consisted of cleanup of the extract on a SPE cartridge followed by reversed-phase LC with amperometic detection. For determination of 1-nitropyrene using GC/MS, the particulate matter was processed by Soxhlet extraction with a 1:1 (volume:volume) mixture of toluene:methanol. The extract was passed through a Florisil SPE cartridge with DCM as the eluent. The sample was then analyzed by GC/MS using a 0.25 mm i.d. x 30 m fused silica capillary column with a 0.25 μm film thickness of 5 % phenyl-substituted methylpolysiloxane phase. The mass spectrometer was operated in the electron impact mode or negative chemical ionization mode with methane as the reagent gas. For both the LC and GC/MS measurements, 1-nitropyrene-d_9 was added prior to solvent extraction for use as an internal standard.

Percent Extractable Mass (Table 3): For the determination of percent extractable mass, six subsamples of approximately 200 mg of SRM 1650a were processed using Soxhlet extraction for 18 hours with DCM. The extract was concentrated to approximately 20 mL and then filtered to remove particulate matter. Aliquots of 100 μL to 150 μL were placed in tared aluminum foil pans; the DCM was evaporated until constant mass to obtain the mass of the residue.

Particle-Size Information (Table 4): Dry particle-size distribution measurements for SRM 1650a were obtained as part of a collaborative effort with Honeywell, Inc. A Microtrac® particle analyzer, which makes use of light-scattering techniques, was used to measure the particle size distribution of SRM 1650a. Briefly, a reference beam is used to penetrate a field of particles and the light that scatters in the forward direction from the field is measured,

and the particle-size as a volume distribution is derived via a computer-assisted analysis. From these data, the total volume, average size, and a characteristic width of the particle-size distribution are calculated. The system has a working range from 0.7 μm to 700 μm.

Mutagenicity Assay (Table 5): The reference values for the mutagenic activity of a dichloromethane extract of SRM 1650a were determined as part of an international collaborative study in 1989 sponsored by the International Programme on Chemical Safety (IPCS). The IPCS is jointly sponsored by the World Health Organization (WHO), the United Nations Environmental Programme (UNEP), and the International Labor Organization (ILO). The program was initiated, supported, and technically coordinated by the US EPA Office of Health Research. Twenty laboratories from North America, Europe, and Japan participated in the study for which a complete summary is available [14,15]. As part of the protocol, each laboratory used DCM to extract the organic material from SRM 1650a. Half of the laboratories used Soxhlet extraction and the other half used ultrasonic extraction procedures. The extracted material was analyzed using the Salmonella/mammalian microsomal plate-incorporation assay using strains TA98 and TA100 [16]. The mean DCM extractable mass determined in the IPCS collaborative study was 17.5 % ± 1.5 %, and in all 20 laboratories, the extract was found to be mutagenic in both strains with and without activation.

Bioassay reference values are provided in Table 5. Each reference value is the best estimate of the mutagenic activity, from the data available, for a DCM extract of SRM 1650a using the protocol specified for the IPCS collaborative study. For the reference values to apply, the sample should be Soxhlet or ultrasonically extracted with DCM. The DCM extract should be evaporated to near dryness and solvent exchanged into dimethylsulfoxide. The bioassay procedure should follow the *Salmonella typhimurium* plate incorporation protocol as described by Marion and Ames [16] and adhere to the guidelines published by Claxton et al. [17]. Minimal media plates should be made of Difco agar and should contain 30 mL ± 1 mL of base layer agar. The exogenous activation system (S9) should be an Aroclor-1254 induced rat liver homogenate as described by Marion and Ames [16]. Duplicate plates should be used for each of the three to five dose levels.

The uncertainty for each mutagenic activity is expressed in two ways. The first uncertainty in the mutagenic activity listed, expressed as the 95 % confidence limits about mean potency value, takes into account both between and within laboratory sources of variation. While these confidence limits represent the uncertainty for the best estimate of the mutagenic activity of SRM 1650a, they do not reflect the variation in the values reported by individual participating laboratories. They should also not be taken to represent the range of mutagenic activity values from other laboratories using the protocol of Marion and Ames [16] with some additional constraints [18]. The second uncertainty, expressed as 80 % tolerance limits, sometimes called prediction limits or control limits [19], are provided to characterize differences in the mutagenic activity reported by the 20 laboratories that participated in the IPCS interlaboratory study and to establish a target range for other laboratories that analyze SRM 1650a using the modified Marion and Ames protocol. Additionally, for the SRM user's values to be assessed using the tolerance limits given, data should be treated using the same or very similar statistical methods as those used in this study [20,21].

The 80 % Tolerance Limit is the range within which 80 % of the mutagenic activity values reported in the interlaboratory study are expected to reside. These limits may be used by all laboratories using the IPCS Salmonella bioassay protocol to determine if their findings are consistent with those reported by the 20 laboratories that participated in the IPCS collaborative study. Although these laboratories may not be representative of all laboratories that conduct the Salmonella bioassay, the tolerance limits given do provide a range of values that all laboratories following the IPCS protocol should strive to obtain. The first set of tolerance limits given are for laboratories that use the same number of replicate extractions and bioassays as were performed in the IPCS collaborative study. The second set of tolerance limits, which are slightly wider, apply to the case where only a single extraction and bioassay are performed.

A personal computer program developed by the US EPA entitled "GeneTox Manager," contains the statistical analysis software developed by several research groups for the Salmonella assay including the program described by Krewski et al. [20,21]. The GeneTox Manager has been described [22] and is available from the US EPA by a written request to: Dr. Larry D. Claxton, Environmental Carcinogenesis Division, MD-68, US Environmental Protection Agency, Research Triangle Park, NC 27711.

Specific Surface Area and Porosity (Table 7): The specific surface area and porosity were determined based on N_2 gas adsorption measurements [23]. The gas adsorption measurements were performed on a NOVA-1200 instrument (Quantachrome Corp., Boynton Beach, FL) at 77 K after the samples were outgassed for 24 hours at 120 °C under vacuum. The N_2 isotherms were analyzed using the Brunauer-Emmet-Teller (BET) equation [24] to obtain the surface area (Table 7) and the Barrett-Joyner-Halenda (BJH) method [25] to obtain the porosity. Based on the BJH method, SRM 1650a shows a wide distribution of mesopores, but with substantial outer area. The pore diameter of the particles in SRM 1650a range from 4 nm to 45 nm with the greater number of particles at about 25 nm.

Table 1. Certified Concentrations for Selected PAHs in SRM 1650a

	Mass Fractions (mg/kg)[a]
Phenanthrene[b,c,d,e,f,g]	68.4 ± 8.5
Fluoranthene[b,c,d,e,f,g]	49.9 ± 2.7
Pyrene[b,c,d,e,f,g]	47.5 ± 2.7
Benz[*a*]anthracene[b,c,d,e,f,g,h]	6.33 ± 0.77
Chrysene[b,h]	14.5 ± 0.8
Triphenylene[b,h]	11.5 ± 1.6
Benzo[*a*]fluoranthene[c,f,g,h]	0.437 ± 0.075
Benzo[*b*]fluoranthene[b,g,h,i]	8.81 ± 0.60
Benzo[*j*]fluoranthene[g,h,i]	3.52 ± 0.40
Benzo[*k*]fluoranthene[b,d,e,f,g,h,i]	2.64 ± 0.31
Benzo[*a*]pyrene[b,c,d,e,f,g,h]	1.33 ± 0.35
Benzo[*e*]pyrene[c,d,e,f,g,h]	7.44 ± 0.53
Benzo[*ghi*]perylene[c,d,f,g]	6.50 ± 0.94
Indeno[1,2,3-*cd*]pyrene[c,d,f,g]	5.62 ± 0.53
Benzo[*b*]chrysene[d,f,g,i]	0.316 ± 0.038
Picene[d,f,g,i]	0.620 ± 0.081
1-Nitropyrene[j]	19 ± 2

[a] Each set of results is expressed as the certified value ± the expanded uncertainty. Each certified value is a mean of the means from two or more analytical methods. For results from two methods, the certified value is the equally weighted mean; for results from three or more methods, the certified value is the mean weighted as described in Paule and Mandel [26]. Each uncertainty, computed according to the CIPM approach as described in the ISO Guide [27], is an expanded uncertainty at the 95 % level of confidence, which includes random sources of uncertainty within each analytical method and among methods, as well as uncertainty due to the variation among the bottles. The expanded uncertainty defines a range of values within which the true value is believed to lie at a level of confidence of approximately 95 %.

[b] LC-FL of PAH fractions isolated by normal-phase LC after Soxhlet extraction with DCM.

[c] GC/MS (Ia) on 5 % phenyl-substituted methylpolysiloxane phase after PFE with toluene:methanol mixture.

[d] GC/MS (II) on 5 % phenyl-substituted methylpolysiloxane phase after PFE with DCM.

[e] GC/MS (III) on 5 % phenyl-substituted methylpolysiloxane phase after Soxhlet extraction with DCM.

[f] GC/MS (IVa) on 5 % phenyl-substituted methylpolysiloxane phase after PFE with DCM.

[g] GC/MS (IVb) on 50 % phenyl-substituted methylpolysiloxane phase of same extract as GC/MS (IVa).

[h] GC/MS (IVc) on a smectic liquid crystalline phase of same extract as GC/MS (IVa).

[i] GC/MS (Ib) on 50 % phenyl-substituted methylpolysiloxane phase of same extracts as GC/MS (Ia).

[j] The certified value and its uncertainty for 1-nitropyrene are as stated in the Certificate of Analysis for SRM 1650 and have not been revised for SRM 1650a; however, the value has been reconfirmed by recent GC/MS analyses. The certified value is based on results obtained by two independent techniques. The uncertainty is two times the standard deviation for the mean value determined using the procedures described by Paule and Mandel [26].

The concentrations for selected PAHs in Table 2 are provided as reference values because either the results have not been confirmed by an independent analytical technique as required for certification or the agreement among results from multiple methods was insufficient for certification. Although bias has not been evaluated for the procedures used, the reference values should be useful for comparison with results obtained using similar procedures.

Table 2. Reference Concentrations for Selected PAHs in SRM 1650a

	Mass Fractions (mg/kg)[a]
Anthracene[b,c,d,e,f,g]	1.5 ± 0.6[h]
1-Methylphenanthrene[c,d,e,f,g]	34 ± 7
2-Methylphenanthrene[c,d,e,f,g]	70 ± 4
3-Methylphenanthrene[c,d,e,f,g]	57 ± 8
4- and 9-Methylphenanthrene[c,d,e,f,g]	33 ± 9
1,2-Dimethylphenanthrene[f,g]	6.3 ± 0.5
1,6-, 2,5-, and 2,9-Dimethylphenanthrene[f]	38 ± 3
1,7-Dimethylphenathrene[f]	16.6 ± 0.7
1,8-Dimethylphenanthrene[f,g]	4.5 ± 0.5
2,6-Dimethylphenanthrene[f,g]	29 ± 2
2,7-Dimethylphenanthrene[f,g]	20 ± 2
3,6-Dimethylphenanthrene[f,g]	23 ± 2
1-, 3-, and 7-Methylfluoranthene[f,g]	12.8 ± 0.6
8-Methylfluoranthene[f,g]	4.9 ± 0.3
1-Methylpyrene[f,g]	3.2 ± 0.2
2-Methylpyrene[f,g]	9.2 ± 0.7
4-Methylpyrene[f,g]	7.3 ± 0.4
Benzo[*ghi*]fluoranthene[i]	12.1 ± 0.3
Benzo[*c*]phenathrene[c,d,e,f,g,i]	2.8 ± 0.6
Perylene[b,c,f,g]	0.16 ± 0.04
Dibenz[*a,c*]anthracene[j]	0.50 ± 0.06
Dibenz[*a,h*]anthracene[g,j]	0.9 ± 0.2
Dibenz[*a,j*]anthracene[f,j]	0.5 ± 0.1
Pentaphene[d,f,g,j]	0.2 ± 0.1
Coronene[f]	2.0 ± 0.1

[a] Each set of results is expressed as the reference value ± the expanded uncertainty. Each reference value is the mean from one analytical method or a mean of the means from two or more analytical methods. For results from two methods, the reference value is the equally weighted mean; for results from three or more methods, the reference value is the mean weighted as described in Paule and Mandel [26]. Each uncertainty, computed according to the CIPM approach as described in the ISO Guide [27], is an expanded uncertainty at the 95 % level of confidence, which includes random sources of uncertainty within each analytical method and among methods, as well as uncertainty due to the variation among the bottles. The expanded uncertainty defines a range of values that contain the best estimate of the true value at a level of confidence of approximately 95 %.

[b] LC-FL of PAH fractions isolated by normal-phase LC after Soxhlet extraction with DCM.

[c] GC/MS (Ia) on 5 % phenyl-substituted methylpolysiloxane phase after PFE with toluene:methanol mixture.

[d] GC/MS (II) on 5 % phenyl-substituted methylpolysiloxane phase after PFE with DCM.

[e] GC/MS (III) on 5 % phenyl-substituted methylpolysiloxane phase after Soxhlet extraction with DCM.

[f] GC/MS (IVa) on 5 % phenyl-substituted methylpolysiloxane phase after PFE with DCM.

[g] GC/MS (IVb) on 50 % phenyl-substituted methylpolysiloxane phase of same extract as GC/MS (IVa).

[h] The reference value for anthracene is valid only for Soxhlet extraction or PFE at 100 °C and 2000 psi. A higher concentration of anthracene has been observed using PFE at 200 °C.

[i] GC/MS (IVc) on a smectic liquid crystalline phase of same extract as GC/MS (IVa).

[j] GC/MS (Ib) on 50 % phenyl-substituted methylpolysiloxane phase of same extracts as GC/MS (Ia).

The total extractable mass in Table 3 is provided as a reference value because the result is method specific as defined by the corresponding procedures described in the Preparation and Analysis section. Although bias has not been evaluated for the procedure used, the reference value should be useful for comparison with results obtained using similar procedures.

Table 3. Reference Value for Total Extractable Mass for SRM 1650a

	Mass Fraction[b]
Total Extractable Mass[a]	20.2 % ± 0.4 %

[a] Extractable mass as determined from Soxhlet extraction using DCM.

[b] This set of results is expressed as the reference value ± the expanded uncertainty. The reference value for the total extractable mass is the mean value of six measurements. The uncertainty, computed according to the CIPM approach as described in the ISO Guide [27], is an expanded uncertainty at the 95 % level of confidence. The expanded uncertainty defines a range of values for the reference value within which the true value is believed to lie, at a level of confidence of 95 %.

Particle size results in Table 4 are provided as reference values because the results are method specific as defined by the corresponding procedures described in the Preparation and Analysis section. Although bias has not been evaluated for the procedure used, the reference values should be useful for comparison with results obtained using similar procedures.

Table 4. Reference Values for Particle-Size Characteristics for SRM 1650a

Particle Measurement	Value[a]
Mean diameter (volume distribution, MV, μm)[b]	25.7 ± 0.6
Mean diameter (area distribution, μm)[c]	10.7 ± 0.1
Mean diameter (number distribution, μm)[d]	1.55 ± 0.04
Surface Area (m^2/cm^3)[e]	0.562 ± 0.007

The following data show the percent of the volume that is smaller than the indicated size:

Percentile	Particle Diameter (μm)[a]
95	81 ± 4
90	53 ± 2
80	35.0 ± 0.9
70	26.4 ± 0.4
60	21.0 ± 0.2
50[f]	17.1 ± 0.1
40	14.0 ± 0.1
30	11.2 ± 0.1
20	8.6 ± 0.1
10	5.6 ± 0.1

[a] The reference value is the mean value of measurements from the analysis of subsamples from four bottles. Each uncertainty, computed according to the CIPM approach as described in the ISO Guide [27], is an expanded uncertainty at the 95 % level of confidence. The expanded uncertainty defines a range of values that contains the best estimate of the true value at a level of confidence of approximately 95 %.

[b] The mean diameter of the volume distribution represents the center of gravity of the distribution and compensates for scattering efficiency and refractive index. This parameter is strongly influenced by coarse particles.

[c] The mean diameter of the area distribution, calculated from the volume distribution with less influence from the presence of coarse particles than the MV parameter.

[d] The mean diameter of the number distribution, calculated from the volume distribution.

[e] Calculated specific surface area assuming solid, spherical particles. This is a computation and should not be interchanged with an adsorption method of surface area determination (see Table 7) as this value does not reflect porosity or topographical characteristics.

[f] Median diameter (50 % of the volume is less than 17.1 μm).

Mutagenic activity results in Table 5 are provided as reference values because the results are method specific as defined by the corresponding procedures described in the Preparation and Analysis section. Although bias has not been evaluated for the procedure used, the reference values should be useful for comparison with results obtained using similar procedures.

Table 5. Reference Values for Ames Bioassay Mutagenic Activity of SRM 1650a[a]

Strain/Activation	Mutagenic Activity[b]	95 % Confidence Limits[c]	80 % Tolerance Limits	
			Multiple Extraction/ Bioassay[d]	Single Extraction/ Bioassay[e]
TA100, +S9	4585 rev/mg	2854 to 7365	1208 to 17402	1177 to 17858
TA100, -S9	3766 rev/mg	2736 to 5182	1516 to 9351	1460 to 9711
TA98, +S9	2265 rev/mg	1484 to 3456	679 to 7550	668 to 7675
TA98, -S9	2794 rev/mg	2066 to 3780	1183 to 6599	1166 to 6698

[a] Results summarized from Claxton et al. [18]. Reference values refer to the mutagenic activity of the dichloromethane extract of SRM 1650a per unit mass of the particulate material extracted. Doses for the IPCS collaborative study were based on the following mg equivalents of SRM 1650a particles: TA100, +/-S9 (0.025, 0.05, 0.100, 0.150, 0.200); TA98, +/-S9 (0.0625, 0.125, 0.250, 0.400, 0.500). Total extractable mass for SRM 1650a with DCM from the IPCS collaborative study in 1988 was 17.5 % ± 1.5 % (mass fraction) as compared with 20.2 % ± 0.4 % as reported in Table 3. The value reported in Table 3 was determined in 1998 using improvements in the Soxlet extraction procedure which may account for the increase in extractable mass.

[b] Geometric mean of all replicate mutagenicity potency values reported by participating laboratories after deleting outlying observations. Results reported as revertants per mg (rev/mg) of SRM 1650a.

[c] Calculated on a logarithmic scale taking into account both inter- and intra-laboratory variation, excluding outliers, and then re-expressed in the original scale by taking antilogs.

[d] Tolerance limits for mutagenic activity determined in a single laboratory using the same number of replicate extracts/bioassays as in the IPCS collaborative trial.

[e] Tolerance limits for mutagenic activity determined in a single laboratory using only one replicate extraction/bioassay.

Table 6. Information Values for Concentrations of Selected Nitro-substituted PAHs and a PAH Quinone in SRM 1650a

	Mass Fraction (mg/kg)
2-Nitrofluorene	0.27
6-Nitrobenzo[*a*]pyrene	1.6
7-Nitrobenz*[a]*anthracene	2.8
9-Fluorenone	33

Table 7. Information Values for Specific Surface Area of SRM 1650a as Determined by Gas Adsorption

Specific Surface Area (S)[a]	108 m^2/g

[a] Specific surface area determined by multi-point N_2 adsorption BET method (see Specific Surface Area and Porosity).

REFERENCES

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