National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 1649

Urban Dust/Organics

This Standard Reference Material (SRM) is intended primarily for use in the evaluation and calibration of analytical methods for the determination of trace level organic constituents on atmospheric particulate matter or on materials with a similar matrix. This material is atmospheric particulate matter collected in an urban location.

Certified values of selected organic constituents, polycyclic aromatic hydrocarbons (PAHs), are shown in Table 1. These certified values are based on results obtained by two different analytical methods utilizing 1-g sample sizes (see Table 2). Noncertified values for selected PAHs, which are provided for information only, are also listed in Table 2. In addition to the values for PAHs, a number of inorganic constituents are listed in Tables 3 and 3a. The inorganic constituent values are included for information only since they were determined using only one analytical technique. Noncertified reference values are also given in Table 4 for the mutagenic activity. The methylene chloride extractable mass was determined to be 5.0% (see section, Reference Values for Mutagenic Activity.)

NOTICE AND WARNINGS TO USER:

This material is a naturally occurring urban dust and may contain a number of chemicals of unknown toxicities; therefore, utmost caution and care should be exercised during its handling and use.

Expiration of Certification: This certification is valid, within the limits certified, for three years from the date of purchase. In the event that the certification should become invalid before then, purchasers will be notified by NIST.

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Consultation on statistical design of the experimental work was provided by K.R. Eberhardt and K. Kafader of the Statistical Engineering Division.

Analytical determinations were performed in the Organic Analytical Research Division by S.N. Chesler, W.F. Cuthrell, W.E. May, R.E. Rebbert, and S.A. Wise, and in the Inorganic Analytical Research Division by R.R. Greenberg and W.F. Koch.

The coordination of technical measurements leading to certification was performed under the direction of S.A. Wise, S.N. Chesler, W.E. May, and H.S. Hertz of the Organic Analytical Research 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 4-8-82)

William P. Reed, Chief
Standard Reference Materials Program

(over)

PREPARATION AND ANALYSIS

This SRM was prepared from atmospheric particulate matter collected in the Washington, DC area using a baghouse specially designed for the purpose. The material was collected over a period in excess of twelve months and, therefore, represents a time-integrated sample. While the sample is not intended to be representative of the area in which it was collected, it should generally typify atmospheric particulate matter obtained from an urban area.

The material was removed from the filter bags by a specially designed vacuum cleaner and combined into a single lot. This lot was screened through a fine-mesh sieve to remove bag fibers and other extraneous materials. The sieved material was then thoroughly mixed in a V-blender, bottled, and sequentially numbered.

The certified values for the PAHs were determined using two different analytical procedures (i.e., extraction solvent, sample clean-up and analysis). Gas chromatography (GC) with flame-ionization detection and liquid chromatography (LC) with selective fluorescence detection were employed for the final quantitative analyses. Ramdonly selected bottles were used for the analytical measurements. Sample aliquants of 1 g were extracted in a Soxhlet extractor for 48h with a cycle time of about 20 min. Quantitation was based on an internal standard (1-methylpyrene for GC analysis and 7-methylfluoranthene or perylene-d_{12} for LC analysis).

The two different methods of analysis included the use of different extraction solvents which are commonly used for the extraction of organic compounds from air particulate material. Although agreement in the results from the different solvents is an indication of "total recovery" of the certified analytes, it cannot necessarily be assumed that the absolute amount of a particular analyte is extractable from the matrix. Therefore, the certified values may be method dependent.

GC ANALYSIS

Samples prepared for GC analysis were extracted with 450 mL of a 1:1 mixture of benzene/methanol. Prior to GC analysis the particulate extract was concentrated in a rotary evaporator, redissolved in cyclohexane, and partitioned with a mixture of N,N-dimethylformamide and water. The liquid-liquid partition the PAH fraction was then isolated by normal-phase LC on an aminosilane column as described previously (1,2). The PAH fraction was concentrated and analyzed by gas chromatography on a fused silica column (30 m x 0.25 mm I.D.) coated with SE-52. A gas chromatogram from the analysis of the PAH fraction is shown in Figure 1.

LC ANALYSIS

The samples for LC analyses were extracted with 450 mL of methylene chloride. Sample clean-up for the LC analysis consisted of concentration of the extract, solvent exchange to cyclohexane, followed by partitioning with nitromethane. The nitromethane solution was concentrated to approximately 1 mL and diluted with 1 mL of tetrahydrofuran. The LC analyses were performed on a 5-μm octadecylsilane (C_{18}) column with a solvent gradient from 40 % acetonitrile in water to 100 % acetonitrile. Fluorescence detection of the liquid chromatographic effluent was used to achieve the selectivity necessary to quantify the individual components in the complex mixture. The various excitation and emission wavelengths used to optimize the selectivity and/or sensitivity for the various PAHs are summarized in Table 4. Three liquid chromatographic runs, each using three or four sets of wavelength conditions, were used for the quantitation of the PAHs (see Figure 2). Further details of the analytical procedures used for the certification of the PAHs in this SRM are described elsewhere [3].

Table 1. Certified Values for Selected Polycyclic Aromatic Hydrocarbons

Compound	Concentration mg/kg[a]
Benz[*a*]anthracene	2.6 ± 0.3
Benzo[*a*]pyrene	2.9 ± 0.5
Benzo[*ghi*]perylene	4.5 ± 1.1
Fluoranthene	7.1 ± 0.5
Indeno[*1,2,3-cd*]pyrene	3.3 ± 0.5

[a]The certified value and the estimated uncertainty listed for a constituent are contained in the union of 95% confidence intervals computed separately for each analytical method and represents an evaluation of the combined effects of method imprecision, possible systematic errors among methods, and material inhomogeneity. The estimated uncertainty is intended to correspond to approximately 95% confidence limits.

Table 2. *Summary of Results by the Various Analytical Methods*

	Concentration mg/kg[a]		
Compound	GC [4][a]	LC-I [18]	LC-II [9]
*Benzo[*a*]anthracene	2.4 ± 0.1 (4)	2.8 ± 0.3 (18)	2.4 ± 0.1 (3)
*Benzo[*a*]pyrene	3.0 ± 0.3 (4)	2.6 ± 0.4 (18)	2.6 ± 0.1 (9)
Benzo[*b*]fluoranthene	--------	6.2 ± 0.3 (18)	--------
Benzo[*e*]pyrene	3.3 ± 0.2 (4)	--------	--------
*Benzo[*ghi*]perylene	4.7 ± 0.2 (4)	3.9 ± 0.8 (12)	5.2 ± 0.6 (9)
Benzo[*k*]fluoranthene	--------	2.0 ± 0.1 (18)	2.1 ± 0.1 (9)
Chrysene	--------	3.5 ± 0.1 (5)	3.7 ± 0.2 (9)
Dibenz[*a,h*]anthracene	--------	--------	0.41 ± 0.07 (9)
*Fluoranthene	7.3 ± 0.2 (4)	7.0 ± 0.5 (24)	6.8 ± 0.4 (9)
*Indeno[*1,2,3-cd*]pyrene	3.3 ± 0.3 (4)	3.4 ± 0.4 (16)	3.6 ± 0.2 (9)
Perylene	0.84 ± 0.09 (4)	0.80 ± 0.04 (17)	0.65 ± 0.02 (9)
Phenanthrene	--------	--------	4.5 ± 0.3[b] (9)[c]
Pyrene	7.2 ± 0.2 (4)	6.3 ± 0.4 (17)	6.2 ± 0.2 (9)
Triphenylene	--------	--------	1.7 ± 0.1 (3)

[a]Number in [ ] indicates number of samples extracted.
[b]Uncertainty is one standard deviation of the mean.
[c]Number in ( ) indicates number of measurements.
*Indicates compounds with certified values in Table 1.
Note: LC-I used 7-methylfluoranthene as the internal standard and LC-II used perylene-d_{12} as the internal standard.

SUPPLEMENTAL INFORMATION

The values listed below are not certified. They are based on measurements made using a single method or technique and are given for *information only*. While no reason exists to suspect systematic bias in these numbers, no attempt was made to evaluate such bias attributable to the methods. *The uncertainties indicated are two times the standard deviation of the individual results (precision).*

Table 3. Inorganic Constituents

Element	Content, mg/kg[a]	Element	Content, mg/kg[a]
Ag	3.5 ± 0.3	Rb	47 ± 5
As	67.0 ± 1.4	S (%)	3.27 ± 0.08
Ba	569 ± 35	Sb	29.9 ± 0.7
Br (%)	0.119 ± 0.001	Sc	8.73 ± 0.08
Cd	18 ± 3	Se	25.6 ± 0.5
Ce	51.6 ± 1.1	Sm	4.71 ± 0.05
Cl (%)	0.282 ± 0.014	Sn	56 ±26
Co	16.4 ± 0.3	Th	6.63 ± 0.14
Cr	211 ± 3	U	2.65 ± 0.16
Cs	2.85 ± 0.10	W	3.8 ± 0.9
Eu	0.87 ± 0.04	Zn (%)	0.167 ± 0.003
Fe (%)	3.00 ± 0.02		
Hf	4.41 ± 0.10		
La	33.3 ± 0.3		
Mo	14 ± 3		

[a]Measurements are based on samples analyzed as received and a minimum sample size of 100 mg.

Table 3a. Leachable Anions[a]

Anion	Content wt.%[b]
Sulfate	9.40 ± 0.22
Nitrate	0.68 ± 0.04
Phosphate	0.32 ± 0.03
Chloride	0.086 ± 0.004

[a]Anions were leached into a dilute carbonate solution by agitation for 15 min in a 55 °C ultrasonic bath.
[b]Measurements are based on samples analyzed as received and a sample size ≥ 250 mg.

Table 4. Fluorescence Conditions for the LC Determination of Selected PAHs in Atmospheric Particulate Matter

	Wavelength (nm) (see Figure 2)		
	Excitation	Emission	PAH Quantified
λ_1	285	450	fluoranthene and 7-methylfluoranthene (I.S.)[a]
λ_2	400	440	perylene-d_{12} (I.S.) and perylene
λ_3	295	405	benzo[*k*]fluoranthene,benzo[*a*]-pyrene, and benzo[*ghi*] perylene
λ_4	330	385	pyrene
λ_5	285	385	benz[*a*]anthracene, dibenz[*a,h*]-anthracene, and benzo[*ghi*] perylene
λ_6	290	360	phenanthrene
λ_7	270	360	chrysene
λ_8	300	500	ideno[*1,2,3-cd*]pyrene

[a]I.S. - internal standard.

REFERENCE VALUES FOR THE MUTAGENIC ACTIVITY OF SRM 1649

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 [4 and 5]. As part of the protocol, each laboratory used methylene chloride to extract the organic material from SRM 1649. 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 5.0 ± 0.4 % 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 a methylene chloride extract of SRM 1949 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 Marion 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 Marion and Ames in [6]. Duplicate 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 1649, 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 [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 1649 using the modified Marion and Ames protocol. Additionally, in order for investigator 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. [8 and 9]. This software is available from the NIST Standard Reference Materials Program for a nominal fee.

Table 4. Reference Values[a] for the Mutagenic Activity of Standard Reference Material 1649

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	102 rev/mg	66-158	30-351	29-365
TA100, -S9	103 rev/mg	73-146	39-275	36-295
TA98, +S9	214 rev/mg	153-299	83-555	80-570
TA98, -S9	237 rev/mg	186-301	119-471	115-488

[a]Refers to the mutagenic activity of a methylene chloride extract of SRM 1649 per unit mass on particulate material extracted. Doses for IPCS collaborative study were based on the following mg equivalents of SRM 1649 particles:

TA100, +/-S9	0.25, 0.5, 1.0, 1.5, 2.0
TA98, +S9	1.0, 2.0, 4.0, 6.0, 8.0
TA98, -S9	1.25, 2.5, 5.0, .5, 10.0

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

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

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

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

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