



Overview of the Draft IRIS Assessment of Benzo[a]pyrene

Presentation for the
Benzo[a]pyrene Augmented Chemical Assessment Advisory Committee of
the Science Advisory Board
March 4, 2015

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This presentation will cover:

- Implementation of 2011 and 2014 NRC recommendations in the benzo[a]pyrene assessment
- General information on benzo[a]pyrene (BaP)
- Overview of the Toxicological Review
- Major public comments and EPA's responses to those comments



Implementation of 2011 and 2014 NRC Recommendations

All IRIS assessments now include:

- Revised document structures to enhance clarity, reduce volume, address redundancies and inconsistencies, and include:
 - Distinct sections for hazard identification and dose-response
 - An executive summary that concisely summarizes major conclusions
 - A preamble that describes IRIS assessment methods
 - A detailed literature search strategy
 - Use of the HERO database
 - Standardized presentation of evidence in tables and arrays
- Assessments will continue to be updated based on feedback.



Advancements of the State of the Science in this Assessment

- A more systematic approach for evidence integration
- Uniform language to describe hazard conclusions for noncancer effects
- Multiple organ/system specific reference values
 - Increases transparency
 - Greater use of entire database
 - Supports cumulative risk assessment
- Prominent use of mechanistic data
 - Cancer descriptor
 - Systematic analysis of transcriptomics data
- Innovative methods to assess dermal cancer risk

- Five-ring polycyclic aromatic hydrocarbon (PAH)
 - Exposure occurs as a mixture of PAHs
 - Most well studied PAH
 - Used as an index chemical for PAHs
- 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, graphite, and coal tar
- Non-occupational exposure:
 - Tobacco products
 - Diet (e.g., barbequed, smoked, or contaminated foods)



Literature Search Strategy

References identified based on initial keyword search (see Table LS-1): **~21,000 references**

Secondary keyword searching (see Table LS-1): **~14,600 references excluded**

References identified based on secondary keyword search (see Table LS-1): **~6,100 references**

30 references submitted by American Petroleum Institute

Manual screen of titles/abstracts: **~4,940 references excluded**

- Not relevant to BaP toxicity in mammals (e.g., toxicity in aquatic species, plants)
- Site-specific risk assessments
- Chemical analytical methods
- Cancer chemotherapy studies

Considered for inclusion in the Toxicological Review: **~ 1,000 references**; references subsequently evaluated based on Preamble Section 3

Manual screen of manuscripts excluded: **~ 600 references**

- Not relevant to BaP toxicity in mammals
- Inadequate basis to infer exposure
- Inadequate reporting of study methods or results
- Animal toxicity studies with mixtures of chemicals
- Abstracts
- Duplicates

Approximately **700 references** cited in the Draft Toxicological Review

- Developmental toxicity: **37 references**
- Reproductive toxicity: **70 references**
- Immunotoxicity: **58 references**
- Other Toxicological Effects: **27 references**
 - Forestomach toxicity: **5 references**
 - Hematological toxicity: **3 references**
 - Liver toxicity: **3 references**
 - Kidney toxicity: **3 references**
 - Cardiovascular toxicity: **11 references**
 - Neurological toxicity: **12 references**
- Carcinogenicity: **171 references**
- Toxicokinetic: **115 references**
- Genotoxicity: **196 references**

Literature search output and references available on HERO (<https://hero.epa.gov>)

[see Figure LS-I of Toxicological Review]

Human data

- Multiple studies of human exposures to PAH mixtures (some with BaP-specific exposure metrics)

Animal data

- Many chronic oral cancer bioassays
- Many dermal cancer bioassays
- One chronic inhalation cancer bioassay
- Several subchronic studies looking at a variety of noncancer endpoints (including reproductive and developmental studies)

Other information

- Toxicokinetics
- Numerous mechanistic studies (including transcriptomics data)



Hazard Identification - noncancer

Developmental	Reproductive	Immunological
decreased body weight decreased fetal survival decreased fertility atrophy of reproductive organs altered neurobehavioral outcomes	decreased sperm parameters decreased reproductive organ weights histological changes hormone alterations	altered immune cell populations decreased immunoglobulin levels histopathological changes in the spleen and thymus
<i>Developmental toxicity is a human hazard of BaP exposure.</i>	<i>Reproductive toxicity is a human hazard of BaP exposure.</i>	<i>Immunotoxicity is a potential human hazard of BaP exposure.</i>

- Human studies involving PAH mixtures report generally analogous effects.
- The vast majority of the available mechanistic data inform the carcinogenic effects of BaP, however some data are available to inform potential mechanisms associated with noncancer effects.
- There is less evidence for effects in other organ/systems (e.g., liver, kidney, and cardiovascular system).



RfD Derivation

Effect	Point of Departure (mg/kg-d)	UF	Chronic RfD (mg/kg-d)	Confidence
Developmental: Neurobehavioral changes Chen et al. (2012) Neurodevelopmental study in rats	BMDL: 0.086	Total UF = 300 $UF_A = 10$ $UF_H = 10$ $UF_{DB} = 3$	3×10^{-4}	Medium
Reproductive: Decreased ovary weight Xu et al. (2010) 60 day reproductive study in adult rats	BMDL: 0.37	Total UF = 1000 $UF_A = 3$ $UF_H = 10$ $UF_S = 10$ $UF_{DB} = 3$	4×10^{-4}	Medium
Immunological: Decreased thymus weight and IgM De Jong et al. (1999) 35 day study in adult rats	BMDL: 1.9	Total UF = 1000 $UF_A = 3$ $UF_H = 10$ $UF_S = 10$ $UF_{DB} = 3$	2×10^{-3}	Low
Proposed Overall Reference Dose (RfD) - Developmental			3×10^{-4}	Medium

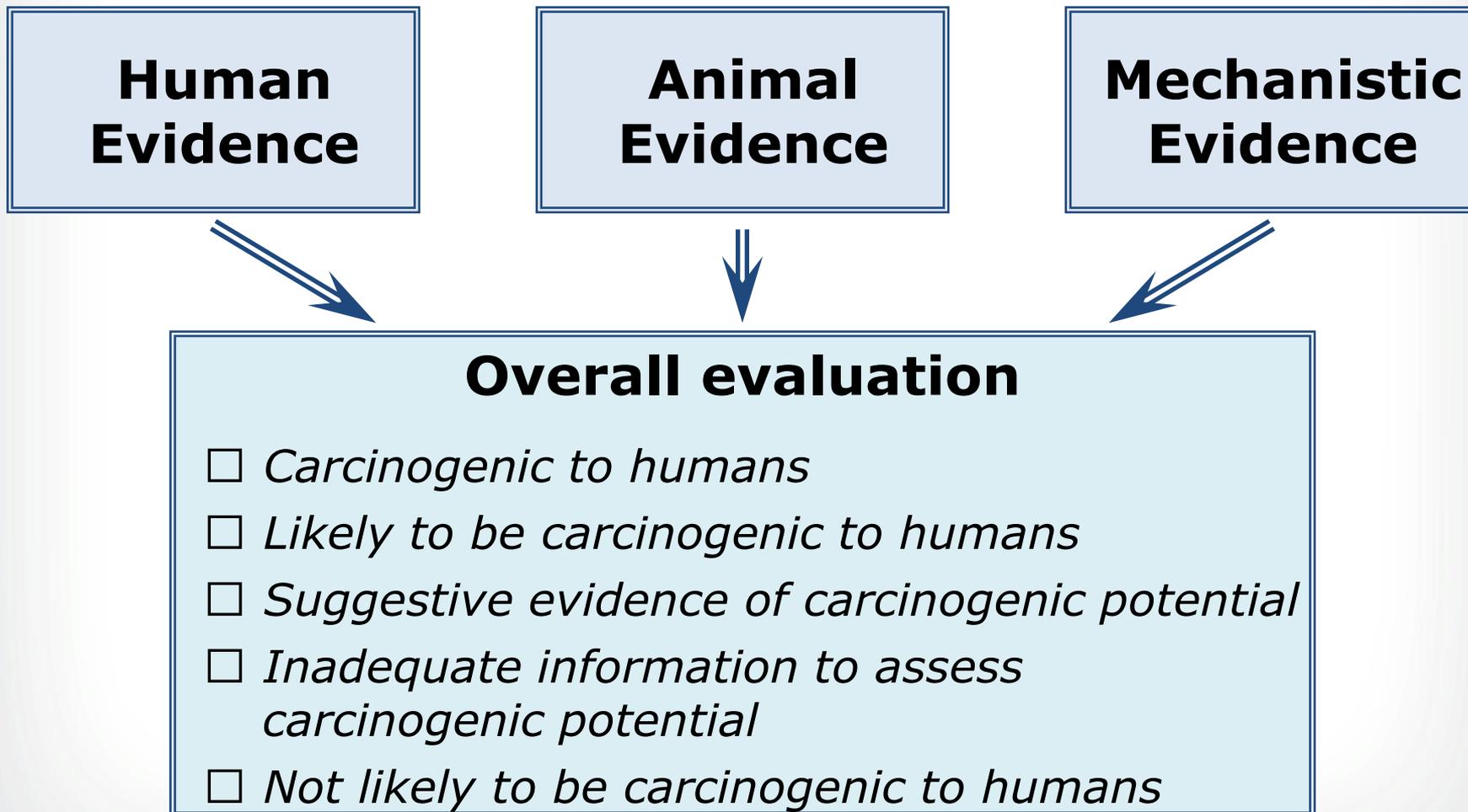
[see Tables 2-1, 2-2, 2-3 of Toxicological Review]



RfC Derivation

Effect	Point of Departure (mg/m ³)	UF	Chronic RfC (mg/m ³)	Confidence
Developmental: Decreased fetal survival Archibong et al. (2002) Developmental study in rats	LOAEL: 0.0046	Total UF = 3000 UF _A = 3 UF _H = 10 UF _L = 10 UF _D = 10	2×10^{-6}	Low-medium
Reproductive: Reductions in testes weight and sperm parameters Archibong et al. (2008); Ramesh et al. (2008) 60 day reproductive study in rats	LOAEL: 0.014	Total UF = 30,000 UF _A = 3 UF _H = 10 UF _L = 10 UF _S = 10 UF _D = 10	Not calculated due to UF >3000	N/A
Proposed Overall Reference Concentration (RfC) - Developmental			2×10^{-6}	Low-medium

- Data were insufficient to derive an organ/system-specific reference value for immunological hazard.





Cancer Descriptor

Supporting evidence for the *carcinogenic to humans* cancer descriptor

Strong human evidence	<ul style="list-style-type: none">-Increased risk of lung, bladder, and skin cancer in humans exposed to complex PAH mixtures containing BaP.-Increased risk of lung cancer with increasing cumulative exposure to BaP.
Extensive animal evidence	<ul style="list-style-type: none">-Tumors in every animal species tested, by all routes of exposure.-Multiple tumor sites (alimentary tract, respiratory tract, skin, liver, kidney and auditory gland).
Strong evidence identifying key precursor events	Formation of DNA-reactive metabolites, DNA damage by the reactive metabolites, formation of DNA adducts, and formation and fixation of DNA mutations (particularly in tumor suppressor genes or oncogenes).
Strong evidence that key precursor events occur in humans	BaP-specific adducts and characteristic mutations (G→T transversions) in highly PAH exposed humans.



Conclusions for the Cancer Mode of Action and Early Life Susceptibility

- **Key events** include:
 - Bioactivation to reactive metabolites
 - DNA damage by reactive metabolites
 - Fixation of DNA mutations, particularly in tumor suppressor genes or oncogenes
 - Clonal expansion of mutated cells
- Other **potential contributing mechanisms**: oxidative stress, inflammation, immune suppression, cytotoxicity and regenerative cell proliferation, and aryl hydrocarbon receptor (AhR) signaling.
- The **overall evidence supports mutagenicity** as the primary mode of action for BaP-induced carcinogenicity.
- According to the 2005 U.S. EPA Supplemental Guidance for Assessing Susceptibility from Early Life Exposure to Carcinogens, individuals exposed during early life to carcinogens with a mutagenic mode of action are assumed to have increased risk for cancer.
- The BaP slope factors are derived for adult exposures, and do not reflect presumed early life susceptibility to this chemical.
- Therefore, EPA's **Age Dependent Adjustment Factors** (ADAFs: 10-, 3-, 1-fold) should be applied to account for increased early life risk.



Summary of the Dose Response Analysis for Oral Cancer Data

Principal Study	Elevated tumor types	Selected model	Oral Slope factor _{HED} (mg/kg-d) ⁻¹
Kroese et al. (2001) male rats	Forestomach and oral cavity squamous cell tumors; hepatocellular adenomas or carcinomas; small intestine adenocarcinomas; Kidney urothelial carcinomas; skin/mammary basal cell and squamous cell tumors	Multistage Weibull	0.5
Kroese et al. (2001) female rats	Forestomach and oral cavity squamous cell tumors; hepatocellular adenomas or carcinomas; small intestine adenocarcinomas;	Multistage Weibull	0.3
Beland and Culp (1998) female mice	Esophagus, tongue, and larynx squamous cell tumors	Multistage Weibull	1

- As data are not available to indicate one slope factor is more relevant for extrapolation to humans, the most sensitive slope factor was used to represent overall risk.

Oral Slope Factor = 1 per mg/kg-day

[see Table 2-7 of Toxicological Review]



Summary of the Dose Response Analysis for Inhalation Cancer Data

Principal Study	Elevated tumor types	Selected model	Inhalation Unit Risk (mg/m ³) ⁻¹
Thyssen et al. (1981) male hamsters	Upper respiratory and digestive tracts tumors (larynx, pharynx, trachea, esophagus, and forestomach)	Multistage Weibull, 2 ^o	0.6

- Only inhalation route cancer bioassay available.
 - Strengths: lifetime duration, nose-only exposure, examination of multiple organs, availability of individual animal pathology reports and weekly air monitoring.
 - Weaknesses: minimal detail about aerosol particle size, variability of chamber concentrations, and use of sodium chloride as carrier particle.
- Interspecies dosimetric adjustments could not be done due to the use of a hygroscopic carrier particle, so it was assumed that equal risk for all species would be associated with equal concentrations in air.

Inhalation Unit Risk = 0.6 per mg/m³



Summary of the Dose Response Analysis for Dermal Cancer Data

Principal Study	Elevated tumor types	Selected model	Dermal Slope factor _{HED} (µg/d) ⁻¹
Sivak et al. (1997); NIOSH (1989) male mice	Skin tumors (papillomas and carcinomas)	Multistage Weibull, 2°	0.006

- No established methodology for interspecies adjustments for dermal toxicity at the point of contact.
 - Several options for interspecies scaling calculations are presented in Appendix E.
 - Allometric scaling using body weight to the ³/₄ power selected based on general differences in dermal toxicokinetics between species.
- Dermal slope factor derived for a local effect (skin cancer) and not intended to estimate systemic risk of cancer following dermal exposure.

Dermal Slope Factor = 0.006 per mg/day

Neurobehavioral endpoint selected as the basis for RfD

Comment: EPA should not consider the neurobehavioral changes observed in the elevated plus maze (described as decreased anxiety-like effects) in adult rodents treated with BaP during development as an adverse effect.

EPA's Response:

- A normal level of anxiety is a protective function of the nervous system.
- A decreased ability of an organism to adapt to the environment is considered to be an adverse effect (US EPA, 1998).
- Any functional alteration resulting from developmental exposure is considered biologically relevant (US EPA 1991).
- See discussion regarding the significance of this endpoint in Sec 2.1.1.

Consideration of human skin graft mouse model

Comment: EPA should increase consideration of studies of PAH exposure in murine models with human skin grafts.

EPA's Response:

- Questions remain regarding the metabolic function, viability, and vascularization of the human skin grafts (some were from cadavers).
- Several potent carcinogens do not cause skin tumors in this model system.
- Mice with PAH-treated human skin grafts were followed for less than 7 months. Human squamous cell carcinoma is estimated to have a latency of > 20 years.
- Additional text regarding uncertainties of this model system and its ability to predict hazard for human skin cancer risk has been added to Section 1.1.5.



Apparent threshold in animal cancer bioassays

Comment: The animal carcinogenicity studies used in the derivation of the oral slope factor, inhalation unit risk, and dermal slope factor demonstrate threshold exposures for BaP.

EPA's Response:

- Animal bioassays cannot identify thresholds due to limited power to detect levels of cancer risk less than 10%. For example:
 - Thyssen et al, 1981: tumor incidence at lowest concentration: 0/19 (95% confidence interval = 0 – 20%)
 - Sivak et al., 1997: tumor incidence at lowest at lowest dose: 0/30 (95% confidence interval = 0 – 13%)
 - Oral bioassays of BaP demonstrated elevated tumor responses at all exposure levels tested.
- Thresholds are more reliably determined through consideration of modes of action and toxicokinetic pathways.



Inclusion of studies of patients therapeutically treated with coal tar

Comment: EPA should include epidemiological studies of skin cancer risk in eczema and psoriasis patients treated therapeutically with dermatological formulations containing coal tar (a PAH mixture).

EPA's Response:

- There are limitations to this body of literature, particularly relating to the level of detail regarding exposure measures, length of exposure, length of follow-up, and ability to address effects attributable to other types of therapies.
- Acute studies of coal tar exposure in patients provide in vivo evidence of BaP-specific genotoxicity (BaP-DNA adducts).
- See increased discussion of these studies in Sec 1.1.5 and Appendix D.3.3.



Validation of dermal slope factor

Comment: EPA should perform calculations to determine if the proposed dermal slope factor is scientifically supportable. These commenters stated that based on their calculations, the current dermal slope factor would indicate that BaP in soil is the cause of 30% of all human skin cancers in the US.

EPA's Response:

- EPA could not reproduce these calculations.
- Example calculations have been performed by EPA using an equation for average daily dose of BaP contacting the skin (not absorbed) and the associated risk at the daily dose.
- Central tendency exposures using BaP soil concentrations of 100 ppb (a central estimate from uncontaminated sites), results in risks in range of 10^{-6} .
- Details of example calculations are provided in Appendix G.

The BaP assessment:

- 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.