DEPARTMENT OF COMMERCE • UNITED STATES OF AMERICA

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1975

Diesel Particulate Extract

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 extracts and similar matrices. In addition to certified and non-certified values for selected PAHs and nitro-substituted PAHs, non-certified values are provided for extract residue mass, and mutagenic activity. All of the chemical constituents for which certified and non-certified values are provided in SRM 1975 were naturally present in the particulate material before extraction. A unit of SRM 1975 consists of four ampoules, each containing approximately 1.2 mL of a dichloromethane extract of diesel particulate matter collected from an industrial diesel-powered forklift.

Diesel particulate matter from the same lot of material that was used to prepare SRM 1975 is also available as SRM 2975 Diesel Particulate Matter (Industrial Forklift) [1].

Certified Mass Fraction Values: Certified values for mass fractions are provided for eight PAHs in Table 1. The certified values for the PAHs are based on the agreement of results obtained at NIST from two or more independent analytical techniques [2]. 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 [2]. Metrological traceability is to the International System of Units (SI) unit of mass expressed as derived unit of mass fraction.

Non-Certified Values: Non-certified values for mass fractions are provided for 23 additional PAHs (some in combination) in Table 2. Non-certified values for mass fractions of 19 nitro-substituted PAHs are provided in Table 3. A non-certified value for the extract residue mass is provided in Table 4. Non-certified values for mutagenicity in the Salmonella plate-incorporation assay are summarized in Table 5. Non-certified values represent best estimates of the true value; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [2]. Non-certified values are traceable to the measurement processes and standards used by NIST.

Period of Validity: **SRM 1975** is valid, with the measurement uncertainty specified, until **01 May 2031**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Use"). The value assignments are nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its validity. 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.

The technical measurements leading to the certification of SRM 1975 was conducted by B. Benner of the NIST Chemical Sciences Division, and M. Schantz, formerly of NIST.

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Consultation on the statistical design of the experimental work and evaluation of the data were provided by S.D. Leigh and M.G. Vangel, formerly of NIST.

The diesel particulate material was provided by M.E. Wright of the Donaldson Company, Inc., Minneapolis, MN. The extract was prepared by M.J. Hays, formerly of NIST.

Analytical measurements for the certification of SRM 1975 were performed at NIST by D.L. Poster and L.C. Sander, and of the NIST Chemical Sciences Division and H.M. Bamford, D. Bezabeh, R. Deisenhofer, M. Lopez de Alda, M.M. Schantz, and P. Schubert, formerly of NIST. Additional analytical measurements for the nitro-substituted PAHs were provided by M. Nishioka of Battelle, Columbus, OH and C. Chiu of the Analysis and Methods Division, Environment Technology Centre, Environment Canada, Ottawa, Canada. Mutagenicity data were provided by T.J. Hughes, J. Lewtas, and L.D. Claxton of the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC. The measurements of the extractable residue mass were provided by L. Gratz of Michigan Technological University, Houghton, MI.

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

INSTRUCTION FOR USE

Samples of the SRM for analysis should be withdrawn from ampoules and used immediately. The certified values are not valid for materials in ampoules that have been stored after opening, even if resealed.

NOTICE AND WARNING TO USERS

Storage: SRM 1975 must be stored in its original ampoule at approximately 25 °C (room temperature) and away from direct sunlight.

Handling: This material is an extract of 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.

PREPARATION AND ANALYSIS(1)

Sample Collection and Preparation: The diesel particulate material was obtained from M.E. Wright of the Donaldson Company, Inc., Minneapolis, MN. The material was collected from a filtering system designed specifically for diesel-powered forklifts [3]. This diesel particulate material was selected based on recommendation by J. Lewtas, U.S. Environmental Protection Agency, Research Triangle Park, NC. The diesel particulate material was received at NIST in a 55-gal drum. The material was removed from the drum and homogenized in a V-blender for 1 h and then stored in polyethylene bags. A total of 13.7 kg of diesel particulate material was homogenized; a total of 5.65 kg of material was extracted for preparation of SRM 1975 and the remaining diesel particulate material was bottled for distribution as SRM 2975 [1]. Subsamples of approximately 38 g of diesel particulate material were extracted for 24 h with 800 mL of dichloromethane (DCM). Six subsamples were extracted per day for 25 days for a total of 5.6 kg of diesel particulate matter extracted. The extracts from each day were combined and concentrated by evaporation under nitrogen. During the 25 days of extracting the diesel particulate material, the concentrated extract was stored at 40 °C. The final combined extract solution was concentrated to approximately 8 L; this solution was then ampouled with 1.2 mL of extract per ampoule.

Polycyclic Aromatic Hydrocarbons (Tables 1 and 2): The general approach used for the value assignment of the PAHs in SRM 1975 was similar to that reported for the recent certification of several environmental matrix SRMs [4–7] and consisted of combining results from analyses using different chromatographic separation and detection techniques. This approach consisted of cleanup of the extracts using different solid phase extraction (SPE) or normal-phase liquid chromatography (LC), followed by analysis using the following techniques: (1) reversed-phase liquid chromatography with fluorescence detection (LC-FL) analysis of the total PAH fraction, (2) reversed-phase LC-FL analysis of isomeric PAH fractions isolated by normal-phase LC (i.e., multidimensional LC), (3) gas chromatography/mass spectrometry (GC/MS) analysis of the PAH fraction on two stationary phases of

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(1) Certain commercial equipment, instrumentation, 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.

different selectivity, i.e., a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase and a smectic liquid crystalline stationary phase. The analysis of SRM 1975 has been described in more detail [6].

Two sets of GC/MS results, designated as GC/MS (I) and GC/MS (Sm), were obtained using two columns with different selectivities for the separation of PAHs. For GC/MS (I) analyses, duplicate subsamples approximately 0.8 g each from nine ampoules of SRM 1975 were analyzed. An internal standard solution (SRM 2269 and SRM 2270) of perdeuterated PAHs was added to the extract subsample, and then the extract was passed through a silica SPE cartridge and eluted with 2 % DCM in hexane. The processed extract was then analyzed by GC/MS using a 0.25 mm i.d. x 30 m fused silica capillary column with a 5 % phenyl-substituted methylpolysiloxane phase (0.25 µm film thickness) (DB-5 MS, Agilent Technologies, Wilmington, DE). For GC/MS (Sm) analyses, 0.8 g subsamples from six ampoules of SRM 1975 were prepared as described above for GC/MS (I) using an aminopropylsilane SPE cartridge. The processed extract was then analyzed by GC/MS using 0.2 mm i.d. × 25 m (0.15 µm film thickness) smectic liquid crystalline phase (SB-Smectic, Dionex, Lee Scientific Division, Salt Lake City, UT).

Two sets of LC-FL results, designated as LC-FL (Total) and LC-FL (Fraction), were used in the certification process. Subsamples of approximately 0.8 g from each of six ampoules of SRM 1975 were spiked with the perdeuterated internal standards (SRM 2269 and SRM 2270) and processed through two aminopropylsilane SPE cartridges connected in series to obtain the total PAH fraction. A second 0.8 g subsample from each of six ampoules was processed as described above and the total PAH fraction was then fractionated further on a semipreparative aminopropylsilane column (µBondapak NH_2, 9 mm i.d. x 30 cm, Waters Associates, Milford, MA) to isolate a four to six aromatic ring PAH fraction as described previously [8–10]. The total PAH fraction and the four to six aromatic ring PAH fractions were analyzed using a 5-µm particle-size polymeric octadecylsilane (C_{18}) column (4.6 mm i.d. × 25 cm, Hypersil-PAH, Keystone Scientific, Inc., Bellefonte, PA) with wavelength programmed fluorescence detection [9,10].

Homogeneity Assessment for PAHs: The homogeneity of SRM 1975 was assessed by analyzing duplicate samples of 0.8 g each from nine bottles selected by stratified random sampling. Samples were processed and analyzed as described above for GC/MS (I). No statistically significant differences among ampoules were observed for the PAHs at the 0.8 g sample amount.

Nitro-Substituted PAHs (Table 3): SRM 1975 was analyzed at NIST and two other laboratories for the determination of nitro-substituted PAHs. At NIST, three sets of three, five, and four samples of SRM 1975 (~0.8 g each) were spiked with the following perdeuterated nitro-PAHs for use as internal standards: 9-nitroanthracence-d_9, 3-nitrofluoranthene-d_9, 1-nitropyrene-d_9, and 6-nitrochrysene-d_{11}. Each sample was divided into two subsamples which were then processed through an aminopropylsilane SPE cartridge using 40 mL of 20 % DCM in hexane. The concentrated eluant was then subjected to normal-phase LC using a semi-preparative amino/cyano phase column with a mobile phase of 20 % DCM in hexane to isolate the nitro-PAH fraction. The nitro-PAH fraction was analyzed by GC with negative chemical ionization mass spectrometry (GC/NCI-MS) using a 0.25 mm i.d. × 30 m fused silica capillary column containing a 5 % diphenyl-substituted dimethylsiloxane phase, 0.25 µm film thickness or a 50 % phenyl-substituted methylpolysiloxane phase, 0.25 µm film thickness.

Additional nitro-PAH measurements were provided by Battelle, Columbus, OH. For the Battelle measurements, five samples were prepared at each of two concentrations of extractable mass (0.2 and 2.0 mg/mL). Each sample was spiked with the following perdeuterated nitro-PAHs: 1-nitronaphthalene-d_7, 3-nitrobiphenyl-d_5, 4-nitrobiphenyl-d_5, 9-nitroanthracene-d_9, and 1-nitropyrene-d_9. Each sample was analyzed by GC/NCI-MS on a 60 m 5 % phenyl-substituted methylpolysiloxane column (32 mm i.d., 0.25 µm film thickness).

Extract Residue Mass (Table 4): The concentration of the extract residue mass was determined from triplicate measurements from each of six ampoules of SRM 1975. A 50 µL subsample of SRM 1975 was allowed to evaporate to dryness; after reaching constant mass, the residue mass was determined.

Mutagenicity Assay (Table 5): The values for the mutagenic activity of SRM 1975 were determined within an Environmental Protection Agency (EPA) laboratory using the *Salmonella typhimurium*/mammalian microsome mutagenicity assay [11–13]. The protocol for this study [14] was a modification of the protocol used in a previous 20 laboratory international study [15,16] on three other SRMs; SRM 1650 (a diesel particulate sample), SRM 1649 (an air particulate sample), and SRM 1597 (a coal tar extract). Modifications were made based upon the recommendations from the participants of the international study [17]. This study used tester strains YG1021 [18], YG1026 [18], TA98NR [19] and TA100NR [19] in addition to strains TA98 and TA100 that were used in the previous studies.

Prior to testing, an aliquot of SRM 1975 was solvent-exchanged into dimethyl sulfoxide (DMSO) to create a starting concentration of 1.0 mg SRM 1975/mL DMSO (equivalent to 1 µg/µL). Adhering to published guidelines [20], the standard *Salmonella typhimurium* plate incorporation assay [11] was used. Modifications that developed from the recommendations noted above included the following: (1) the base layer agar contained trace amounts of histidine and biotin rather than being incorporated in the overlay agar, (2) the plates were incubated for 72 h, (3) the colony counter was calibrated before each use with both hand-counted plates and a template disk with a known number of dots which simulated colonies, (4) each test round had duplicate plates per dose and 10 arithmetically-spaced doses that were contained within one order of magnitude, and (5) four test rounds were conducted. The GeneTox Manager software [21] was used to record the data and to generate comparative slope values using three different statistical programs [22–24]. Table 5 provides the average slope values and the percent coefficient of variation for these values for the four experiments with each condition. A more thorough presentation of methods, results, and conclusions for the mutagenicity assay of SRM 1975 has been published [14].

Although the EPA laboratory providing the mutagenicity data may not be representative of all laboratories that conduct the Salmonella assay, this laboratory, which was established in 1980, has been involved in a variety of collaborative studies, and is well published in the use of the assay. Results, therefore, can be said to come from a well-established laboratory and are likely to represent "typical" bioassay results for SRM 1975.

Table 1. Certified Mass Fractions for Selected PAHs in SRM 1975

	Mass Fractions (mg/kg)(a)	
Phenanthrene(b,c)	8.00	± 0.20
Fluoranthene(b,c)	13.5	± 0.6
Benz[*a*]anthracene(b,d,e)	0.092	± 0.015
Chrysene(c,d,e)	1.95	± 0.07
Triphenylene(c,d,e)	2.38	± 0.10
Benzo[*b*]fluoranthene(c,d,e)	3.20	± 0.10
Benzo[*k*]fluoranthene(c,d)	0.174	± 0.050
Benzo[*e*]pyrene(b,d)	0.268	± 0.023

(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 [25]. Each uncertainty 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.

(b) GC/MS on 5 % phenyl-substituted methylpolysiloxane phase

(c) LC-FL of total PAH fraction

(d) GC/MS using a smectic liquid crystalline phase

(e) LC-FL of the four to six aromatic ring PAH fraction

Table 2. Non-Certified Mass Fractions for Selected PAHs in SRM 1975

	Mass Fractions (mg/kg)[(a)]		
Naphthalene[(b)]	0.67	±	0.01
1-Methylnaphthalene[(b)]	0.39	±	0.01
2-Methylnaphthalene[(b)]	0.69	±	0.02
Biphenyl[(b)]	0.24	±	0.01
Fluorene[(b)]	0.110	±	0.003
1-Methylphenanthrene[(b)]	0.50	±	0.04
2-Methylphenanthrene[(b)]	1.7	±	0.1
3-Methylphenanthrene[(b)]	0.88	±	0.07
4- and 9-Methylphenanthrene[(b)]	0.25	±	0.03
1,2-Dimethylphenanthrene[(b)]	0.017	±	0.002
1,6-, 2,5-, and 2,9-Dimethylphenanthrene[(b)]	0.14	±	0.01
1,7-Dimethylphenanthrene[(b)]	0.122	±	0.008
1,8-Dimethylphenanthrene[(b)]	0.026	±	0.003
2,6-Dimethylphenanthrene[(b)]	0.14	±	0.01
2,7-Dimethylphenanthrene[(b)]	0.127	±	0.008
3,5 and 3,6-Dimethylphenanthrene[(b)]	0.090	±	0.009
Benzo[*ghi*]fluoranthene[(c)]	4.3	±	0.1
Pyrene[(b,d)]	0.42	±	0.13
1-, 3-, and 7-Methylfluoranthene[(b)]	0.092	±	0.004
Benzo[*c*]phenanthrene[(b,c)]	0.51	±	0.07
Benzo[*ghi*]perylene[(b)]	0.038	±	0.006
Indeno[1,2,3-*cd*]pyrene[(b)]	0.12	±	0.01
Dibenz[*a,c*]anthracene and Dibenz[*a,h*]anthracene[(b)]	0.079	±	0.013

[(a)] Each set of results is expressed as the non-certified value ± the expanded uncertainty. Each non-certified value is the mean of the results from one analytical method or the equally weighted mean of the means of two analytical methods. Each uncertainty is an expanded uncertainty at the 95 % level of confidence, which includes random sources of uncertainty within each analytical method and between methods.

[(b)] GC/MS on 5 % phenyl-substituted methylpolysiloxane phase

[(c)] GC/MS using a smectic liquid crystalline phase

[(d)] LC-FL of the four to six aromatic ring PAH fraction

Table 3. Non-Certified Mass Fractions for Selected Nitro-substituted PAHs in SRM 1975

	Mass Fractions (mg/kg)	
1-Nitronaphthalene(a)	0.013	± 0.001(b)
2-Nitronaphthalene(a)	0.039	± 0.005(c)
3-Nitrophenanthrene(d)	0.078	± 0.006(c)
9-Nitrophenanthrene(a)	0.217	± 0.051(c)
9-Nitroanthracene(d)	1.244	± 0.088(b)
1-Nitrofluoranthene(e)	0.015	± 0.005(c)
2-Nitrofluoranthene(a)	0.055	± 0.014(c)
3-Nitrofluoranthene(e)	1.35	± 0.44(c)
8-Nitrofluoranthene(a)	0.231	± 0.041(b)
1-Nitropyrene(d)	16.59	± 0.44(b)
4-Nitropyrene(a)	0.067	± 0.006(b)
6-Nitrochrysene(d)	0.85	± 0.12(b)
7-Nitrobenz[*a*]anthracene(f)	1.68	± 0.35(b)
6-Nitrobenzo[*a*]pyrene(f)	0.49	± 0.11(b)
1-Nitrobenzo[*e*]pyrene(e)	0.75	± 0.10(c)
3-Nitrobenzo[*e*]pyrene(e)	2.01	± 0.24(c)
1,3-Dinitropyrene(d)	0.606	± 0.055(b)
1,6-Dinitropyrene(f)	1.09	± 0.20(b)
1,8-Dinitropyrene(f)	1.29	± 0.22(b)

(a) Non-certified value is based on three data sets from methods performed at NIST.

(b) The non-certified value is a weighted mean of the results from three or four data sets [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 incorporating inter-method bias with a pooled, within-method variance following the ISO and NIST Guides [27].

(c) The non-certified value is an unweighted mean of the results from two or three data sets. 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 [28] with a pooled, within-method variance following the ISO and NIST Guides [27].

(d) Non-certified mass fractions are based on three data sets from NIST and one data set from Battelle.

(e) Non-certified value is based on two data sets from methods performed at NIST

(f) Non-certified value is based on four data sets from three methods performed at NIST and one method performed at Battelle.

Table 4. Non-Certified Value for Extract Residue Mass in SRM 1975

Residue Mass	19.3 ml/mL ± 0.2 mg/mL(a)

(a) This set of results is expressed as the non-certified value ± the expanded uncertainty. The non-certified value for the extract residue mass is the mean value of 18 measurements. Each uncertainty is an expanded uncertainty at the 95 % level of confidence.

Table 5. Non-Certified Values for Mutagenicity (revertants/µg of organic extract) of SRM 1975[(a)]

Strain	Average Without S9 Activation	% CV	Average With S9 Activation	% CV
Results based on Bernstein et al. [22] model slope values				
TA98	462	8.6	125	13.7
TA100	68	16.8	65	15.2
TA98NR	158	37.8	50	48.5
TA100NR	45	6.5	18	18.7
YG1021	612	8.4	226	5.4
YG1026	137	18.5	121	8.7
Results based on Krewski et al. [23] model slope values				
TA98	465	3.7	121	14.5
TA100	111	28.2	65	16.8
TA98NR	181	42.4	44	44.8
TA100NR	46	9.1	18	18.9
YG1021	630	5.0	224	6.5
YG1026	158	17.4	126	8.8
Results based on Stead et al. [24] model slope values				
TA98	587	20.2	147	15.9
TA100	120	23.7	72	15.6
TA98NR	205	42.9	80	58.3
TA100NR	48	11.5	28	16.1
YG1021	672	11.8	257	9.5
YG1026	178	31.5	136	7.8

(a) A detailed description of the determination of the mutagenicity values for SRM 1975 is provided in Hughes et al. [14].

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Certificate Revision History: 02 July 2021 (Change of expiration date; change of nitro-PAHs certified values to non-certified values due to incomplete stability testing from lack of appropriate calibrants; all other measurands previously labeled as reference or information values were converted to non-certified values; removed Information Concentrations for Selected PAHs of Molecular Mass 300 and 302; editorial changes); 01 April 2008 (Update concentration values for nitro-PAHs and add information concentration values for PAHs of molecular mass 300 and 302; extend expiration date); 19 December 2000 (References corrected in Table 2); 07 November 2000 (Original certificate date).

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