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Toxicological Review of Benzo[a]pyrene

Executive Summary

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EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

Benzo[a]pyrene is a five-ring polycyclic aromatic hydrocarbon (PAH). Benzo[a]pyrene (along with other PAHs) is released into the atmosphere as a component of smoke from forest fires, industrial processes, vehicle exhaust, cigarettes, and through the burning of fuel (such as wood, coal, and petroleum products). Oral exposure to benzo[a]pyrene can occur by eating certain food products, such as charred meats, where benzo[a]pyrene is formed during the cooking process, or by eating foods grown in areas contaminated with benzo[a]pyrene (from the air and soil). Dermal exposure may occur from contact with soils or materials that contain soot, tar, or crude petroleum products or by using certain pharmaceutical products containing coal tars, such as those used to treat the skin conditions, eczema and psoriasis. The magnitude of human exposure to benzo[a]pyrene and other PAHs depends on factors such as lifestyle (e.g., diet, tobacco smoking), occupation, and living conditions (e.g., urban versus rural setting, domestic heating, and cooking methods).

Animal studies demonstrate that exposure to benzo[a]pyrene is associated with developmental (including developmental neurotoxicity), reproductive, and immunological effects. In addition, epidemiology studies involving exposure to PAH mixtures have reported associations between internal biomarkers of exposure to benzo[a]pyrene (benzo[a]pyrene diol epoxide-DNA adducts) and adverse birth outcomes (including reduced birth weight, postnatal body weight, and head circumference), neurobehavioral effects, and decreased fertility.

Studies in multiple animal species demonstrate that benzo[a]pyrene is carcinogenic at multiple tumor sites (alimentary tract, liver, kidney, respiratory tract, pharynx, and skin) by all routes of exposure. In addition, there is strong evidence of carcinogenicity in occupations involving exposure to PAH mixtures containing benzo[a]pyrene, such as aluminum production, chimney sweeping, coal gasification, coal-tar distillation, coke production, iron and steel founding, and paving and roofing with coal tar pitch. An increasing number of occupational studies demonstrate a positive exposure-response relationship with cumulative benzo[a]pyrene exposure and lung cancer.

Effects Other Than Cancer Observed Following Oral Exposure

In animals, oral exposure to benzo[a]pyrene has been shown to result in developmental toxicity (including developmental neurotoxicity), reproductive toxicity, and immunotoxicity. Developmental effects in rats and mice include neurobehavioral changes and cardiovascular effects following gestational exposures. Reproductive and immune effects include decreased sperm counts, ovary weight, and follicle numbers, and decreased immunoglobulin and B cell numbers and thymus weight following oral exposures in adult animals. In humans, benzo[a]pyrene exposure occurs in conjunction with other PAHs and, as such, attributing the observed effects to

benzo[a]pyrene is complicated. However, some human studies report associations between particular health endpoints and internal measures of exposure, such as benzo[a]pyrene-deoxyribonucleic acid (DNA) adducts, or external measures of benzo[a]pyrene exposure. Overall, the human studies report developmental, neurobehavioral, reproductive, and immune effects that are generally analogous to those observed in animals, and provide qualitative, supportive evidence for hazards associated with benzo[a]pyrene exposure.

Oral Reference Dose (RfD) for Effects Other Than Cancer

Organ- or system-specific RfDs were derived for hazards associated with benzo[a]pyrene exposure where data were amenable (see Table ES-1). These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Developmental toxicity, represented by neurobehavioral changes persisting into adulthood, was chosen as the basis for the overall oral RfD as the available data indicate that developmental neurotoxicity represents the most sensitive hazard of benzo[a]pyrene exposure. The neurodevelopmental study by Chen et al. (2012) was used to derive the RfD. Altered responses in three behavioral tests (i.e., Morris water maze, elevated plus maze, and open field tests) were selected to represent the critical effect of abnormal behavior, due to the consistency (i.e., each of these responses were affected in two separate cohorts of rats, including testing as juveniles and as adults; similar effects in these behavioral tests were observed across studies) and sensitivity of these responses, and the observed dose-response relationship of effects across dose groups. Benchmark dose (BMD) modeling for each of the three endpoints resulted in $BMDL_{1SD}$ values that clustered in the range 0.092–0.16 mg/kg-day. The lower end of this range of BMDLs, 0.092 mg/kg-day, was selected to represent the point of departure (POD) from these three endpoints for RfD derivation.

The overall RfD was calculated by dividing the POD for altered behavior in three tests of nervous system function by a composite uncertainty factor (UF) of 300 to account for the extrapolation from animals to humans (10), for interindividual differences in human susceptibility (10), and for deficiencies in the toxicity database (3).

Table ES-1. Organ/system-specific RfDs and overall RfD for benzo[a]pyrene

Effect	Basis	RfD (mg/kg-d)	Confidence
Developmental	Neurobehavioral changes Gavage neurodevelopmental study in rats (postnatal days [PNDs] 5–11) Chen et al. (2012)	3×10^{-4}	Medium
Reproductive	Decreased ovarian follicles and ovary weight Gavage subchronic (60 d) reproductive toxicity study in rats Xu et al. (2010)	4×10^{-4}	Medium
Immunological	Decreased thymus weight and serum IgM Gavage subchronic (35 d) study in rats De Jong et al. (1999) and Kroese et al. (2001)	2×10^{-3}	Low
Overall RfD	**Developmental toxicity (including developmental neurotoxicity)**	3×10^{-4}	**Medium**

Confidence in the Overall Oral RfD

The overall confidence in the RfD is medium. Confidence in the principal study (Chen et al., 2012) is medium. The design, conduct, and reporting of this neurodevelopmental study was good and a wide variety of neurotoxicity endpoints were measured across 40 litters of rats. However, some uncertainty exists regarding the authors' use of dam rotation across litters (an attempt to reduce potential nurturing bias) and a within-litter dosing design, by potentially introducing maternal stress or other unanticipated consequences in the pups, and some informative experimental details were omitted, including the sensitivity of some assays at the indicated developmental ages and lack of reporting of individual animal- or gender-specific data for all outcomes. Several subchronic and developmental studies covering a wide variety of endpoints are also available; however, a multigeneration toxicity study with exposure throughout development and across generations is not available, and the available neurotoxicity studies did not comprehensively evaluate all potentially vulnerable lifestages of nervous system development. Therefore, confidence in the database is medium.

Effects Other Than Cancer Observed Following Inhalation Exposure

In animals, inhalation exposure to benzo[a]pyrene has been shown to result in developmental and reproductive toxicity. Studies in rats following inhalation exposure show decreased embryo/fetal survival and nervous system effects in offspring, and decreased testes weight and sperm counts in adult animals. Overall, the available human PAH mixtures studies report developmental and reproductive effects that are generally analogous to those observed in animals, and provide qualitative, supportive evidence for the hazards associated with benzo[a]pyrene exposure.

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

An attempt was made to derive organ- or system-specific RfCs for hazards associated with benzo[a]pyrene exposure where data were amenable (see Table ES-2). These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Developmental toxicity, represented by decreased embryo/fetal survival, was chosen as the basis for the proposed inhalation RfC as the available data indicate that developmental effects represent a sensitive hazard of benzo[a]pyrene exposure. The developmental inhalation study in rats by Archibong et al. (2002) and the observed decreased embryo/fetal survival (i.e., increased resorptions) following exposure to benzo[a]pyrene on gestation days (GDs) 11–20 were used to derive the overall RfC. The lowest-observed-adverse-effect level (LOAEL) of 25 μg/m^3 based on decreased embryo/fetal survival was selected as the POD. The LOAEL was adjusted to account for the discontinuous daily exposure to derive the POD_{ADJ} and the human equivalent concentration (HEC) was calculated from the POD_{ADJ} by multiplying by the regional deposited dose ratio ($RDDR_{ER}$) for extrarespiratory (i.e., systemic) effects, as described in *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b). These adjustments resulted in a POD_{HEC} of 4.6 μg/m^3, which was used as the POD for RfC derivation.

The RfC was calculated by dividing the POD by a composite UF of 3,000 to account for toxicodynamic differences between animals and humans (3), interindividual differences in human susceptibility (10), LOAEL-to-no-observed-adverse-effect level (NOAEL) extrapolation (10), and deficiencies in the toxicity database (10).

Table ES-2. Organ/system-specific RfCs and overall RfC for benzo[a]pyrene

Effect	Basis	RfC (mg/m^3)	Confidence
Developmental	Decreased embryo/fetal survival Developmental toxicity study in rats (GDs 11–20) Archibong et al. (2002)	2×10^{-6}	Low-medium
Reproductive	Reduced ovulation rate and ovary weight Premating study in rats (14 d) Archibong et al. (2012)	3×10^{-6}	Low-medium
Overall RfC	**Developmental toxicity**	2×10^{-6}	Low-medium

Confidence in the Overall Inhalation RfC

The overall confidence in the RfC is low-to-medium. Confidence in the principal study (Archibong et al., 2002) is medium. The conduct and reporting of this developmental inhalation study were adequate; however, a NOAEL was not identified. Confidence in the database is low due to the lack of a multigeneration toxicity study and the lack of information on varied toxicity

endpoints following subchronic and chronic inhalation exposure. However, confidence in the RfC is bolstered by consistent systemic effects observed by the oral route (including reproductive and developmental effects) and similar effects observed in human populations exposed to PAH mixtures.

Evidence for Human Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), benzo[a]pyrene is "carcinogenic to humans" based on strong and consistent evidence in animals and humans. The evidence includes an extensive number of studies demonstrating carcinogenicity in multiple animal species exposed via all routes of administration and increased cancer risks, particularly in the lung and skin, in humans exposed to different PAH mixtures containing benzo[a]pyrene. Mechanistic studies provide strong supporting evidence that links the metabolism of benzo[a]pyrene to DNA-reactive agents with key mutational events in genes that can lead to tumor development. These events include formation of specific DNA adducts and characteristic mutations in oncogenes and tumor suppressor genes that have been observed in humans exposed to PAH mixtures. This combination of human, animal, and mechanistic evidence provides the basis for characterizing benzo[a]pyrene as "carcinogenic to humans."

Quantitative Estimate of Carcinogenic Risk From Oral Exposure

Lifetime oral exposure to benzo[a]pyrene has been associated with forestomach, liver, oral cavity, jejunum or duodenum, and auditory canal tumors in male and female Wistar rats, forestomach tumors in male and female Sprague-Dawley rats, and forestomach, esophagus, tongue, and larynx tumors in female $B6C3F_1$ mice (male mice were not tested). Less-than-lifetime oral exposure to benzo[a]pyrene has also been associated with forestomach tumors in more than 10 additional bioassays with several strains of mice. The Kroese et al. (2001) and Beland and Culp (1998) studies were selected as the best available studies for dose-response analysis and extrapolation to lifetime cancer risk following oral exposure to benzo[a]pyrene. These studies included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated animals for up to 2 years, and included detailed reporting methods and results (including individual animal data).

Time-weighted average (TWA) daily doses were converted to human equivalent doses (HEDs) on the basis of (body weight [BW])$^{3/4}$ scaling (U.S. EPA, 1992). EPA then used the multistage-Weibull model for the derivation of the oral slope factor. This model was used because it incorporates the time at which death-with-tumor occurred and can account for differences in mortality observed between the exposure groups. Using linear extrapolation from the $BMDL_{10}$, human equivalent oral slope factors were derived for each gender/tumor site combination (slope factor = $0.1/BMDL_{10}$) reported by Kroese et al. (2001) and Beland and Culp (1998). The oral slope factor of **1 per mg/kg-day** based on the tumor response in the alimentary tract (forestomach,

esophagus, tongue, and larynx) of female B6C3F$_1$ mice (Beland and Culp, 1998) was selected as the factor with the highest value (most sensitive) among a range of slope factors derived.

Quantitative Estimate of Carcinogenic Risk From Inhalation Exposure

Inhalation exposure to benzo[a]pyrene has been associated with squamous cell neoplasia in the larynx, pharynx, trachea, nasal cavity, esophagus, and forestomach of male Syrian golden hamsters exposed for up to 130 weeks to benzo[a]pyrene condensed onto sodium chloride particles (Thyssen et al., 1981). Supportive evidence for the carcinogenicity of inhaled benzo[a]pyrene comes from additional studies with hamsters exposed to benzo[a]pyrene via intratracheal instillation. The Thyssen et al. (1981) bioassay represents the only study of lifetime exposure to inhaled benzo[a]pyrene.

A time-to-tumor dose-response model was fit to the TWA continuous exposure concentrations and the individual animal incidence data for the overall incidence of tumors in the upper respiratory tract or pharynx. The inhalation unit risk of **6×10^{-4} per µg/m^3** was calculated by linear extrapolation (slope factor = 0.1/BMCL$_{10}$) from a BMCL$_{10}$ of 0.16 mg/m^3 for the occurrence of upper respiratory and upper digestive tract (forestomach) tumors in male hamsters chronically exposed by inhalation to benzo[a]pyrene (Thyssen et al., 1981).

Quantitative Estimate of Carcinogenic Risk From Dermal Exposure

Skin cancer in humans has been documented to result from occupational exposure to complex mixtures of PAHs including benzo[a]pyrene, such as coal tar, coal tar pitches, unrefined mineral oils, shale oils, and soot. In animal models, numerous dermal bioassays have demonstrated an increased incidence of skin tumors with increasing dermal exposure of benzo[a]pyrene in all species tested, although most benzo[a]pyrene bioassays have been conducted in mice.

Carcinogenicity studies in animals by the dermal route of exposure are available for benzo[a]pyrene and are supportive of the overall cancer hazard. A quantitative estimate of skin cancer risk from dermal exposure is not included in this assessment, as methodology for interspecies extrapolation of dermal toxicokinetics and carcinogenicity are still under development.

Susceptible Populations and Lifestages

Benzo[a]pyrene has been determined to be carcinogenic by a mutagenic mode of action in this assessment. According to the *Supplemental Guidance for Assessing Susceptibility from Early Life Exposure to Carcinogens* (U.S. EPA, 2005b), individuals exposed during early life to carcinogens with a mutagenic mode of action are assumed to have an increased risk for cancer. The oral slope factor of 1 per mg/kg-day and inhalation unit risk of 0.0006 per µg/m^3, calculated from data applicable to adult exposures, do not reflect presumed early life susceptibility to this chemical. Although some chemical-specific data exist for benzo[a]pyrene that demonstrate increased early life susceptibility to cancer, these data were not considered sufficient to develop separate risk estimates for childhood exposure. In the absence of adequate chemical-specific data to evaluate differences in

age-specific susceptibility, the *Supplemental Guidance* (U.S. EPA, 2005b) recommends that age-dependent adjustment factors (ADAFs) be applied in estimating cancer risk. The ADAFs are 10- and 3-fold adjustments that are combined with age specific exposure estimates when estimating cancer risks from early life (<16 years of age) exposures to benzo[a]pyrene.

Regarding effects other than cancer, there are epidemiological studies that report associations between developmental effects (decreased postnatal growth, decreased head circumference, and neurodevelopmental delays), reproductive effects, and internal biomarkers of exposure to benzo[a]pyrene. Studies in animals also indicate alterations in neurological development and heightened susceptibility to reproductive effects following gestational or early postnatal exposure to benzo[a]pyrene. More preliminary data suggest that effects on cardiovascular, kidney, pulmonary, and immune system development may result from early life exposures, although few in vivo developmental studies exist to confirm these findings.

Key Issues Addressed in Assessment

The overall RfD and RfC were developed based on effects observed following exposure to benzo[a]pyrene during a critical window of development. The derivation of a general population toxicity value based on exposure during development has implications regarding the evaluation of populations exposed outside of the developmental period and the averaging of exposure to durations outside of the critical window of susceptibility. Discussion of these considerations is provided in Sections 2.1.5 and 2.2.5.

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