# National Institute of Standards & Technology

# Certificate of Analysis

## Standard Reference Material 1597

### Complex Mixture of Polycyclic Aromatic Hydrocarbons from Coal Tar

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

Certified and Noncertified Concentrations of Polycyclic Aromatic Hydrocarbons: Certified values for the concentrations of selected PAHs are provided in Table 1 and are based on data obtained by using two different analytical techniques, i.e., gas chromatography with flame ionization detection and liquid chromatography with fluorescence detection. Noncertified concentrations of other PAHs are listed in Table 2 and are provided for information only. The results obtained from the gas chromatographic and liquid chromatographic analyses are summarized and compared in Table 3. The major components of SRM 1597 are identified in Table 4 based on GC retention indices and molecular weight data. Noncertified reference values are given in Table 5 for the mutagenic activity of SRM 1597. (see Section, Reference Values for Mutagenic Activity).

NOTICE AND WARNING TO USER:

Handling: This material contains PAHs, many of which have been reported to have mutagenic and/or carcinogenic properties, and should be handled with care. Use approved methods of disposal.

Expiration of Certification: The certified values are valid, within the limits specified, for three years from the date of shipment. In the event that the certification should become invalid before then, purchaser will be notified by NIST.

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

Use: Sample aliquots for analysis should be withdrawn immediately after opening the ampoules and should be processed without delay for the certified values in Table 1 to be valid within the stated uncertainty. Certified values are not applicable to material stored in ampoules that have been opened, even if they are resealed.

Preparation and analytical determinations were performed in the Organic Analytical Research Division by B. A. Benner, Jr., G.D. Byrd, R.E. Rebbert, M.M. Schantz, and S.A. Wise.

The coordination of the technical measurements leading to the certification of this SRM was under the direction of S.A. Wise, R.E. Rebbert, S.N. Chesler, and W.E. May of the Organic Analytical Research Division.

Gaithersburg, MD 20899
May 11, 1992
(Revision of certificate dated 9-29-87)

William P. Reed, Chief
Standard Reference Materials Program

(over)

Consultation on the 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.

## PREPARATION AND ANALYSIS

Sample Preparation: The coal tar used in the preparation of this SRM 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 eluent 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 5-mL amber ampoules. Each ampoule contains approximately 1.3 mL of solution. A unit consists of four ampoules.

Gas Chromatography Analysis: Twelve randomly selected ampoules of SRM 1597 were analyzed in duplicate by capillary column gas chromatography (GC) on a 5% phenyl-substituted methylpolysiloxane stationary phase (DB-5) with flame ionization detection. Aliquots of SRM 1597 were analyzed directly by GC with no sample cleanup. Four internal standards were added to each sample for quantification purposes: acenaphthene, 1-methylphenanthrene, 1-ethylpyrene, and m-tetraphenyl. Corrections were applied to account for the small amounts of these compounds naturally present in the sample. A chromatogram from the GC analysis of SRM 1597 is shown in Figure 1. Retention indices were determined for the major peaks in the chromatogram using the method of Lee et al. [1,2]. The major peaks in the GC analysis of SRM 1597 were identified based on retention indices and molecular weight data as listed in Table 4. The PAH isomers of molecular weight 302 were identified using LC, GC, and fluorescence spectroscopy as described previously (5). Data for triphenylene and chrysene were obtained by GC analysis of the SRM using a liquid crystalline stationary phase since these two isomers are not separated on the nonpolar stationary phase.

Liquid Chromatography Analysis: Six randomly selected ampoules were analyzed by reversed-phase liquid chromatography (LC) on a $C_{18}$ column. Wavelength-programmed fluorescence detection was used to optimize the sensitivity and selectivity for the compounds determined [3]. Aliquots of SRM 1597 were analyzed directly by LC with no sample cleanup. Quantitation was based on comparison with three internal standards (phenanthrene-$d_{12}$, and perylene-$d_{12}$) which were added to the SRM 1597 immediately prior to analysis. For the determination of triphenylene and benzo[*ghi*]perylene, fractions containing isomeric PAHs were isolated by normal-phase LC [3,4]. These fractions were then analyzed by reversed-phase LC on a $C_{18}$ column. The analytical methods and results of the certification measurements have been previously described in detail [6].

## REFERENCE VALUES FOR THE MUTAGENIC ACTIVITY OF SRM 1597

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 [7 and 8] or from the NIST Standard Reference Materials Program upon request. As part of the protocol, each laboratory solvent-exchanged an aliquot of SRM 1597 into dimethylsulfoxide prior to Salmonella/mammalian microsomal plate-incorporation bioassay using strains TA98 and TA100 [9].

The suggested Bioassay Reference Values are given in Table 5 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 1597 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 Maron and Ames [9] and adhere to the guidelines published by Claxton et al. [10]. 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 [9]. 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 1597, 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 [9] with some additional constraints [11]. Tolerance limits, sometimes called prediction limits or control limits [12] 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 1597 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 [13 and 14][a].

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.

[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. [13 and 14]. This software is available from the NIST Standard Reference Materials Program for a nominal fee.

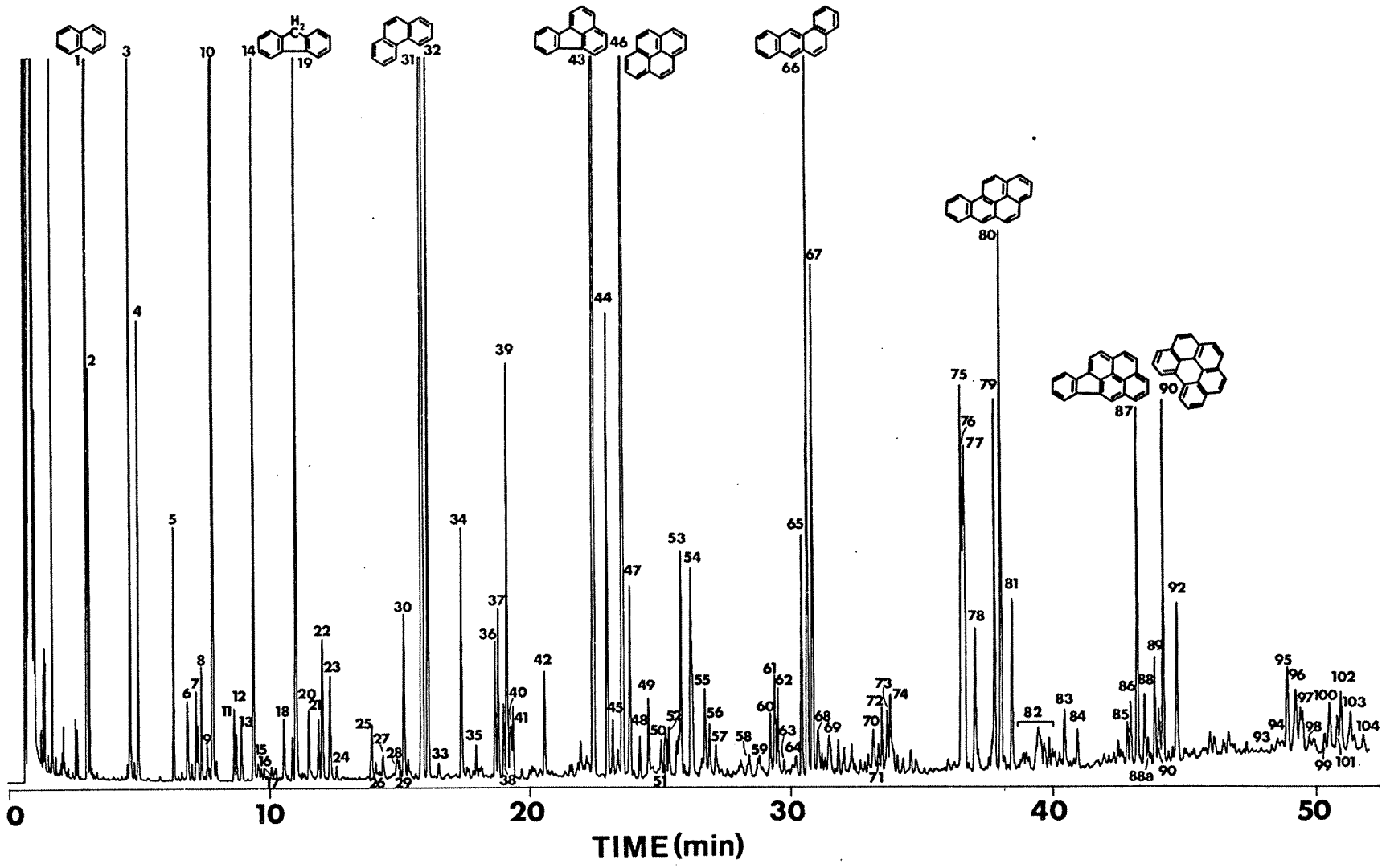


Figure 1. Capillary column gas chromatogram from the analysis of SRM 1597 using hydrogen carrier gas and temperature programmed from an initial temperature of 100 °C for 2 min to 280 °C at 4 °C/min.

Table 1. Certified Concentrations of Selected Polycyclic Aromatic Hydrocarbons in SRM 1597

| Compound[a] | Concentration mg/kg[b] | Concentration μg/mL[c] |
|---|---|---|
| Naphthalene | 1160 ± 50 | 1000 ± 50 |
| Phenanthrene | 462 ± 3 | 400 ± 4 |
| Anthracene | 101 ± 2 | 87.4 ± 2.4 |
| Fluoranthene | 322 ± 4 | 278 ± 4 |
| Pyrene | 235 ± 2 | 204 ± 3 |
| Benz[*a*]anthracene | 98.6 ± 3.6 | 85.3 ± 3.4 |
| Chrysene | 71.7 ± 1.0 | 62.0 ± 1.1 |
| Triphenylene | 12.1 ± 0.4 | 10.5 ± 0.4 |
| Benzo[*a*]pyrene | 95.8 ± 5.8 | 82.9 ± 5.3 |
| Perylene | 26.1 ± 1.0 | 22.6 ± 1.0 |
| Indeno[1,2,3-*cd*]pyrene | 60.2 ± 4.4 | 52.1 ± 4.0 |
| Benzo[*ghi*]perylene | 53.7 ± 7.6 | 46.5 ± 6.7 |

[a]Compounds are listed in order of GC elution.
[b]The certified values were obtained from the combined analytical results of the GC and LC analyses. The associated uncertainties are expressed as two standard deviations of the mean values for the two techniques.
[c]The values listed in μg/mL are computed from the certified concentration in mg/kg multiplied by the density at 23 °C. For these values, the associated uncertainties have been expanded by an additional 0.3% of the certified concentration to enable use of SRM 1597 certificate values (μg/mL) over the temperature range 20 - 26 °C.

Table 2. Noncertified Concentrations of Polycyclic Aromatic Compounds in SRM 1597

NOTE: The values shown in this table are not certified because they are not based on agreement of results from two independent methods. These results are included for information only and are provided with only two significant figures.

| Compound[a] | Concentration mg/kg | Concentration μg/mL |
|---|---|---|
| Benzothiophene | (28)[b] | (24) |
| 2-Methylnaphthalene | (97)[b] | (84) |
| 1-Methylnaphthalene | (47)[c] | (41) |
| Biphenyl | (27)[b] | (24) |
| Acenaphthylene | (250)[b] | (220) |
| Dibenzofuran | (89)[b] | (77) |
| Fluorene | (140)[c] | (120) |
| Dibenzothiophene | (23)[b] | (20) |
| Carbazole | (39)[c] | (34) |
| 4H-Cyclopenta[*def*]phenanthrene | (51)[b] | (44) |
| Acephenanthrylene | (60)[b] | (52) |
| Cyclopenta[*cd*]pyrene | (38)[b] | (33) |
| Benzo[*b*]fluoranthene | (66)[d] | (57) |
| Benzo[*k*]fluoranthene | (43)[d] | (37) |
| Benzo[*e*]pyrene | (57)[c] | (49) |
| Picene | (12)[b] | (10) |
| Anthanthrene | (29)[e] | (25) |
| Coronene | (11)[b] | (10) |

[a]Compounds listed in order of GC elution.
[b]Value determined by GC with response factor assumed to be unity.
[c]Value determined by GC; response factor determined using standard of known purity.
[d]Value is the mean of the LC and GC (liquid crystalline phase) analyses; LC and GC response factors determined using standard of known purity.
[e]Value is the mean of the LC and GC analyses.

Table 3. Summary of the Results by the Analytical Techniques Used to Characterize SRM 1597

| Compound[d] | Concentration, mg/kg[a] GC[c] | | LC-Fluorescence[d] | |
|---|---|---|---|---|
| Naphthalene | 1130 ± 20 | | 1180 ± 20 | |
| 2-Methylnaphthalene | 97.1 ± 1.1[e] | | | |
| 1-Methylnaphthalene | 47.0 ± 0.6 | | | |
| Biphenyl | 27.4 ± 0.3[e] | | | |
| Acenaphtylene | 252 ± 1[e] | | | |
| Dibenzofuran | 88.9 ± 0.5[e] | | | |
| Fluorene | 136 ± 1 | | | |
| Dibenzothiophene | 23.0 ± 0.4[e] | | | |
| Phenanthrene | 461 ± 5 | | 463 ± 4 | |
| Anthracene | 102 ± 1 | | 99.7 ± 1.4 | |
| Carbazole | 39.0 ± 0.4 | | | |
| 4H-Cyclopenta[*def*]phenanthrene | 51.3 ± 0.3[e] | | | |
| Fluoranthene | 320 ± 4 | | 324 ± 5 | |
| Acephenanthrylene | 59.5 ± 0.9[e] | | | |
| Pyrene | 234 ± 3 | | 237 ± 3 | |
| Cyclopenta[*cd*]pyrene | 38.0 ± 0.6[e] | | | |
| Benz[*a*]anthracene | 102 ± 1 | | 96.2 ± 2.5 | (97.4 ± 0.6)[i] |
| Chrysene | 79.3 ± 1.0[f] | (70.7 ± 2.9)[h] | 72.0 ± 1.4 | (72.4 ± 0.9)[i] |
| Triphenylene | | (11.9 ± 1.0)[h] | | (12.2 ± 0.1)[i] |
| Benzo[*j*]fluoranthene | | (36.9 ± 3.4)[h] | | |
| Benzo[*b*]fluoranthene | | (70.1 ± 4.2)[h] | 61.4 ± 1.7 | |
| Benzo[*k*]fluoranthene | | (47.4 ± 4.1)[h] | 38.8 ± 1.3 | |
| Benzo[*e*]pyrene | 57.1 ± 0.5 | (53.8 ± 3.1)[h] | | |
| Benzo[*a*]pyrene | 98.7 ± 0.8 | | 92.8 ± 2.8 | |
| Perylene | 26.7 ± 0.9 | | 25.6 ± 0.2 | |
| Indeno[1,2,3-*cd*]pyrene | 60.3 ± 1.3 | | 63.9 ± 3.7 | (56.3 ± 0.1)[i] |
| Picene | 12.1 ± 0.7[e] | | | |
| Benzo[*ghi*]perylene | 57.5 ± 1.3 | | 49.9 ± 0.3[i] | |
| Anthanthrene | 31.8[e] | | 26.7 ± 3.2[i] | |
| Coronene | 11.3 ± 1.3[e] | | | |

[a]Uncertainty is expressed as ± one standard deviation of a single measurement. These values are not certified and are provided only as a summary and comparison of the data from the various analytical techniques used in the certification measurements.
[b]Compounds listed in order of GC elution.
[c]GC values based on response factors determined using a standard of known purity; twelve ampoules analyzed in duplicate.
[d]LC values based on response factors determined using a standard of known purity; six ampoules analyzed in triplicate.
[e]GC value based on a response factor assumed to be unity.
[f]GC value is the total concentration of chrysene and triphenylene.
[g]GC value is the total concentration of benzo[j]fluoranthene, benzo[b]fluoranthene, and benzo[k]fluoranthene.
[h]Value determined by GC on liquid crystalline stationary phase; five ampoules were analyzed five times each.
[i]Value determined after normal-phase LC fractionation to isolate isomeric group [3,4]; samples from three normal-phase LC fractionations analyzed in triplicate.

Table 4. Identification of Components in SRM 1597 by Gas Chromatography-Mass Spectrometry

| Peak No.[a] | RI[b] | RI[c] | Mol. Wt.[d] | Identification |
|---|---|---|---|---|
| 1 | 200.00 | 200.00 | 128 | Naphthalene |
| 2 | | | 134 | Benzothiophene |
| 3 | 200.47 ± 0.24 | 220.22 ± 0.23 | 142 | 2-Methylnaphthalene |
| 4 | 223.42 ± 0.27 | 223.01 ± 0.28 | 142 | 1-Methylnaphthalene |
| 5 | 236.39 ± 0.31 | 236.44 ± 0.19 | 154 | Biphenyl |
| 6 | | | 156 | |
| 7 | | | 156 | |
| 8 | | | 154 | |
| 9 | | | 156 | |
| 10 | 247.65 ± 0.16 | 246.92 ± 0.31 | 152 | Acenaphthylene |
| 11 | | | 168 | |
| 12 | 253.67 ± 0.28 | 253.14 ± 0.28 | 154 | Acenaphthene |
| 13 | | | 153 | |
| 14 | 259.07 ± 0.24 | 258.77 ± 0.24 | 168 | Dibenzofuran |
| 15 | | | 170 | |
| 16 | | | 168 | |
| 17 | | | 170 | |
| 18 | | | 166 | |
| 19 | 269.94 ± 0.17 | 269.73 ± 0.30 | 166 | Fluorene |
| 20 | | | 166 | |
| 21 | | | 182 | |
| 22 | | | 166 | |
| 23 | | | 182 | |
| 24 | | | 182 | |
| 25 | 288.29 ± 0.15 | 288.42 ± 0.15 | 180 | 2-Methylfluorene |
| 26 | 289.14 ± 0.17 | 289.20 ± 0.21 | 180,196 | 1-Methylfluorene/Unknown |
| 27 | 290.78 ± 0.13 | 290.83 ± 0.22 | 180 | Methylfluorene |
| 28 | | | 196 | |
| 29 | | | 196 | |
| 30 | 295.59 ± 0.17 | 295.17 ± 0.17 | 184 | Dibenzothiophene |
| 31 | 300.00 | 300.00 | 178 | Phenanthrene |
| 32 | 301.38 ± 0.11 | 301.18 ± 0.11 | 178 | Anthracene |
| 33 | | | 184 | |
| 34 | 310.35 ± 0.19 | 311.71 ± 0.19 | 167 | Carbazole |
| 35 | | | 204 | |
| 36 | 318.93 ± 0.16 | 319.19 ± 0.24 | 192 | 3-Methylphenanthrene |
| 37 | 319.76 ± 0.17 | 319.93 ± 0.20 | 192 | 2-Methylphenanthrene |
| 38 | 321.14 ± 0.14 | 321.57 ± 0.12[e] | 192 | 2-Methylanthracene |
| 39 | 321.96 ± 0.16 | 321.77 ± 0.12 | 190 | 4H-Cyclopenta[*def*]phenanthrene |
| 40 | 322.78 ± 0.20 | 322.81 ± 0.21 | 192 | 4-and/or 9-Methylphenanthrene |
| 41 | 323.52 ± 0.19 | 323.64 ± 0.42 | 192 | 1-Methylphenanthrene |
| 42 | | | 204 | |
| 43 | 344.49 ± 0.07 | 344.51 ± 0.06 | 202 | Fluoranthene |
| 44 | 347.82 ± 0.12 | 347.67 ± 0.08 | 202 | Acephenanthrylene |
| 45 | 349.17 ± 0.11 | 348.75 ± 0.12 | 208 | Phenanthro[4,5-*bcd*]thiophene |
| 46 | 351.91 ± 0.15 | 351.51 ± 0.15 | 202 | Pyrene |

Table 4. Continued

| | | | | |
|---|---|---|---|---|
| 50 | | | 218 | |
| 51 | | | 216 | |
| 52 | 362.42 ± 0.14 | 363.92 ± 0.10 | 191 | 4H-Benzo[*def*]carbazole |
| 53 | 366.54 ± 0.11 | 366.72 ± 0.10 | 216 | Benzo[*a*]fluorene |
| 54 | 369.05 ± 0.12 | 369.40 ± 0.10 | 216 | Benzo[*b*]fluorene/Methylpyrene |
| 55 | | | 232,216 | |
| 56 | 373.72 ± 0.17 | 373.45 ± 13 | 216 | 1-Methylpyrene |
| 57 | | | 216 | |
| 58 | | | 230 | |
| 59 | | | 230 | |
| 60 | 389.16 ± 0.12 | 389.09 ± 0.09 | 234 | Benzo[*b*]naphthol[2,1-*d*]thiophene |
| 61 | 390.28 ± 0.15 | 389.92 ± 0.11 | 226 | Benzo[*ghi*]fluoranthene |
| 62 | 391.07 ± 0.13 | 391.24 ± 0.10 | 226 | Benzo[*c*]phenanthrene |
| 63 | 392.51 ± 0.17 | 392.59 ± 0.10 | 234 | Benzo[*b*]naphthol[1,2-*d*]thiophene |
| 64 | 395.59 ± 0.14 | 395.61 ± 0.10 | 234 | Benzo[*b*]naphthol[2,3-*d*]thiophene |
| 65 | 397.15 ± 0.17 | 396.55 ± 0.17 | 226 | 4H-Cyclopental[*cd*]pyrene |
| 66 | 398.69 ± 0.04 | 398.76 ± 0.04 | 228 | Benz[*a*]anthracene |
| 67 | 400.00 | 400.00 | 228 | Chrysene/Triphenylene |
| 68 | | | 242 | |
| 69 | | | 258 | |
| 70 | | | 242 | |
| 71 | | | 242 | |
| 72 | | | 240 | |
| 73 | | | 240 | |
| 74 | | | 240 | |
| 75 | 443.11 ± 0.05 | 443.13 ± 0.11 | 252 | Benzo[*b*]fluoranthene |
| 76 | 443.64 ± 0.09 | | 252 | Benzo[*j*]fluoranthene |
| 77 | 444.06 ± 0.07 | 444.02 ± 0.07 | 252,268 | Benzo[*k*]fluoranthene |
| 78 | 446.88 ± 0.06 | | 252,268 | Benzo[*a*]fluoranthene |
| 79 | 452.70 ± 0.06 | 452.29 | 252 | Benzo[*e*]pyrene |
| 80 | 454.57 ± 0.09 | 454.02 ± 0.07 | 252 | Benzo[*a*]pyrene |
| 81 | 457.63 ± 0.07 | 457.17 ± 0.06 | 252 | Perylene |
| 82 | | | 266 | |
| 83 | | | 264 | |
| 84 | | | 264 | |
| 85 | | | 276 | Indeno[7,1,2,3-*cdef*]chrysene |
| 86 | | | 278 | Dibenz[*a,j*]anthracene |
| 87 | 493.88 ± 0.11 | 493.88 ± 0.09 | 276 | Indeno[1,2,3-*cd*]pyrene |
| 88 | 495.92 ± 0.11 | 496.20 ± 0.30 | 278 | Dibenzo[*a,h*]anthracene |
| 88a | 496.83 ± 0.12 | | 278 | Pentaphene |
| 89 | 498.84 ± 0.06 | 498.90 ± 0.23 | 278 | Benzo[*b*]chrysene |
| 90 | 500.00 | 500.00 | 278 | Picene |
| 91 | 501.38 ± 0.10 | 501.32 ± 0.18[e] | 276 | Benzo[*ghi*]perylene |
| 92 | 505.29 ± 0.10 | | 276 | Anthanthrene |
| 93 | | | 302 | |
| 94 | | | 302 | Dibenzo[*b,e*]fluoranthene[g] |
| 95 | 536.89 ± 0.10 | | 302 | Naphthol[1,2-*k*]fluoranthene[g] |
| 96 | | | 302 | Dibenzo[*b,k*]fluoranthene[g] |
| 97 | | | 302 | |
| 98 | | | 300,302 | Unknown/Naphthol[2,3-*k*]fluoranthene[g] |
| 99 | | | | |
| 100 | 549.07 ± 0.10 | | 300 | Coronene |

Table 4. Continued

| | | |
|---|---|---|
| 101 | 302 | Dibenzo[*a,e*]pyrene/dibenzo[*e,l*]pyrene[g] |
| 102 | 302,316 | Naphthol[2,1-*a*]pyrene/Benzo[*b*]perylene/ Dibenzo[2,3-*a*]pyrene/ Dibenzo[*a,i*]pyrene[g] |
| 103 | 302,316 | Dibenzo[*a,h*]pyrene[g] |

[a]See Figure 1.
[b]Retention Index (RI) value is the average of four to seven experiments and the uncertainty ± one standard deviation of the mean.
[c]RI values from reference [2].
[d]Molecular weight assignment based on highest mass ion of significant relative abundance observed in electron impact mass spectrum.
[e]RI value from reference[1].
[f]Identification based on RI data in reference [2].
[g]Identifications based on data from reference [5]; other isomers may also by present.

Table 5. Reference Values[a] for the Mutagenic Activity of Standard Reference Material 1597

| 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 | 144 rev/μL | 100-208 | 51-411 | 50-416 |
| | 166 rev/mg | 116-240 | 59-475 | 58-481 |
| TA98, +S9 | 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, 1.25, 2.5, 4.0 and 5.0 μL of SRM 1597 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 inter- and intralaboratory 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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