



Draft IRIS Assessment of Benzo[a]pyrene

Presentation for the
Benzo[a]pyrene Augmented Chemical Assessment Advisory Committee of
the Science Advisory Board
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This presentation will:

- Briefly review key aspects of the Benzo[a]pyrene Toxicological Review.
- Address questions raised by CAAC panel members and public commenters at the teleconference held on March 4, 2015.
- Note several key assumptions in the derivation of the dermal slope factor.



General Information

- Draft Toxicological Review is a re-assessment of the 1987 BaP assessment on IRIS
 - 1987 IRIS assessment contains an oral slope factor and cancer descriptor (probable human carcinogen)
- Five-ring polycyclic aromatic hydrocarbon (PAH)
 - Exposure occurs as a mixture of PAHs
 - Most well studied PAH
 - Used as an index chemical for PAH mixtures
- Major sources of environmental exposure:
 - Burning of fossil fuels (especially wood and coal), motor vehicle exhaust, power plants, and various industrial combustion processes
 - Natural sources include forest fires and volcanoes
- Occupational exposure:
 - Production of aluminum, coke, tar, shale oil, and carbon black; coal gasification, iron and steel foundries, wood impregnation, roofing, road paving, chimney sweeping, etc.
- Non-occupational exposure:
 - Tobacco products
 - Diet (e.g., barbequed, smoked, or contaminated foods)
 - Topical therapies for psoriasis/eczema containing coal tar



Health Hazards Identified

EFFECTS OTHER THAN CANCER

- Animal studies indicate there is evidence for potential hazards, i.e., **developmental, reproductive, and immune system toxicity**.
- Human studies report effects that are generally analogous to the effects observed in animal toxicological studies, and provide supportive evidence.

CANCER

- Under EPA's *Guidelines for Carcinogen Risk Assessment* (2005) BaP is "**carcinogenic to humans**" based on strong and consistent evidence in humans and animals, including mechanistic data.
- The overall evidence supports **mutagenicity** as the primary mode of action for BaP-induced carcinogenicity.



Reference Values Derived

Chronic RfD	Endpoint	mg/kg-d
Developmental: Chen et al. (2012) Neurodevelopmental study in rats	Neurobehavioral changes	3×10^{-4}
Reproductive: Xu et al. (2010) 60 day reproductive study in adult rats	Decreased ovary weight	4×10^{-4}
Immunological: De Jong et al. (1999) 35 day study in adult rats	Decreased thymus weight and IgM	2×10^{-3}

Chronic RfC		mg/m ³
Developmental: Archibong et al. (2002) Developmental study in rats	Decreased fetal survival	2×10^{-6}



Summary of the Cancer Risk Values

	Principal study	Elevated tumor types	Cancer risk values
Oral Slope Factor	Beland and Culp (1998) female mice	Esophagus, tongue, and larynx squamous cell tumors	1 (mg/kg-d) ⁻¹
Inhalation Unit Risk	Thyssen et al. (1981) male hamsters	Upper respiratory and digestive tract tumors (larynx, pharynx, trachea, esophagus, and forestomach)	0.6 (mg/m ³) ⁻¹
Dermal Slope Factor	Sivak et al. (1997); NIOSH (1989) male mice	Skin tumors (papillomas and carcinomas)	0.006 (μg/d) ⁻¹



Summary of Questions Raised During March 4th Teleconference

- Clarification of literature search details [panel]
- Question of whether BaP adducts are quantitatively associated with cancer risk [panel]
- Question regarding use of rat versus mouse in characterizing immunotoxicity [panel]
- Potential sex-related differences in BaP dermal carcinogenicity [panel]
- Request to include additional therapeutic coal tar references [public comment]
- Question regarding the use of the human studies of PAH mixtures [public comment]
- Question about validity of dermal slope factor considering commenter's estimated dermal dose from urban soil exposure and associated risk [public comment]

Additional details regarding the BaP literature search were requested during the teleconference. [panel]

- Front matter of the Toxicological Review includes a Literature Search Strategy/Study Selection Section and a summary flow diagram.
- Comprehensive literature search up through Feb 2012
- Literature search updated through August 2014
- Databases searched: Pubmed, Toxcenter, Toxline, TSCATS, ChemID, Chemfinder, CCRIS, HSDB, GENETOX, RTECS
 - Primary and secondary keywords used for the databases can be found in Appendix C of the Supplemental Information.



Objectives of Literature Search

Identify primary sources of health effects data in humans and animals in order to evaluate potential human health effects associated with chronic exposure to BaP.

- Focus on publicly available, peer-reviewed literature.
- Scope includes epidemiological, experimental animal, and mechanistic data via oral, inhalation and dermal routes of exposure.

- Exposure is to BaP (or PAH mixtures with a measure of BaP).
- Exposure is measured in environmental/biological media or tissues.
- Study includes a measure of one or more primary health effect endpoints.
- Study includes a measure of one or more secondary health effect endpoints (e.g., genotoxicity, oxidative stress, inflammation, etc) evaluating cellular, biochemical, or molecular effects relevant to mode of action.



Exclusion Criteria

- References not relevant to BaP toxicity in mammals (e.g., toxicity in aquatic species, plants).
- References not pertinent to evaluating primary sources of potential health effects (e.g., site-specific risk assessments, chemical analytical method studies, review articles, editorials, and environmental fate and transport studies).
- References in which BaP is used as the positive control for evaluation of carcinogenicity/genotoxicity of other chemicals.
- References with an inadequate basis to infer BaP exposure.
- References that inadequately report study methods or results.
- References evaluating animal toxicity of chemical mixtures; less relevant for evaluating BaP-specific effects.
- References available only as an abstract.



BaP-DNA Adducts

Question of whether BaP DNA adducts are quantitatively associated with cancer risk in humans. [panel]

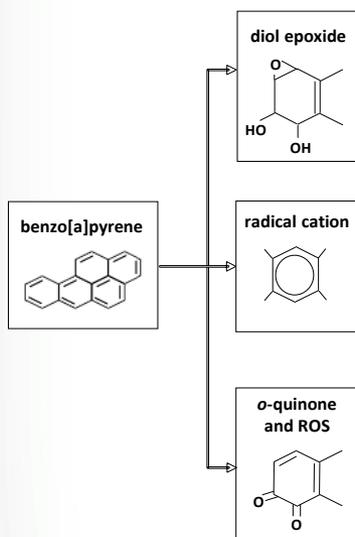
- DNA adducts not used in a quantitative manner in this assessment.
- DNA adducts are sensitive biomarkers of PAH exposure in humans.
- BPDE-DNA adducts are specific to BaP exposures within PAH mixtures.
- These adducts are known to lead to signature mutations (G→T transversions).
- Unique mutational spectra resulting from BPDE-DNA adducts found in PAH-associated tumors in humans at mutational hotspots (p53, K-ras).
- Specific distribution of BPDE-DNA adducts to these same p53 hotspots are observed following in vitro exposures to BaP in human cells.

Exposure-Response:

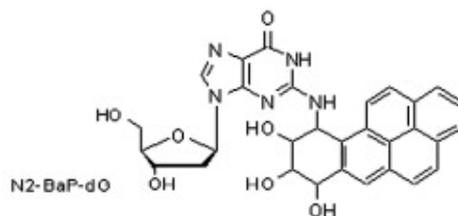
- Clear association between exposure to BaP and increased DNA adducts and tumor incidence in animal models.
- BPDE-DNA adducts are significantly increased in cancer patients who were smokers or occupationally exposed to PAHs; adduct levels highly correlated with increased CYP1A1 activity and/or GSTM1 null genotypes.
- Human PAH exposure correlates with increased BaP adducts and HPRT mutations.

There is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information

Bioactivation



DNA adduct formation



Mutation

Predominantly
G:C to T:A
transversions

- Polymorphisms in CYP or AhR (phase I) or GST (phase II) genes lead to increased adduct formation and cancer risk

- BaP-specific adducts detected at significantly higher levels in cancer patients previously exposed to PAHs

- BaP-specific mutational spectra identified in K-ras and p53 in PAH-associated tumors in humans



Animal Model for Characterizing Immunotoxicity

Question was raised regarding the use of rats (rather than mice) to characterize immunotoxicity. [panel]

- Available studies measuring immune endpoints (oral, no inhalation):
 - Kroese et al., 2001; 90d gavage study Wistar rats
 - DeJong et al., 1999; 35d gavage study Wistar rats
- No subchronic or chronic studies identified in mice (no oral, no inhalation).
 - One intratracheal administration study (Schnizlein et al., 1987)
 - Several injection studies in mice included in hazard discussion:
 - In adult mice (Lyte and Bick, 1985; Dean et al., 1983; Munson and White, 1983; Temple et al., 1993)
 - In utero (Holladay and Smith, 1995, 1994; Urso and Johnson, 1988)
 - SRBC assay using mice and rats (Temple et al., 1993)
- Available mice studies provide support for immunotoxicity effects observed in rats.
- Environmental route of exposure (i.e., oral or inhalation) and \geq subchronic duration preferred for RfD/RfC derivation; thus rat study selected for derivation of immune RfD.



Potential Sex Differences in Dermal Carcinogenesis

Question regarding whether sex-specific differences in BaP-induced dermal carcinogenicity are apparent across the database. [panel]

- Several lifetime mouse studies modeled to inform low dose dermal cancer risk.
 - Individual studies included only one sex (2 data sets in males; 8 datasets in females).
 - Not possible to evaluate the relative sensitivity of male and female mice.
 - Dermal slope factor derived from data in male mice (Sivak et al. 1997/NIOSH 1989).
- Less than lifetime studies may inform sex related differences in dermal carcinogenicity.
 - Wilson and Holland (1988): 10 month study in C3H/He male and female mice
 - No difference in tumor incidence or multiplicity between sexes.
 - Earlier time to tumor for female mice.
 - High response observed at lowest dose (95%); subtle low dose effects may be masked.
 - Nesnow et al. (1983): 1 year study in SENCAR male and female mice
 - No difference in tumor incidence or multiplicity between sexes.
 - Low response at lowest doses tested; subtle low dose effects likely not masked.
- Available BaP dermal carcinogenesis studies do not indicate clear sex-specific differences.



Inclusion of Additional Coal Tar studies

Several studies regarding therapeutic use of coal tar not cited in the Toxicological Review of Benzo[a]pyrene. [public comment]

- Prior to peer review, public comments from American Coke and Coal Chemicals Institute (submitted to EPA in November 2013) included a list and table of 20 citations pertaining to pharmaceutical use of coal tar (pp 102-107 of those comments).
- In response, EPA included a number of these studies in the assessment.
 - Increased discussion of coal tar studies in the carcinogenicity hazard section.
 - Added a discussion of studies that evaluated non-melanoma skin cancer risk in patients treated with coal tar in the Appendix (pp D-33 to D-38).
 - Case reports, reviews, and studies that did not include a measure of coal tar use were not included in the assessment.
- Provided a written response to the public comments on this topic in Appendix D.3.3.



Additional Coal Tar studies (cont)

Clarification was requested regarding whether these studies are included in EPA's HERO database so that panel members may access them.

- 1/3 were already cited in assessment (and therefore included in HERO)
- 1/3 were reviews or letters to the editors (not primary health effects data, thus not included)
- Remaining studies were added to HERO, but have various limitations of design
 - Did not analyze skin cancer risk in relation to coal tar exposure
 - No information to characterize exposure level of coal tar (e.g., duration or number of treatments)
 - No information on length of follow up
 - Or authors expressed low confidence in registry data used to assess incidence of non-melanoma skin cancer

Question regarding the discussion of PAH Mixtures studies in humans, as PAH mixtures contain many components considered to be carcinogenic. [public comment]

- Environmental exposure to BaP occurs as a complex mixture of PAHs and other components, therefore it is difficult to attribute effects to any one component (as noted in the Preface, Literature Search/Study Selection chapter, and Section 1.1).
- The discussion of the human evidence for carcinogenicity primarily focuses on epidemiologic studies that include a direct measure of BaP exposure (see Section 1.1.5).
- Human studies involving exposure to PAH mixtures provide mechanistic data supporting key precursor events for carcinogenicity identified in animals.
- The few human studies of noncancer effects highlighted in evidence tables include a measure of BaP exposure.
 - Noncancer health effects in populations highly exposed to PAH mixtures (but with no measure of BaP exposure) mentioned briefly in hazard section along with a disclaimer regarding inability to attribute effects to any one component.
- All proposed toxicity values in this draft assessment are derived from animal studies using BaP only, not PAH mixture studies.



Dermal Slope Factor: Estimating Soil Exposure and Associated risk

Commenter estimated urban soil exposure and associated risk to perform a “real world validation” to determine if the proposed dermal slope factor is scientifically supportable. [public comment]

- In response to previous public comments, and because the BaP assessment contains a novel derivation of a dermal slope factor, EPA provided illustrative example calculations (to demonstrate a theoretical implementation) to estimate average daily dose of BaP contacting the skin (not absorbed) through soil exposure and the estimated increased cancer risk at that dose.
 - Example calculation in appendix calculated a point estimate of exposure using:
 - Exposure factors for a “central tendency exposure”
 - An average (not high-end) soil concentration (100ppb) from uncontaminated sites measured in studies cited in Appendix A
 - At this point estimate of exposure, associated risk was 7×10^{-6} .
 - Details provided in Appendix G as part of the response to comments.
 - Example calculations not intended to be a validation exercise.
 - Probabilistic methods could yield more representative estimates of exposure.
- Public Commenter estimated urban soil exposure and associated risk
 - Exposure calculated using high-end exposure assumptions (70 years of exposure; adult gardener scenario)
 - Resulting high-end exposure estimate and associated risk extrapolated to the entire urban US population



Dermal Slope Factor –Key Assumptions

Dose metric

- DSF expressed in terms of risk per ug/day of BaP
 - DSF was derived based on applied, not absorbed dose of BaP
 - Modeled studies did not quantify the actual cm² of dorsal skin treated
 - It is assumed that risk at *low doses* of BaP is dependent on absolute dermal dose
 - Skin surface area exposed to BaP is an important variable, considered as part of exposure assessment

Interspecies scaling

- Established methodology does not exist to adjust for interspecies differences at the point of contact
 - BaP metabolism is known to occur in the dermal layer
 - Viewing skin as an organ, and without evidence to the contrary, metabolic processes were assumed to scale allometrically

Adjustment for decreased bioavailability in soil

- Animal studies applied BaP in solvent
 - Suggested adjustment for soil exposure (25%) calculated based on study of BaP dermal exposure in monkeys (Wester et al. 1990).
 - Added as part of example exposure equation (see Appendix G).



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BaP Assessment Represents a Significant Advancement for the IRIS Program

- Contains the first non-cancer reference values for BaP on IRIS.
- Derives multiple organ/system-specific reference values (to facilitate subsequent risk assessments of multiple chemicals).
- Provides an updated oral slope factor for BaP.
- Provides an inhalation unit risk for BaP.
- Proposes the first dermal slope factor for any agent on IRIS.
- Is the first characterization on IRIS of a chemical as “carcinogenic to humans” based in part on mechanistic data.
- Contains the first systematic analysis of transcriptomics data in an IRIS assessment.
- Addresses public comments.
- Represents a significant advance for the IRIS Program in implementing the 2011 and 2014 NRC recommendations.