National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 1650

Diesel Particulate Matter

This Standard Reference Material (SRM) is intended primarily for use in the evaluation of analytical methods used for the determination of trace concentration levels of polycyclic aromatic hydrocarbons (PAHs) and nitro-polynuclear aromatic hydrocarbons (nitro-PAHs) on diesel particulate matter or on materials with a similar matrix. The particulate matter was obtained from several four-cycle diesel engines (see section, Sample Preparation and Analysis).

Certified values for five PAHs and one nitro-PAH are shown in Table 1. These certified values are based on results obtained by two or more different analytical methods. Noncertified values for seven PAHs and 2 selected nitro-PAHs, provided for information only, are given in Table 2. A summary of the analytical results by the various methods is shown in Table 3. Noncertified reference values for the mutagenic activity of SRM 1650 are given in Table 4. The methylene chloride extractable mass was determined to be 17.5% (see Section, Reference Values for the Mutagenic Activity).

Notice and Warnings to Users

This diesel particulate material may contain a number of toxic compounds. Therefore, each sample should be treated as a potential human health hazard and care should be exercised during its handling and use.

Expiration of Certification: The certified values are valid, within the limits specified, for three years from the date of shipment. NIST will monitor the stability of this SRM, and if any of the certified values change significantly, customers will be notified.

Storage: The SRM vials should be stored in the dark at temperatures between 4 and 30 °C.

Use: The minimum sample size for analysis is 50 mg for the certified values in Table 1 to be valid within the stated uncertainties. It is recommended that samples for analysis be withdrawn from the vial immediately after opening and processed without delay.

Analytical determinations were performed at NIST in the Organic Analytical Research Division by H.X. Gu, L.R. Hilpert, W.F. Kline, W.E. May, and W.A. MacCrehan.

The coordination of technical measurements leading to certification was performed under the direction of W.E. May, Chief of the Organic Analytical Research Division.

Consultation on statistical design of the experimental work was provided by R.C. Paule of the Statistical Engineering Division.

The technical and support aspects involved in the preparation, certification, revision, update, and issuance of this Standard Reference Material were coordinated through the Standard Reference Materials Program by T.E. Gills.

Gaithersburg, MD 20899
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(Revision of certificate dated 2-11-85)

William P. Reed, Chief
Standard Reference Materials Program

(over)

Sample Preparation and Analysis

The diesel particulate material used to prepare this SRM was obtained through the Coordinating Research Council, Inc., Atlanta, GA. The particulate matter was collected from the heat exchangers of a dilution tube facility, following 200 engine hours of particle 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 condition, it should be representative of heavy-duty diesel engine particulate emissions.

The certified values shown in Table 1 represent the results from at least two different analytical procedures. Randomly selected vials from the entire sample set of approximately 1200 vials were used for the analytical determinations.

The two or more independent methods of analysis used for the certification measurements included the use of different extraction solvents that are commonly used for the extraction of organic compounds from particulate material. While the agreement in results from different methods appears to indicate "total recovery" of the certified analytes, such an indication is not sufficient evidence that all of an individual analyte has been recovered. Consequently, the certified values may be method dependent.

GC/MS Analysis

Samples (50 mg) of the diesel particulate material were Soxhlet extracted with 40 mL of a 1:1 mixture of toluene/methanol. A solution of deuterated internal standards was added prior to the extraction step. The Soxhlet extracts were worked up by concentration under N_2 and filtration through a Florisil SepPak*. The desired fraction was eluted with methylene chloride, concentrated under N_2, and analyzed by GC/MS. Gas chromatographic separations were performed on a 30 m x 0.25 mm I.D. fused silica column coated with a 0.25 μm film of DB-5* liquid phase. The mass spectrometer was operated in either the electron impact (EI) or negative ion chemical ionization (NICI) modes. Methane was used as the reagent gas for the NICI measurements. Response factors for the analytes relative to the deuterated internal standards were determined by analyzing a solution of the analytes and internal standards prepared to mimic the concentrations of the analytes in the sample. Quantitative results for the GC/MS measurements are shown in Table 3.

HPLC Analysis

Samples (100-150 mg) of the diesel particulate material were Soxhlet extracted with 450 mL of methylene chloride. A solution of deuterated internal standards was added prior to the extraction step. The Soxhlet extracts were concentrated to approximately 5 mL and filtered through a silica SepPak*. The solvent was changed to isooctane and the extracts were fractionated on a semipreparative aminosilane column using 3 percent methylene chloride in hexane as the mobile phase. The volumes of the fractions were reduced and the solvent changed to acetonitrile prior to quantitative analyses by reversed-phase liquid chromatography on a 5 μm Vydac TP201* column with fluorescence detection using wavelength programming [1].

The nitro-PAH was determined by two methods, the details of which have been published elsewhere [2]: (1) fractionation of the extract on a propylaminocyano column followed by quantification using reversed-phase liquid chromatography with wavelength programmed fluorescence detection after postcolumn, on-line conversion of the nitro-PAH to the corresponding amine, or (2) filtration through C-18 SepPak* followed by reversed-phase liquid chromatographic quantification with potential programmed differential pulse detection at a gold-mercury electrode. Results from the HPLC analyses are given in Table 3.

Statistical Analyses of Analytical Data

Statistical analyses [3] were performed on the data obtained from the various analytical procedures previously described. GC/MS electron impact measurements were used to study sample vial homogeneity. No significant vial-to-vial variability was observed for five of the six compounds certified. Statistically significant vial-to-vial variability was observed for benzo[ghi]perylene, and its certified value includes an allowance for that variability.

*Certain commercial equipment, instruments, or materials are identified to specify adequately the experimental procedures used. 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.

Table 1
Certified Values for Organic Compound Concentrations in Diesel Particulate SRM 1650

Compound	Concentration (μg/g)[a]
Benz[a]anthracene	6.5 ± 1.1
Benzo[a]pyrene	1.2 ± 0.3
Benzo[ghi]perylene	2.4 ± 0.6
Fluoranthene	51 ± 4
1-Nitropyrene	19 ± 2
Pyrene	48 ± 4

[a]The listed values are average values obtained from at least two independent analytical techniques. The listed uncertainties are two times the standard deviations for the average values and were calculated using procedures outlined by Paule and Mandel [3].

Table 2
Non-Certified Values for Organic Compound Concentrations in Diesel Particulate SRM 1650

Compound	Concentration (μg/g)[a]
Benzo[e]pyrene	9.6
Benzo[k]fluoranthene	2.1
Chrysene	22
9-Fluorenone	33
Indeno[1,2,3-cd]pyrene	2.3
7-Nitrobenz[a]anthrene	2.8
6-Nitrobenzo[a]pyrene	1.6
2-Nitrofluorene	0.27
Perylene	0.13
Phenanthrene	71

[a]The values shown in this table are not certified because they are not based on the concordant results from two independent methods. These values are provided for information only.

Table 3

Summary of Results for Diesel Particulate Analysis by Various Analytical Methods[a]

(Concentrations in $\mu g/g$)

	----GC/MS----		-----HPLC-----	
Compound	EI	NICI	Fluorescence	Electrochemical
Benz[a]anthracene*	6.0 ± 0.1		7.1 ± 0.3	
Benzo[a]pyrene*	1.3 ± 0.1	0.9 ± 0.1	1.4 ± 0.1	
Benzo[e]pyrene	9.6 ± 0.3			
Benzo[ghi]perylene*	2.3 ± 0.1	2.6 ± 0.1	2.4 ± 0.4	
Benzo[k]fluoranthene			2.1 ± 0.2	
Chrysene			22 ± 1	
Fluoranthene*	48.5 ± 1	54.5 ± 1	49.8 ± 0.3	
9-Fluorenone	33 ± 1			
Indeno[1,2,3-cd]pyrene	1.8 ± 0.1	2.1 ± 0.1	3.2 ± 0.3	
7-Nitrobenz[a]anthracene			2.8	
6-Nitrobenzo[a]pyrene			1.6	
2-Nitrofluorene			0.27	
1-Nitropyrene*	19.9 ± 0.5	19.3 ± 0.6	19.9 ± 0.4	16.8 ± 0.6
Perylene			0.13 ± 0.02	
Phenanthrene	79 ± 1		63 ± 2	
Pyrene*	49.0 ± 0.7		45.5 ± 1.7	

[a]The summary of results given above is presented as background information. The listed uncertainties represent one standard deviation for these results and recognize only the within-method variability. They, in general, grossly underestimate the true uncertainties of the concentrations.

*Indicates compounds with certified values in Table 1.

REFERENCE VALUES FOR THE MUTAGENIC ACTIVITY OF SRM 1650

The reference values for the mutagenic activity of this SRM were determined as part of an international collaborative study 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 U.S. Environmental Protection Agency's Office of Health Research. Twenty laboratories from North America, Europe, and Japan participated in the study for which a complete summary is available in [4 and 5] or from the NIST Standard Reference Materials Program upon request. As part of the protocol, each laboratory used methylene chloride to extract the organic material from SRM 1650. Half of the laboratories used Soxhlet extraction and the other half used ultrasonication extraction procedures. The extracted material was analyzed using the Salmonella/mammalian microsomal plate-incorporation assay using strains TA98 and TA100 [6]. The mean methylene chloride extractable mass was 17.5 ± 1.5 % and was mutagenic in both strains with and without activation in all 20 laboratories.

The suggested Bioassay Reference Values are given in Table 4. Two types of reference values are provided. The first value is the best estimate of the mutagenic activity, from the data available for methylene chloride extract of SRM 1650 using the protocol specified for the IPCS collaborative study. For the Reference Values to apply, the sample should be Soxhlet or ultrasonically extracted with methylene chloride. The methylene chloride 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 Maron and Ames [6] and adhere to the guidelines published by Claxton et al.[7]. Minimal media plates should be made of Difco agar and should contain 30 ± 1 mL of base layer agar. The exogenous activation system (S9) should be an Aroclor-1254 induced rat liver homogenate as described by Maron and Ames in [6]. Duplicates plates should be used for each of 3-5 dose levels.

The uncertainty in the mutagenic activity, 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 1650, 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 Maron and Ames [6] with some additional constraints [8]. Tolerance limits, sometimes called prediction limits or control limits [9] 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 1650 using the modified Maron and Ames protocol. Additionally, in order for investigator'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 [10 and 11][a].

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 for the 20 laboratories that participated in the IPCS 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 was 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 is performed.

[a] A personal computer program developed by the U. S. Environmental Protection Agency to run under MS-DOS entitled **GeneTox Manager** contains the statistical analysis software developed by Krewski, et al. [10 and 11]. This software is available from the NIST Standard Reference Materials Program for a nominal fee.

Table A. Reference Values[a] for the Mutagenic Activity of Standard Reference Material 1650

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

[a]Refers to the mutagenic activity of a methylene chloride extract of SRM 1650 per mass of particulate material extracted. Doses for IPCS collaborative study were based on the following mg equivalents of SRM 1650:

TA100, +/-S9	0.025, 0.050, 0.100, 0.150, 0.200
TA98, +/-S9	0.0625, 0.125, 0.250, 0.400, 0.500

[b]Geometric mean of all replicate mutagenic activity values reported by participating laboratories after excluding outlying observations.

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