



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 1597a

Complex Mixture of Polycyclic Aromatic Hydrocarbons from Coal Tar

This Standard Reference Material (SRM) is intended for use in the evaluation and validation of analytical methods for the determination of a natural, combustion-related mixture of polycyclic aromatic hydrocarbons (PAHs). SRM 1597a is isolated from a coal tar sample and dissolved in toluene. It is suitable for direct analysis (i.e., without sample cleanup or concentration) in the determination of PAHs using analytical techniques such as gas chromatography (GC), liquid chromatography (LC), or gas chromatography/mass spectrometry (GC/MS). This SRM may also be used to evaluate procedures for measurement of mutagenic activity of combustion-related mixtures of PAHs and related compounds. A unit of SRM 1597a consists of one 5 mL ampoule, containing 1.3 mL of material.

Certified Mass Fraction Values: Certified values for concentrations, expressed as mass fractions, for 34 PAHs are provided in Table 1. The certified values are based on the agreement of results obtained at NIST from two or more chemically independent analytical techniques [1,2]. 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.

Reference Mass Fraction Values: Reference values for concentrations, expressed as mass fractions, are provided for 36 additional PAHs in Table 2 and for 10 polycyclic aromatic sulfur heterocycles (PASH) in Table 3. Reference values are given in Table 4 for the mutagenic activity of SRM 1597a. 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.

Expiration of Certification: The certification of **SRM 1597a** is valid, within the measurement uncertainty specified, until **01 October 2028**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Handling, Storage, and Use"). The certification is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur which affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The coordination of the technical measurements leading to the certification of this material was under the leadership of M.M. Schantz and S.A. Wise of the NIST Chemical Sciences Division.

The preparation of SRM 1597a was performed by S.A. Wise of the NIST Chemical Sciences Division.

Analytical measurements for the certification of SRM 1597a were performed by A. Deissler, M.J. Lopez de Alda, S. Mössner, D.L. Poster, L.C. Sander, M.M. Schantz, P. Schubert, and S.A. Wise of the NIST Chemical Sciences Division.

Consultation on the statistical design of the experimental work and evaluation of the data were provided by S.D. Leigh of the NIST Statistical Engineering Division.

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Certificate Issue Date: 17 December 2012
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Robert L. Watters, Jr., Director
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Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Handling: This material contains polycyclic aromatic hydrocarbon compounds (many of which have been reported to have mutagenic and/or carcinogenic properties) in toluene and should be handled with care. Use proper disposal methods. See Material Safety Data Sheet for safe handling instructions.

Storage: The sealed ampoule, as received, should be stored in the dark at temperatures between 10 °C and 30 °C.

Use: Sample aliquots for analysis should be withdrawn at 20 °C to 25 °C immediately after opening the ampoule and should be processed without delay for the certified values in Table 1 to be valid within the stated uncertainty. Because of the volatility of toluene, certified values are not applicable to material stored in an ampoule that has been opened for more than 5 min, even if it is resealed. Material may be transferred to a vial that can be sealed to prevent evaporation of the solvent.

PREPARATION AND ANALYSIS⁽¹⁾

Sample Preparation: The coal tar used in the preparation of SRM 1597a is a medium crude coke oven tar. Approximately 36 g of the coal tar sample was processed through a liquid chromatographic column containing attapulgus clay to remove the highly polar constituents in the sample. The column was eluted with approximately 7 L of 10 % methylene chloride in *n*-pentane, and the eluant was collected and concentrated until a thick orange syrup remained. This material was then dissolved in approximately 4.5 L of toluene and aliquoted into a 5 mL amber ampoule. An ampoule contains approximately 1.3 mL of solution, and the density of the solution at 23 °C is approximately 0.866 g/mL. This material was originally issued in 1988 as SRM 1597 [3].

Polycyclic Aromatic Hydrocarbons: The general approach used for the value assignment of the PAHs in SRM 1597a is similar to that reported for the recent certification of several environmental matrix SRMs [4–8] and consisted of combining results from analyses using various combinations of different chromatographic separation and detection techniques. The methods utilized analysis by GC/MS on four stationary phases of different selectivity, i.e., a 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase, a 50 % phenyl-substituted methylpolysiloxane phase, a relatively non-polar proprietary phase, and a liquid crystalline phase and analysis by liquid chromatography with fluorescence detection (LC-FL).

Seven sets of GC/MS results, designated as GC/MS (Ia), (Ib), (IIa), (IIb), (IIIa), (IIIb), and (IV) were obtained using four columns with different selectivities for the separation of PAHs. For the GC/MS (Ia), (Ib), (IIa), (IIb), (IIIa), and (IIIb) analyses, subsamples of approximately 0.5 mL (exact mass known) were used for analysis. For GC/MS (IV), subsamples of approximately 0.25 mL (exact mass known) were used for analysis. For methods GC/MS (Ia), (Ib), (IIIa), and (IIIb), the samples were analyzed without a pretreatment step. For methods GC/MS (IIa) and (IIb), the coal tar samples were fractionated on a semi-preparative aminopropylsilane column (μ Bondapak NH₂, 9 mm i.d. \times 30 cm, Waters Associates, Milford, MA) to isolate isomeric PAH fractions as described [3,9–11].

The samples were then analyzed by GC/MS using the following methods. Methods designated as GC/MS (Ia), GC/MS (IIa), GC/MS (IIIa), and GC/MS (IV) used a 0.25 mm i.d. \times 60 m fused silica capillary column with 50 % (mole fraction) phenyl-substituted methylpolysiloxane phase (0.25 μ m film thickness) (DB-17MS, J&W Scientific, Folsom, CA). For GC/MS (Ib), samples were analyzed by GC/MS using a 0.25 mm i.d. \times 15 m fused silica capillary column with dimethyl 50 % (mole fraction) liquid crystalline polysiloxane phase (0.25 μ m film thickness) (LC-50, J&K Environmental, Sydney, Nova Scotia, Canada). The GC/MS (IIb) analyses were performed using a 0.25 mm i.d. \times 60 m fused silica capillary column with 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase (0.25 μ m film thickness) (DB-5MS, J&W Scientific, Folsom, CA). The GC/MS (IIIb) analyses were performed using 0.25 mm i.d. \times 60 m fused silica capillary column with a relatively non-polar proprietary phase (0.25 μ m film thickness) (DB-XLB, J&W Scientific, Folsom, CA).

For the GC/MS measurements described above, selected perdeuterated PAHs were added to the coal tar for use as internal standards for quantification purposes.

⁽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.

In addition to the GC/MS methods, one LC-FL method was used [11] for the determination of PAHs of molecular mass 278 and 302. For the LC-FL method, subsamples of 1 mL from four ampoules were spiked with an internal standard solution containing selected perdeuterated PAHs. These samples were fractionated using the semi-preparative aminopropylsilane column described above. The reversed-phase LC analyses of the fractions were performed on a polymeric C₁₈ column (Vydac 201TP, The Separations Group, Hesperia, CA).

Polycyclic Aromatic Sulfur Heterocycles: The methods used for the value assignment of the PASHs in SRM 1597a are summarized in detail in Mössner and Wise [12]. In summary, 1 g samples of SRM 1597a were diluted in dichloromethane (DCM), perdeuterated internal standard solutions were added, and the mixtures were sonicated. These samples were fractionated using the semi-preparative aminopropylsilane column described above. Two subfractions were collected, one containing the dibenzothiophenes and the other containing the benzonaphthothiophenes. The samples were then analyzed by GC with atomic emission detection (GC-AED) and GC/MS using a 0.25 mm i.d. × 60 m fused silica capillary column with 5 % (mole fraction) phenyl-substituted methylpolysiloxane phase (0.25 μm film thickness) (DB-5MS) and a 0.25 mm i.d. × 60 m fused silica capillary column with 50 % (mole fraction) phenyl-substituted methylpolysiloxane phase (0.25 μm film thickness) (DB-17MS).

Mutagenic Activity of SRM 1597a: 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 study 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 references 13 and 14. As part of the protocol, each laboratory solvent-exchanged an aliquot of SRM 1597a into dimethylsulfoxide prior to *Salmonella*/mammalian microsomal plate-incorporation bioassay using strains TA98 and TA100 [15].

The suggested bioassay reference values are given in Table 4 in both rev/μL and rev/mg units. 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 1597a using the protocol specified for the IPCS collaborative study. For the reference values to apply, the bioassay procedure should follow the *Salmonella typhimurium* plate incorporation protocol as described by Marion and Ames [15] and adhere to the guidelines published by Claxton et al. [16].

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 1597a, they do not reflect the variation in the values reported by individual participating laboratories. They also do not represent the range of mutagenic activity values from other laboratories using the protocol of Maron and Ames [15] with some additional constraints [17]. Tolerance (prediction or control) limits [18] 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 1597a using the modified Maron and Ames protocol. Additionally, to assess values using the tolerance limits given, data should be treated using the same or very similar statistical methods as those used in this study [19,20].

The "80 % Tolerance Limits" 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.

Table 1. Certified Mass Fractions for Selected PAHs in SRM 1597a

	Mass Fraction (mg/kg) ^(a)	
Naphthalene ^(c,d,e,f)	1030	± 100
Biphenyl ^(c,d,e,f)	27.6	± 0.4
Acenaphthylene ^(c,d,e,f)	263	± 7
Acenaphthene ^(c,d,e,f)	7.63	± 0.26
Fluorene ^(c,d,e,f)	145	± 4
Phenanthrene ^(c,d,e,f,g)	454	± 7
Anthracene ^(c,d,e,f,g)	107	± 3
3-Methylphenanthrene ^(c,d,e,f,g)	15.8	± 0.8
2-Methylphenanthrene ^(c,d,e,f,g)	19.1	± 1.1
9-Methylphenanthrene ^(c,d,f)	5.31	± 0.50 ^(b)
1-Methylphenanthrene ^(c,d,e,f,g)	9.23	± 0.22
Fluoranthene ^(c,d,e,f,g)	327	± 7
Pyrene ^(c,d,e,f,g)	240	± 7
Benzo[ghi]fluoranthene ^(c,f,g)	13.5	± 0.2 ^(b)
Benzo[c]phenanthrene ^(c,d,e,f,g)	11.0	± 0.5
Benzo[a]anthracene ^(c,d,e,f,g)	98.1	± 2.3
Chrysene ^(g,h)	66.2	± 5.3 ^(b)
Triphenylene ^(g,h)	12.1	± 0.6 ^(b)
Benzo[b]fluoranthene ^(c,d,f,g)	66.1	± 4.4
Benzo[j]fluoranthene ^(c,d,f)	36.5	± 2.4 ^(b)
Benzo[k]fluoranthene ^(c,d,e,f,g)	41.2	± 0.4
Benzo[e]pyrene ^(c,d,e,f,g)	50.4	± 1.0
Benzo[a]pyrene ^(c,d,e,f,g)	93.5	± 1.4
Perylene ^(c,d,e,f,g)	24.6	± 0.9
Benzo[ghi]perylene ^(c,d,e,f,g,i)	50.5	± 0.6
Indeno[1,2,3-cd]pyrene ^(c,d,e,f,g)	55.5	± 0.8
Dibenz[a,j]anthracene ^(c,e,f,g,i)	6.80	± 0.46
Dibenz[a,c]anthracene ^(c,f,g,i)	4.35	± 0.21
Dibenz[a,h]anthracene ^(c,d,f,g,i)	6.93	± 0.40
Benzo[b]chrysene ^(c,d,e,f,g,i)	10.8	± 0.4
Picene ^(c,d,e,f,g,i)	6.59	± 0.22
Dibenzo[b,k]fluoranthene ^(c,f,i,j)	11.2	± 0.8
Dibenzo[a,e]pyrene ^(c,f,i,j)	9.08	± 0.39
Dibenzo[a,h]pyrene ^(c,f,g,i,j)	2.57	± 0.30

^(a) Certified values are weighted means of the results from four to six analytical methods [21]. The uncertainty listed with each value is an expanded uncertainty about the mean, with coverage factor 2 (approximately 95 % confidence), except for benzo[k]fluoranthene for which a coverage factor of 8 was used, calculated by combining a between-method variance incorporating between-method bias with a pooled within-source variance following the ISO Guide [2].

^(b) Certified values are unweighted means of the results from two or three analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [22] with a pooled, within-method variance following the ISO Guide [2].

^(c) GC/MS (Ia) on a 50 % phenyl-substituted methylpolysiloxane phase.

^(d) GC/MS (IIa) on a 50 % phenyl-substituted methylpolysiloxane phase.

^(e) GC/MS (IIb) on a 5 % phenyl-substituted methylpolysiloxane phase.

^(f) GC/MS (IIIa) on a 50 % phenyl-substituted methylpolysiloxane phase.

^(g) GC/MS (Ib) dimethyl 50 % liquid crystalline polysiloxane phase.

^(h) GC/MS (IIIb) on a relatively non-polar proprietary phase.

⁽ⁱ⁾ LC-FL.

^(j) GC/MS (IV) on a 50 % phenyl-substituted methylpolysiloxane phase.

Table 2. Reference Mass Fractions for Selected PAHs in SRM 1597a

	Mass Fraction (mg/kg)
1-Methylnaphthalene ^(c,d,e,f)	43.9 ± 1.8 ^(a)
2-Methylnaphthalene ^(c,d,e,f)	95.0 ± 2.9 ^(a)
2,6-Dimethylnaphthalene ^(c,d,e)	5.75 ± 0.63 ^(b)
2-Methylanthracene ^(c,g)	10.4 ± 0.2 ^(b)
4-Methylphenanthrene ^(c,d)	1.04 ± 0.13 ^(b)
1,7-Dimethylphenanthrene ^(c,g)	1.43 ± 0.10 ^(b)
2,6-Dimethylphenanthrene ^(d,e)	1.06 ± 0.24 ^(b)
2,7-Dimethylphenanthrene ^(d,e)	0.99 ± 0.23 ^(b)
3,9-Dimethylphenanthrene ^(d,e)	2.51 ± 0.39 ^(b)
1,8-Dimethylphenanthrene ^(d,e)	0.26 ± 0.05 ^(b)
4-Methylpyrene ^(c,d,e,g)	5.13 ± 0.36 ^(a)
1-Methylpyrene ^(d,e,g)	4.6 ± 1.1 ^(b)
2-Methylpyrene ^(c,d,e)	9.0 ± 2.1 ^(b)
8-Methylfluoranthene ^(c,d,e)	6.33 ± 0.78 ^(b)
3-Methylchrysene ^(c,g)	2.57 ± 0.03 ^(b)
Dibenzothiophene ^(c,f)	17.7 ± 0.4 ^(b)
4H-cyclopenta[<i>def</i>]phenanthrene ^(c,f)	52.4 ± 4.5 ^(b)
Cyclopenta[<i>cd</i>]pyrene ^(c,d,e,g)	37.6 ± 3.4 ^(a)
Benzo[<i>a</i>]fluoranthene ^(c,d,e,f)	20.5 ± 4.9 ^(a)
Pentaphene ^(d,e,h)	4.6 ± 1.5 ^(b)
Anthanthrene ^(c,f,g)	26.6 ± 1.4 ^(b)
Coronene ^(c,e,i)	8.7 ± 1.8 ^(b)
Dibenzo[<i>b,e</i>]fluoranthene ^(c,g,i)	0.98 ± 0.02 ^(b)
Naphtho[1,2- <i>b</i>]fluoranthene ^(c,f,g,i)	8.6 ± 2.0 ^(a)
Naphtho[1,2- <i>k</i>]fluoranthene ^(c,f,h,i)	10.7 ± 1.2 ^(a)
Naphtho[2,3- <i>b</i>]fluoranthene ^(c,f,h,i)	3.52 ± 0.30 ^(a)
Dibenzo[<i>a,k</i>]fluoranthene ^(c,f,g,i)	3.21 ± 0.31 ^(a)
Dibenzo[<i>j,l</i>]fluoranthene ^(c,f,g,i)	6.5 ± 1.4 ^(a)
Dibenzo[<i>a,l</i>]pyrene ^(c,i)	1.12 ± 0.17 ^(b)
Naphtho[2,3- <i>e</i>]pyrene ^(c,f,h,i)	4.31 ± 0.44 ^(a)
Naphtho[2,1- <i>a</i>]pyrene ^(c,g,i)	10.2 ± 0.9 ^(b)
Dibenzo[<i>e,l</i>]pyrene ^(c,g,i)	2.72 ± 0.17 ^(b)
Naphtho[2,3- <i>a</i>]pyrene ^(c,f,g,h,i)	4.29 ± 0.89 ^(a)
Benzo[<i>b</i>]perylene ^(f,g,i)	9.04 ± 0.99 ^(b)
Naphtho[2,3- <i>k</i>]fluoranthene ^(g,h,i)	2.07 ± 0.06 ^(b)
Dibenzo[<i>a,i</i>]pyrene ^(f,g,h,i)	3.87 ± 0.34 ^(a)

^(a) Reference values are weighted means of the results from four to six analytical methods [21]. 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 between-method bias with a pooled within-source variance following the ISO Guide [2].

^(b) Reference values are unweighted means of the results from two or three analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [22] with a pooled, within-method variance following the ISO Guide [2].

^(c) GC/MS (Ia) on a 50 % phenyl-substituted methylpolysiloxane phase.

^(d) GC/MS (IIa) on a 50 % phenyl-substituted methylpolysiloxane phase.

^(e) GC/MS (IIb) on a 5 % phenyl-substituted methylpolysiloxane phase.

^(f) GC/MS (IIIa) on a 50 % phenyl-substituted methylpolysiloxane phase.

^(g) GC/MS (Ib) dimethyl 50 % liquid crystalline polysiloxane phase.

^(h) LC-FL.

⁽ⁱ⁾ GC/MS (IV) on a 50 % phenyl-substituted methylpolysiloxane phase.

Table 3. Reference Mass Fractions for Selected PASHs in SRM 1597a

	Mass Fraction (mg/kg) ^(a)	
Naphtho[1,2- <i>b</i>]thiophene ^(b,c)	8.0	± 2.0
Naphtho[2,1- <i>b</i>]thiophene ^(b,d)	6.05	± 0.21
Naphtho[2,3- <i>b</i>]thiophene ^(b,d)	2.87	± 0.56
4-Methyldibenzothiophene ^(b,c,d,e)	1.37	± 0.08
2-Methyldibenzothiophene ^(b,c,d,e)	1.57	± 0.43
3-Methyldibenzothiophene ^(b,c)	0.94	± 0.12
1-Methyldibenzothiophene ^(b,c,d,e)	0.28	± 0.04
Benzo[<i>b</i>]naphtho[2,1- <i>d</i>]thiophene ^(b,c,d,e)	10.1	± 0.42
Benzo[<i>b</i>]naphtho[1,2- <i>d</i>]thiophene ^(b,c,d,e)	2.41	± 0.21
Benzo[<i>b</i>]naphtho[2,3- <i>d</i>]thiophene ^(b,c,d,e)	3.68	± 0.59

^(a) Reference values are unweighted means of the results from two to four analytical methods. The uncertainty listed with the value is an expanded uncertainty about the mean, with coverage factor 2, calculated by combining a between-method variance [22] with a pooled, within-method variance following the ISO Guide [2].

^(b) GC/MS on a 50 % phenyl-substituted methylpolysiloxane phase.

^(c) GC-AED on a 50 % phenyl-substituted methylpolysiloxane phase.

^(d) GC/MS on a 5 % phenyl-substituted methylpolysiloxane phase.

^(e) GC-AED on a 5 % phenyl-substituted methylpolysiloxane phase.

Table 4. Reference Values^(a) for the Mutagenic Activity of SRM 1597a

Strain/ Activation	Mutagenic Activity ^(b)	95 % Confidence Limits ^(c)	80 % Tolerance Limits	
			Multiple Extraction Bioassay ^(d)	Single Extraction Bioassay ^(e)
TA100, +59	144 rev/μL	100 – 208	51 – 411	50 – 416
	166 rev/mg	116 – 240	59 – 475	58 – 481
TA98, +59	60 rev/μL	46 – 79	28 – 132	26 – 137
	69 rev/mg	53 – 91	32 – 153	30 – 158

^(a) Doses for IPCS collaborative study were 0.625 μL, 1.25 μL, 2.5 μL, 4.0 μL, and 5.0 μL of SRM 1597a solution.

^(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 between- and within-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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