



Designation: D7771 – 13

# Standard Test Method for Determination of Benzo- $\alpha$ -Pyrene (BaP) Content in Carbon Black<sup>1</sup>

This standard is issued under the fixed designation D7771; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This test method covers the qualitative and quantitative determination of only benzo- $\alpha$ -pyrene (BaP), a specific polycyclic aromatic hydrocarbon (PAH), on carbon black. The procedure involves Soxhlet extraction with toluene and analysis by gas chromatography with mass spectrometry (GC/MS). This method is not intended to test for U.S. Food and Drug Administration (FDA 21 CFR 178.3297) compliance of carbon blacks used for indirect food contact applications.

1.2 *Units*—The values stated in SI units are to be regarded as the standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

## 2. Referenced Documents

2.1 *ASTM Standards*:<sup>2</sup>

D4483 Practice for Evaluating Precision for Test Method Standards in the Rubber and Carbon Black Manufacturing Industries

2.2 *EPA Standard*:<sup>3</sup>

Method 8270D Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee D24 on Carbon Black and is the direct responsibility of Subcommittee D24.66 on Environment, Health, and Safety.

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<sup>2</sup> For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

<sup>3</sup> Available from United States Environmental Protection Agency (EPA), Ariel Rios Bldg., 1200 Pennsylvania Ave., NW, Washington, DC 20004, http://www.epa.gov/epaoswer/hazwaste/test/new-meth.htm#8270D.

2.3 *Federal Standard*:

21 CFR 178.3297 Indirect Food Additives: Adjuvants, Production Aids, and Sanitizers, Colorants for Polymers<sup>4</sup>

## 3. Terminology

3.1 *Definitions of Terms Specific to This Standard*:

3.1.1 *benzo- $\alpha$ -pyrene, BaP, n*—also known as 3,4-benzopyrene or benzo-a-pyrene is a specific polycyclic aromatic hydrocarbon (PAH) or polynuclear aromatic hydrocarbon that consist of fused aromatic rings with no heteroatom or substituent.

3.1.1.1 *Discussion*—PAHs naturally occur in oil, coal, and tar deposits; are produced by the incomplete combustion of hydrocarbons; and occur in many other products and processes. BaP is a pentacyclic PAH with the formula C<sub>20</sub>H<sub>12</sub>, related to pyrene by fusion of a phenylene group in the alpha position.

## 4. Summary of Test Method

4.1 A portion of carbon black is Soxhlet extracted with toluene for a specified period of time. The resultant extract is purified by solid-phase extraction (SPE), concentrated down to a known volume, and subsequently analyzed for BaP by gas chromatography with mass spectrometry (GC/MS). The BaP quantification is performed by the means of a deuterated internal standard (isotope dilution).

## 5. Significance and Use

5.1 This test procedure is used to determine the concentration of BaP extracted from carbon black by the means of a Soxhlet extraction apparatus with toluene.

## 6. Apparatus

6.1 *Soxhlet Extractor with Reflux Condenser*, 50- or 100-cm<sup>3</sup> capacity.

6.2 *Extraction Thimbles*, glass or cellulose, approximately 50- to 70-cm<sup>3</sup> capacity. For instance, glass extraction thimble

<sup>4</sup> Available from the U.S. Government Printing Office, Superintendent of Documents, 732 N. Capital St., NW., Washington, DC 20402-0001.

of 35-mm diameter by 90-mm height with coarse porosity (70 to 100  $\mu\text{m}$  similar to Ace Glass Size C, Porosity B, Code-14).

6.3 *Heating Mantle*, compatible with boiling flask described in 6.4.

6.4 *Boiling Flasks for Soxhlet*, for example, 250  $\text{cm}^3$ .

6.5 *Glass Beads*, approximately 180 to 250  $\mu\text{m}$  (60/80 mesh) if glass thimbles are used. Need to be very clean or toluene extracted.

6.6 *PTFE or Glass Boiling Beads*, approximately 3 mm.

6.7 *Glass Wool*, need to be very clean or toluene extracted.

6.8 *Rotary Evaporator*, with temperature-controlled water bath, automatic pressure regulation, and solvent-proof membrane vacuum pump.

6.9 *Nitrogen Blow-Down Apparatus*, equipped with a controlled water bath and nitrogen pressure control.

6.10 *Pear-Shaped Flasks for Rotary Evaporator*, for example, 25, 50, and 100  $\text{cm}^3$ .

6.11 *GC/MS*, with autosampler.

6.11.1 MS with electron impact (EI) capability and single-ion monitoring (SIM) mode.

6.11.2 *GC Capillary Column*, usually a nonpolar GC column composed of 5 % phenyl-methyl silicone coating is used for PAH analysis.

6.11.3 *Deactivated Straight Borosilicate Liner with Small Piece of Deactivated Glass Wool*—This liner may be used as long as peak resolution is satisfactory.

6.11.4 Alternative liner is a split/splitless nondeactivated liner with glass wool (4-mm internal diameter, straight liner). This shall be deactivated with a silanizing agent before use. Another alternative is a split/splitless liner with fluorocarbon liner seals. Such a liner will already contain conditioned silanized glass wool. Other liners can be used if they produce acceptable results.

6.11.5 Gold-plated seal for GC injector port or similar nonreactive seal.

6.11.6 GC/MS amber autosampler vials with polytetrafluoroethylene (PTFE)-coated caps.

6.11.7 Crimping tool.

6.12 *Adjustable Micropipettes*, 1000, 200, and 20  $\mu\text{L}$ .

6.13 *Microlitre Syringes of Different Volumes*, for example, 10 and 100  $\mu\text{L}$ .

6.14 *Amber Glass Vials*, approximately 4  $\text{cm}^3$  with caps (rubber with PTFE back).

6.15 *Amber Volumetric Flasks*, 2, 3, 5, 10, 25, and 100  $\text{cm}^3$ .

6.16 *Analytical Balance*, with an accuracy of 0.01 mg.

6.17 *Drying Oven*, gravity convection type, capable of maintaining  $40 \pm 10^\circ\text{C}$ , used for slowing down the cooling when the glassware is taken out of the muffle furnace.

6.18 *Furnace*, capable of temperature regulation of  $500 \pm 25^\circ\text{C}$ , used to burn off organic contamination from glass surfaces.

**TABLE 1 Native and Deuterated BaP Compounds**

PAH	CAS#	Deuterated PAH	CAS#
Benzo(a)pyrene	50-32-8	$\text{d}_{12}$ -Benzo(a)pyrene	63466-71-7

6.19 *Manometer*, capable of pressure readings in the range of  $5 \pm 0.3$  kPa.

## 7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.<sup>5</sup> Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 Separate stock solutions of the deuterated internal standard (IS) and of the nondeuterated (native) BaP standards can be purchased prepared from solid materials or individual solutions (Table 1). A concentration range of 80 to 100  $\mu\text{g}/\text{cm}^3$  for both the native BaP and  $\text{d}_{12}$ -benzo(a)pyrene is recommended. All purchased BaP standard materials shall be 98 % pure or better and certified in respect of their purity, concentration level, and authenticity by the manufacturer.

7.2.1 Follow the manufacturer's recommendation on how best to store the standard stock solutions. It is generally accepted to keep them protected from light. They should be checked frequently for signs of degradation or evaporation, especially just before preparing calibration standards from them. The BaP standard stock solutions shall be replaced/recertified on a yearly basis or sooner if comparisons with quality control (QC) samples indicates a problem.

7.3 *Toluene*, suitable for high resolution gas chromatography analysis (99.99% pure).

7.4 *Acetone*, suitable for high resolution gas chromatography analysis (99.99% pure).

7.5 *Helium*, GC/MS purity grade.

7.6 *Nitrogen*, analytical purity grade.

7.7 *Silica Solid-Phase Extractor Cartridges*, single-use application, having a volume capacity of approximately 5  $\text{cm}^3$  or 2- to 3-cm length by 1-cm diameter.<sup>6</sup>

## 8. Hazards

8.1 This test involves hazardous materials, operations, and equipment. This procedure does not attempt to address the

<sup>5</sup> *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial convention, Inc. (USPC), Rockville, MD.

<sup>6</sup> The sole source of supply of the Sep-Pak cartridges known to the committee at this time is Waters, 34 Maple Street, Milford, MA 01757 (www.waters.com). If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,<sup>1</sup> which you may attend.

safety problems associated with this test. A hazards review shall be conducted by all personnel performing the test. It is the responsibility of the user to review all material safety data sheets (MSDS), manuals, and hazards procedures and establish the appropriate safety measures. Some PAH compounds have been shown to possess mutagenic as well as carcinogenic and teratogenic properties. As such, concentrated extracts of carbon blacks containing PAHs also may possess the same harmful properties. Solvents used are flammable. Appropriate personal protection equipment (PPE) shall be used.

## 9. Preparation of Standard Solutions

9.1 The GC/MS instrument is calibrated through five mixtures of the native BaP and deuterated IS. The recommended BaP concentrations are to cover a range of approximately 0.01 to 0.8  $\mu\text{g}/\text{cm}^3$  (ppm) while the IS concentration is kept constant at around 0.3  $\mu\text{g}/\text{cm}^3$ . A lower BaP concentration range can be used for the case of high-purity carbon blacks, but the difference between any two concentration levels shall not exceed a factor of four. Furthermore, the IS concentration should maintain an S/N ratio of at least 15/1 for routine instrument performance. Subsections 9.2 – 9.5 describe the preparation of the various solutions required.

9.2 *Preparation of Native BaP Standard Solutions for Calibration*—Using the native BaP standard stock solution described in 7.2, prepare at least 10  $\text{cm}^3$  of five toluene solutions in amber glassware at the concentrations suggested in the following. Cap securely the standard solutions, mix thoroughly, and label.

Native BaP Standard 5	0.85 $\mu\text{g}/\text{cm}^3$
Native BaP Standard 4	0.28 $\mu\text{g}/\text{cm}^3$
Native BaP Standard 3	0.09 $\mu\text{g}/\text{cm}^3$
Native BaP Standard 2	0.03 $\mu\text{g}/\text{cm}^3$
Native BaP Standard 1	0.01 $\mu\text{g}/\text{cm}^3$

9.3 *Preparation of the Deuterated IS Solution*—Using the deuterated IS stock solution described in 7.1, prepare at least 50  $\text{cm}^3$  of a diluted toluene solution in amber glassware at the concentration suggested in the following. Cap securely the standard solutions, mix thoroughly, and label. This recommendation on the use of an internal standard is consistent with the practice described in EPA Method 8270D.

IS Standard	3.3 $\mu\text{g}/\text{cm}^3$
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9.4 *Preparation of the BaP Calibration Solutions*—Into five separate amber GC/MS vials, pipette 1.000  $\text{cm}^3$  of the native BaP standards prepared in 9.2 and 0.100  $\text{cm}^3$  of the IS standard solution prepared in 9.3. Cap securely the amber GC/MS vials, mix thoroughly, and label. The following is obtained:

Cal BaP 5	0.773 $\mu\text{g}/\text{cm}^3$ native BaP + 0.300 $\mu\text{g}/\text{cm}^3$ IS
Cal BaP 4	0.255 $\mu\text{g}/\text{cm}^3$ native BaP + 0.300 $\mu\text{g}/\text{cm}^3$ IS
Cal BaP 3	0.082 $\mu\text{g}/\text{cm}^3$ native BaP + 0.300 $\mu\text{g}/\text{cm}^3$ IS
Cal BaP 2	0.027 $\mu\text{g}/\text{cm}^3$ native BaP + 0.300 $\mu\text{g}/\text{cm}^3$ IS
Cal BaP 1	0.009 $\mu\text{g}/\text{cm}^3$ native BaP + 0.300 $\mu\text{g}/\text{cm}^3$ IS

9.5 All standard solutions shall be stored in amber glassware and kept in a refrigerator ( $<6^\circ\text{C}$ ) when not in use. Care has to be taken not to exceed their shelf life. If any indication on degradation is perceived, the standard has to be prepared anew.

## 10. Carbon Black Sample Preparation and Extraction

10.1 All glassware parts coming into contact with the sample shall be BaP free on the basis of the limits of quantification. It is strongly recommended to use separate glassware and extraction units for high-purity carbon blacks and carbon blacks in which higher BaP levels are expected. Blanks should be run on a regular basis.

10.2 Glassware should be rinsed with toluene and acetone after use. The glassware is then dried at  $150^\circ\text{C}$  in a laboratory drying oven.

10.3 For low BaP carbon blacks, a pre-extraction of the extraction unit for at least 4 h is recommended. If glass thimbles are not baked in a furnace as described in 10.5, then the thimbles should be included in the 4 h pre-extraction. Cellulose thimbles should be included in the 4 h pre-extraction. In this case, the thimbles are further dried, for example, in a vacuum drying oven prior to use.

10.4 Disposable devices such as cellulose thimbles are rinsed with toluene and dried prior to use, for example, in a vacuum oven.

10.5 If repeated cleanings and extractions do not produce clean blanks, certain parts of the glassware may also be baked for at least 6 h in a furnace at, for example,  $500^\circ\text{C}$ . This is also valid for glass extraction thimbles if used. It shall be determined with the manufacturer if the glass parts can sustain such temperature—for example, it is unlikely the Soxhlet can sustain this.

10.6 If glass extraction thimbles are used, they should be checked for their drain rate at room temperature by pouring 50  $\text{cm}^3$  of toluene inside the thimble. The time for 40  $\text{cm}^3$  to drip out of the thimble should not exceed 95 s. Otherwise, the thimble is to be discarded. Dry the thimbles with clean nitrogen.

10.7 If glass extraction thimbles are used, pour the 60/80-mesh glass beads into the thimbles to a depth of 1 cm. Sizes other than 60/80 mesh may be used.

10.8 Weigh  $10 \pm 0.1$  g to the nearest 1 mg of the beaded carbon black sample into the dried extraction thimble. Record the exact value as  $W_{CB}$ .

10.8.1 Fluffy or powder carbon black should be densified with toluene before extraction. This is accomplished by weighing  $10 \pm 0.1$  g of carbon black into a beaker and adding toluene in approximately 1- $\text{cm}^3$  aliquots and mixing the toluene into the carbon black with a spatula after each aliquot is added. This densifies the carbon black and forms pellets. Add sufficient toluene to densify the entire sample into crude pellets. The beakers shall then be left in the hood overnight to evaporate the solvent. Once the solvent has evaporated, break up the larger of the carbon black pellets with a spatula. The entire amount of pelletized black is then transferred to the thimble.

10.9 Place a plug of glass wool or fold the top of the cellulose thimble above the black. The plug of wool should be placed such that the glass wool is in contact with the carbon



black. This facilitates drainage of solvent through the glass wool and into the carbon black bed.

10.10 Add a few glass boiling beads into the clean 250-cm<sup>3</sup> boiling flask and pour approximately 150 cm<sup>3</sup> of toluene. Assemble the extraction apparatus. To maintain temperature uniformity throughout the system, wrap the Soxhlet extractor and the boiling flask with aluminum foil. Record the Soxhlet position number, thimble identification (for glass thimbles), and extraction date.

#### 10.11 *Extraction Settings and Conditions:*

10.11.1 Samples are extracted for 16 h.

NOTE 1—Hamm et al<sup>7</sup> demonstrated that 16 h were sufficient to collect over 95 % of the BaP extractable in 48 h from a relatively high PAH content carbon black.

10.11.2 Turn on the water flow. It is recommended that a water-flow monitor, a solenoid valve to switch the water flow on or off, and a timer be assembled such that:

10.11.2.1 An interruption in the water flow triggers the timer to stop, the heating mantles to turn off, and the solenoid valve to turn off; and

10.11.2.2 A power outage stops the timer, switches off the solenoid valve, and prevents the heating mantles from turning back on once power is restored.

10.12 Nitrogen should continuously flow through a manifold connected to the condensers throughout the entire extraction period at a very low flow rate of approximately 10 to 15 sccm (standard cubic centimetres per minute). If the flow rate is too high, toluene may evaporate from the Soxhlet.

10.13 Turn the heating mantles on low.

10.14 After 5 to 20 min, the flasks should be on the verge of boiling. Gently agitate the apparatus; this should initiate the toluene boiling. Once this occurs, turn the heat to a higher setting. After about 30 min, check to ensure that the toluene is dripping from the condenser.

10.15 After at least 4 h of extraction, check and adjust the heater such that:

10.15.1 The cycle time of each Soxhlet is less than 6 min (approximately ten cycles per hour). This is the length of time for the Soxhlet to fill and the solvent to siphon out through the siphon arm. Note that this is not the drain time. Drain time is described in 10.15.2.

10.15.2 If glass extraction thimbles are used, the drain time of the thimbles should be less than 15 min. This is the time for the solvent to drain out of the thimble. This is measured by lowering the heat (to stop the solvent dripping from the condenser) and visually measuring the length of time for most of the solvent to drain through the thimble. If the drain time deviates from the above value, replace the thimble and restart the extraction.

10.16 Extract samples continuously for 16 h and the toluene level should be checked from time to time for potential losses

through evaporation. To avoid degradation of the extracted BaP by overheating of glass walls, a sufficient level of toluene shall always remain in the boiling flask especially at the point at which the Soxhlet is filled and the solvent is starting to siphon out.

10.17 If the remaining solvent volume becomes too small, the extraction has to be interrupted and fresh toluene be added to the extractor after cool down. The nitrogen flow should be checked and adjusted, if necessary. The extraction is subsequently continued to complete the required extraction time.

## 11. Extract Preparation before the GC/MS Analysis

11.1 Once the set extraction time has elapsed, the thimble is removed.

11.2 Depending upon the expected level of BaP in the carbon black sample, the raw toluene extract is processed differently.

11.3 For some extracts that contain very low levels of BaP, the IS solution prepared in 9.3 is added to the extract before the SPE and concentration steps. This is to correct for any potential loss of BaP during the SPE and rotary evaporation steps. In this case, add 200 µL of the IS solution prepared in 9.3 to the extract and swirl the boiling flask to mix in the IS solution. Then pass the entire extract through the silica SPE into the rotary evaporation flask. Rinse the boiling flask with a few cubic centimeters of fresh toluene through the SEP and into the rotary evaporation flask three times. Concentrate the extract under the conditions from 11.4 to about 0.5 cm<sup>3</sup>, then quantitatively transfer the concentrated extract into a 2-cm<sup>3</sup> amber volumetric flask, rinsing twice with about 0.5 cm<sup>3</sup> of fresh toluene. Bring the toluene level to exactly 2.0 cm<sup>3</sup> with toluene, cap securely, mix thoroughly, and label. Record the final extract volume,  $V_f$ , as 2.0 cm<sup>3</sup>. Retain in the 2-cm<sup>3</sup> amber volumetric flask or transfer to a 4-cm<sup>3</sup> vial with PTFE/rubber cap (PTFE side faces the toluene). An aliquot of at least 0.6 cm<sup>3</sup> can now be transferred to the amber GC/MS vial and analyzed by GC/MS as described in Section 12. For the IS standard example given in 9.3, the GC/MS vial allegedly contains 0.33 µg/cm<sup>3</sup> of the IS (CIS = 0.33 µg/cm<sup>3</sup>).

11.4 For all other cases, pass the entire extract through the silica SPE into the rotary evaporator flask. Rinse the boiling flask with fresh toluene and pass this through the SPE three times. For cases where it is known that the extract does not require concentration, it is acceptable to measure the volume of the extract to  $\pm 0.5$  cm<sup>3</sup>, record the volume as  $V_i$  (the initial extract volume), and proceed to 11.6. Otherwise, concentrate the toluene extract on the rotary evaporator down to about 50 cm<sup>3</sup> in the boiling flask. The rotary evaporator shall be set to a maximum bath temperature of  $40 \pm 2$  °C and a vacuum of  $5 \pm 0.3$  kPa. These parameters shall be monitored carefully during all evaporation steps as BaP losses may occur. Alternative evaporators or vacuum and temperature settings or both may be used provided recovery studies are conducted and results of these studies are acceptable.

NOTE 2—The purpose of the SPE is to remove some highly polar material that can contaminate the GC column and cause a problem with peak shape. The traditional way of performing SPE would be first to concentrate the extract to a much lower volume (2 to 3 cm<sup>3</sup>), then pour it

<sup>7</sup> Hamm et al, "Investigations on the extraction and migration behavior of polycyclic aromatic hydrocarbons (PAHs) from cured rubber formulations containing carbon black as reinforcing agent," *Rubber Chemistry & Technology*, Vol 82, Issue 2, 2009.